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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
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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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# 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 adjuvant |
| 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) |
| $GA_3$ | Gibberellic acid $A_3$ |
| 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 |
| $P_{max}$ | 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 |

## 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

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 $ng.hour^{-1}.fruit^{-1}$, but diffusible IAA 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 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-4-pyridyl_-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.

# 1. General Introduction

## 1.1 Kiwifruit as a Horticultural Crop

### 1.1.1 The kiwifruit industry in New Zealand

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*) as it is now recognised internationally, originated in the temperate forests of south-western China (Ferguson, 1990a). The plant formerly known as 'chinese gooseberry', was introduced to the western world at the beginning of the 20th century. Plant specimens and seeds of *Actinidia chinensis and Actinidia deliciosa* were distributed to Europe, the United states and New Zealand by missionaries and other botanical enthusiasts (Ferguson and Bollard, 1990). The cultivar 'Hayward' which is the main commercial cultivar throughout the world, was a selection made from seedlings by the nurseryman Hayward Wright (Ferguson and Bollard, 1990). However 'Hayward' did not become predominant until New Zealand's export markets showed a preference for this cultivar in the 1970's (Sale, 1992). By 1980, 98.5% of kiwifruit planted in the Bay of Plenty (the main kiwifruit growing region of New Zealand) was 'Hayward' (Hall and Kernohan, 1980) cited in Ferguson and Bollard (1990). 'Hayward' has become the industry standard due to a number of characters of the fruit such as large size, good flavour, long storage potential and its comparatively lower vigour (Ferguson et al., 1990), although it tends to produce less fruit than other cultivars (Sale and Lyford, 1990). However reliance on a single cultivar can be unwise because of the possibilities of pest or disease outbreaks, changes in consumer desires and loss of competitive advantage in the market. Thus the development of new cultivars of kiwifruit with different agronomic and consumer values is of major importance and the New Zealand kiwifruit industry has identified a need for new cultivars of kiwifruit. An important requirement for new kiwifruit varieties is an improvement in the size of fruit, as most other species of *Actinidia* have fruits considerably smaller than 'Hayward'.

Control over fruit size is an essential feature of orchard management, as there is almost always a trade-off between total yield and fruit size (Cooper and Marshall, 1992). In recent years the New Zealand kiwifruit marketing board (NZKMB) placed considerable emphasis on marketing larger fruit sizes and has avoided marketing the smallest size grades altogether. This has partially been a response to an over-supply of fruit both in the world market and from New Zealand kiwifruit growers, as well as a response to the demand for larger fruit. In addition, there is also a demand in certain markets for very large fruits. Financial encouragement by means of a premium price for large size fruit has been provided to growers from the NZKMB. For example, returns to growers per fruit from the larger fruit sizes (25-30 count) were approximately double that of small fruit sizes (39-42 count) in 1993 (NZKMB, 1994a). In addition a large market potential has been identified for jumbo sized fruit (NZKMB, 1994b).

Commercial plantings of kiwifruit in New Zealand were made in the early 1930's and the popularity of the fruit increased gradually until the early 1970's when kiwifruit plantings increased exponentially in response to a lucrative export market. Following this commercial success, kiwifruit production began in earnest in several other countries most notably Italy, France, Chile, Japan and USA. Despite recent problems in the industry, kiwifruit is still an important fresh fruit commodity. In 1994/95 world production was estimated at around 800 000 tonnes (Strzelecki, 1995) and 1996 export returns for New Zealand kiwifruit were estimated at NZ$ 271.5 million (Anon., 1997).

### 1.1.2 Taxonomy

The genus *Actinidia* consists of a group of perennial deciduous plants originating from eastern or southern Asia (Ferguson, 1984). Actinidia has been placed in the family Actinidaceae with a closely related genera, Saurauia, probably in the order Theales (Ferguson, 1984). There are more than 50 species and 100 taxa in the genus *Actinidia*, all of which have a climbing habit and are commonly found growing wild as part of the forest under-storey in temperate forests of mountains and hills in south western China (Ferguson, 1990a).

The two main economically important species of Actinidia are *A. deliciosa* and *A. chinensis*. *A. deliciosa* is the species known around the world as kiwifruit and is commercially grown in many countries for domestic consumption and export. There are a large number of named cultivars of *A. deliciosa* including 'Allison', 'Bruno', 'Constricted', 'Elmwood', 'Gracie', 'Hayward', 'Monty' and 'Skelton' (Thorp et al., 1990; Allan, 1982; Anon., 1991; Zhang and Thorp, 1986).

*A. chinensis* is the second species of importance. Large quantities of *A. chinensis* fruit are collected from the wild in China for domestic consumption (Ferguson, 1990c) and considerable effort is being made to select *A. chinensis* cultivars with desirable characteristics for commercial production (Seal, 1992). *A. chinensis* fruit are generally smaller than those of *A. deliciosa* fruit. For example, five selections from the HortResearch breeding program studied by Clayton (1996) had a final mean fruit weight of between 50 and 70g (values estimated visually from graphs), whereas 'Hayward' averages around 100g (Thorp et al., 1990). However some *A. chinensis* selections in New Zealand and China are reported to be of a similar size or larger than 'Hayward' kiwifruit (Ferguson, 1992b). *A. chinensis* may also have a number of advantages over *A. deliciosa* including a high yield, sweeter 'subtropical' flavour and earlier maturity (Ferguson, 1992b) and several new cultivars have been released for grower trials in New Zealand (A. Seal, Personal communication 1996) .

### 1.1.3 Horticultural characteristics

Most members of the *Actinidia* genus are functionally dioeceous (Schmid, 1978; Ferguson, 1984). Although pistillate flowers on female vines produce pollen it is nonviable, while ovaries present on flowers from male vines do not contain viable ovules (Schmid, 1978). Thus there is a requirement for both male and female vines in order for cross pollination to occur. As very high numbers of seeds (700+) are required to attain export sized 'Hayward' kiwifruit (Hopping and Hacking, 1983), pollination is a high priority for growers (see section 1.2.4).

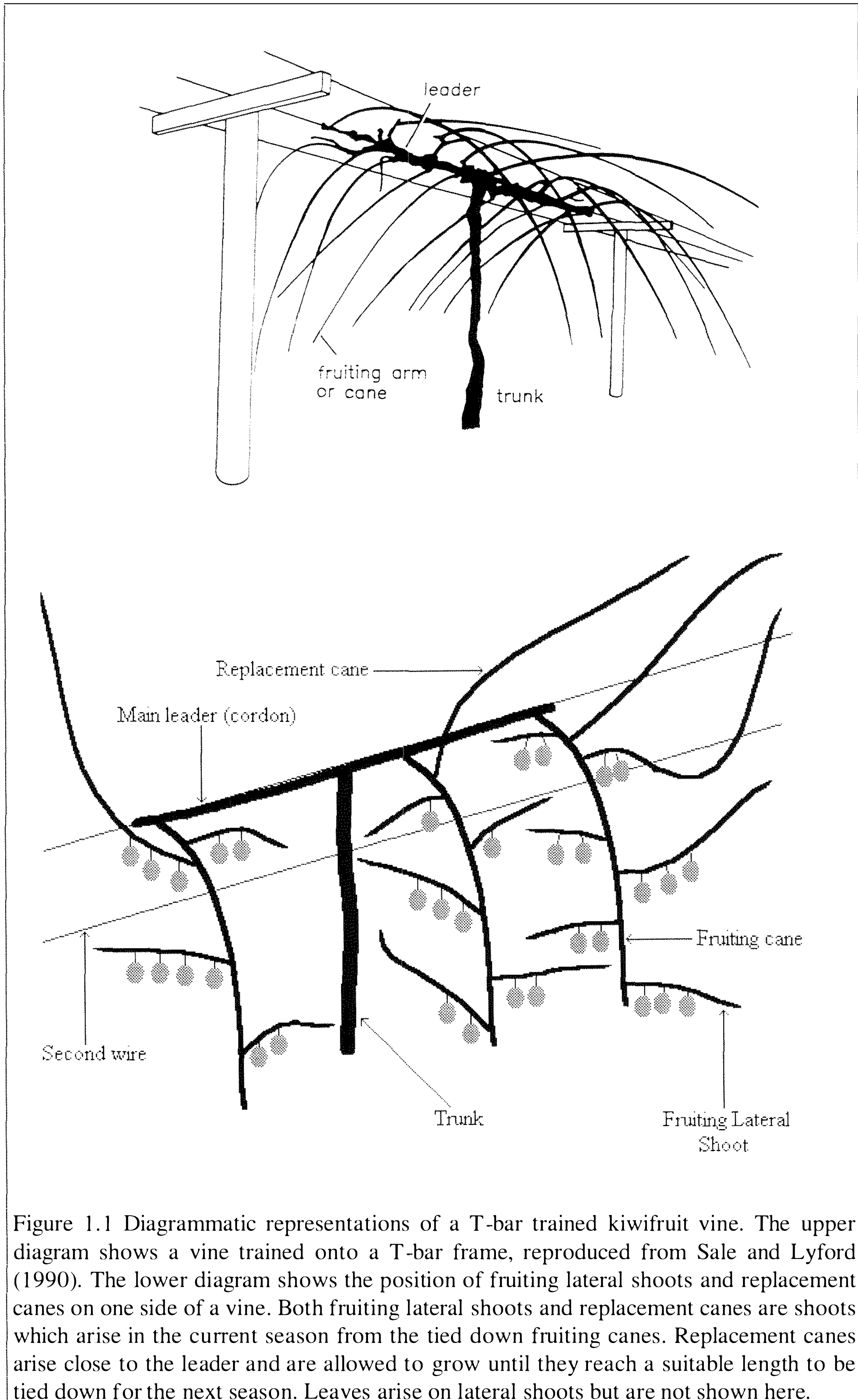


Figure 1.1 Diagrammatic representations of a T-bar trained kiwifruit vine. The upper diagram shows a vine trained onto a T-bar frame, reproduced from Sale and Lyford (1990). The lower diagram shows the position of fruiting lateral shoots and replacement canes on one side of a vine. Both fruiting lateral shoots and replacement canes are shoots which arise in the current season from the tied down fruiting canes. Replacement canes arise close to the leader and are allowed to grow until they reach a suitable length to be tied down for the next season. Leaves arise on lateral shoots but are not shown here.

The specific growing requirements of kiwifruit are free draining soils, an adequate supply of moisture particularly when young, relatively high atmospheric humidity and a period of winter chilling (Sale and Lyford, 1990). *A. deliciosa* are frost tender when in leaf (Sale and Lyford, 1990), although other species, particularly *A. kolomikta* (Warren Wilson, 1972) and *A. arguta* (Ferguson, 1991), are frost hardy and can survive winter temperatures of as low as -40°C.

Most commercial plantings are based on rows of plants trained on either a T-bar or a pergola structure (Sale, 1982) and these structures have been found to promote high yields of export grade fruit, while minimising pruning time (Hopping et al., 1993). The structures support the weight of the crop, as well as allowing accurate canopy management to restrict vegetative growth which if unchecked may become excessively vigorous at the expense of fruit growth. Vines are grown as a single straight trunk and upon reaching the top of the structure, a single permanent leader or 'cordon' is allowed to grow in each direction along a central wire (Figure 1.1). One year old canes are selected each year on the basis of length, thickness and closeness to the main leader and are tied down onto the support wires (Figure 1.1). In spring, bud-break occurs followed by leaf expansion and the development of flower buds on these shoots (Brundell, 1975a). As flowers arise only from the current seasons growth that originates from the one year old canes (Sale and Lyford, 1990), bud-break is of critical importance for overall yield. Although a lack of winter chilling often limits bud-break (Lionakis and Schwabe, 1984; McPherson et al., 1995), the requirement for winter chilling can be overcome to some extent by the application of the dormancy breaking chemical, hydrogen cyanamide (Lionakis and Schwabe, 1984).

Kiwifruit vines display strongly vigorous vegetative growth and intensive summer pruning is required in order to control it and maintain vines in a manageable state (Sale, 1981). Summer pruning involves the removal of actively growing shoot tips from fruiting lateral shoots, removal of re-growth from lateral buds and removal of tangled growth from around fruits, canes and lateral shoots. Summer pruning may be required several times during the season, particularly during mid-summer when vegetative growth is extremely rapid (Sale and Lyford, 1990). In winter, when vines are in a dormant state, tied down canes which have provided fruiting lateral shoots in the previous season are

pruned off as close to the leader as possible. These are replaced with new 'replacement canes' which have arisen as vigorous new growth close to the leader during the previous season (Figure 1.1).

Commercial kiwifruit orchards have traditionally used 'Hayward' scion grafted onto seedling 'Bruno' rootstock. Seedling rootstocks are used because of the low cost of propagation and success of grafting, rather than productive advantages of seedling rootstocks (Lawes, 1990). Lawes et al. (1990) reported that the initial growth of 'Hayward' clones on their own roots was as good as or better than grafted plants. However growers may be reluctant to use cutting grown plants due to a perception that they may be inferior (Lawes, 1990). Several authors have demonstrated that kiwifruit rootstock selections have the potential to affect fruit growth and vine productivity. Cruz-Castillo (1994) found in a trial of different *A. deliciosa* rootstocks selected from commercial orchards, that the performance of rootstocks prior to selection for the trial was in most instances correlated with their performance in the rootstock trial. This demonstrates that the selection of kiwifruit rootstocks based on field performance can be transferred to clonally propagated rootstocks for future plantings. 'Hayward' scion grafted to a Te Puke (New Zealand) selection of *A. hemsleyana* (TR2, Kaimai) has been reported to allow production of around twice as many flowers as seedling rootstocks and produced a similar average fruit size despite carrying a 20% higher crop load (Lowe et al., 1992). Rootstock selections of *A. eriantha and A. rufa* also showed increased flowering, whereas *A. chinensis* showed decreased flowering (Lowe et al., 1992; Wang et al., 1994). The increase in flowering was found to be due to decreased floral abortion in spring of flower primordia (Wang et al., 1994), possibly due to increased mobilisation of carbohydrates to shoots at this time (Lowe et al., 1992).

### 1.1.4 Floral and fruit Anatomy

Pistillate kiwifruit flowers are considered to be a compound dichasium, comprising a terminal (king) flower and up to two smaller lateral flowers (Hopping, 1990) (Figure 1.2). The occurrence of lateral flowers varies between cultivars due to abortion of these

flowers during development (Ferguson, 1984). For example, Brundell (1975b) found that 94% of 'Hayward' inflorescences were single flowers, while in 'Allison' and 'Monty' only 46% and 6% respectively were single. As fruit arising from lateral flowers are of inferior size, these are usually removed in commercial orchards prior to pollination.

The anatomy of pistillate flowers and fruit of *A. deliciosa* have been described by Habart (1974), Hopping (1976b) and Schmid (1978), although these studies referred to the fruit as *A. chinensis* (var *hispida*). Re-classification of *A. chinensis* var. *hispida* to *A. deliciosa* has subsequently been made (Ferguson, 1984). Almost all histological information is based on observations made on transverse fruit sections (Habart, 1974; Schmid, 1978; Hopping, 1976b; Gould et al., 1992; Woolley et al., 1992; Cruz-Castillo, 1994; Patterson et al., 1993). Longitudinal description of vascularisation have been made by Habart (1974) and Schmid (1978).

Fruit develop from a superior, syncarpous ovary (Hopping, 1976b). Locule numbers vary between *Actinidia* species and cultivars. For example *A. deliciosa* 'Hayward' and 'Bruno' contain an average of 40 and 31 locules respectively (Thorp et al., 1990), while *A. chinensis* selections contained between 24 and 32 locules (Harvey and Fraser, 1988). However up to 46 locules have been reported in 'Hayward' kiwifruit (Trustrum, 1983). Each locule contains two rows of up to twenty anatropous unitegmic ovules (Schmid, 1978), though the number of ovules per locule is considerably lower in *A. chinensis* than in *A. deliciosa* (Harvey and Fraser, 1988). *A. deliciosa* 'Hayward' fruit with up to 1861 seeds have been reported (Pyke and Alspach, 1986), although most well pollinated fruit will contain less than 1400 seeds (Hopping, 1976a).

The post anthesis growth of seeds begins within 96 hours of fertilisation characterised by cell division in the endosperm (Harvey and Fraser, 1988) and seed size continues to increase for 80 days in *A. deliciosa* (Hopping, 1976b). The embryo remains in a resting stage in the uninucleate stage for eight to ten weeks after fertilisation in both *A. deliciosa* and *A. chinensis* (Harvey and Fraser, 1988) and thereafter commences cell division and growth reaching full size and development at 110 days (Hopping, 1976b; Harvey and Fraser, 1988). As seed development continues, the locules become progressively filled with large, thin-walled placental cells which surround the seeds (Hopping, 1976b).

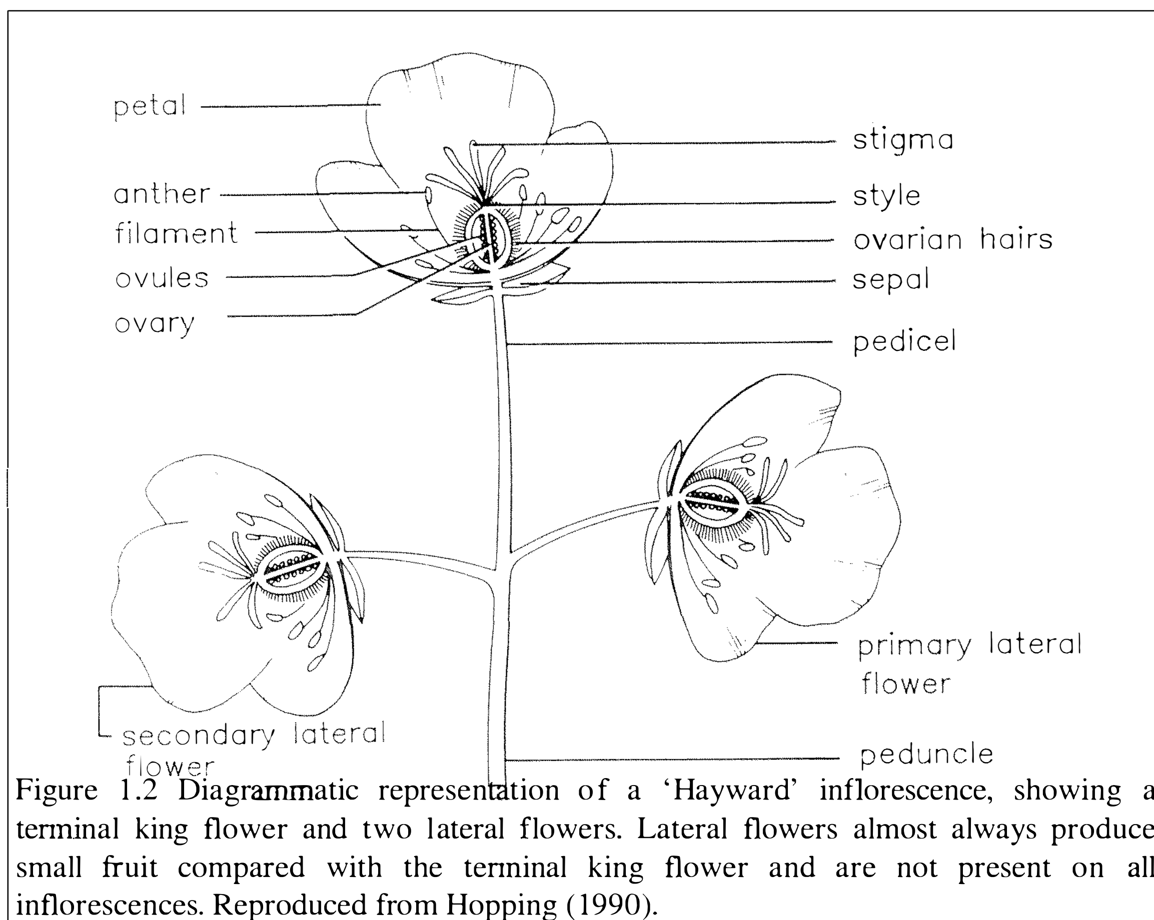


Figure 1.2 Diagrammatic representation of a 'Hayward' inflorescence, showing a terminal king flower and two lateral flowers. Lateral flowers almost always produce small fruit compared with the terminal king flower and are not present on all inflorescences. Reproduced from Hopping (1990).

The ovary is surrounded by a thin epidermal layer of between one and four cells (Habart, 1974; Hopping, 1976b). At anthesis, the pericarp of the fruit consists of relatively uniform parenchymatous cells which extend from the epidermis to a distinct core (Plate 1.2, Plate 1.3) (Schmid, 1978), although the existence of a distinct hypodermal layer of suberised cells is suggested by some authors (Habart, 1974; Hopping, 1976b). The pericarp can be conveniently divided into an inner and outer pericarp by a series of vascular bundles (Plate 1.2) (Hopping, 1976b), defined as dorsal carpellary bundles (Schmid, 1978). As fruit develop, the structure of both inner and outer pericarp becomes considerably more complex. In the outer pericarp, some cells remain comparatively small (small cells) and accumulate starch grains (Gould et al., 1992). Other cells increase several fold in diameter (large cells), do not accumulate starch at all, and have been termed 'juice cells' (Gould et al., 1992). The inner pericarp surrounds the seeds and extends to an inner ring of vascular bundles (Plate 1.3), defined as the ventral carpellary bundles (Schmid, 1978). Inner pericarp tissue cells become elongated in the radial direction, particularly between locules in the locule wall, although more tangential

enlargement occurs immediately beneath the outer pericarp margin (Hopping, 1976b). Inside the inner pericarp is a central core or columnella (Habart, 1974) which is composed of a homogeneous population of parenchymatous cells (Plate 1.1).

### 1.1.5 Potential for fruit growth in the genus *Actinidia*

All aspects of fruiting are subject to genetic regulation (Bringhurst et al., 1989). Regulation may be via the intrinsic characteristics of the fruit itself, or indirectly by the root-system or canopy characteristics. For example, the major difference in characteristics such as colour, flavour and texture between a 'Red Delicious' and 'Granny Smith' apple are inherently due to the fruit scion itself, however these scions grafted to a dwarfing root-stock such as M9 can produce fruit of a larger size than if non-dwarfing or seedling rootstocks are used. Genetic factors which may contribute to increased fruit size include increased fruit cell numbers (Bohner and Bangerth, 1988a), improved source-sink partitioning toward fruit growth (Hanson, 1977), improved canopy light interception (Palmer, 1989), higher levels of floral initiation and set, disease resistance, tolerance to climatic extremes and pollinator compatibility (Alston and Tobutt, 1989).

Comparatively, the kiwifruit industry is based on a very narrow genetic base. Although there are around 50 different species in the genus *Actinidia*, world production is almost exclusively based on the pistillate cultivar 'Hayward' (Ferguson, 1990b). In recent years considerable effort has been made towards selecting new cultivars with characteristics such as increased productivity (Lowe et al., 1992; Seal, 1992), fruit with different shape, colour or flavour (Seal, 1992), tolerance to adverse environmental conditions (Viti et al., 1990; Chat, 1995), hermaphroditism (Bellini et al., 1991; Messina et al., 1990) and early fruit maturity (Pringle et al., 1992). There is considerable variation in fruit size within the *Actinidia* genus. Within the *species A. deliciosa*, the average weight of different cultivars range between 65 and 100g (Zhang and Thorp, 1986). Fruit of *A. chinensis* are smaller than *A. deliciosa* weighing around 50g, although selection of larger fruit has been made by plant breeders (Xie et al., 1992). At the other end of the size spectrum, *A. arguta* average 5 to 10g (Ferguson, 1991) and *A. kolomikta* 2 to 5g (Ferguson, 1992a).

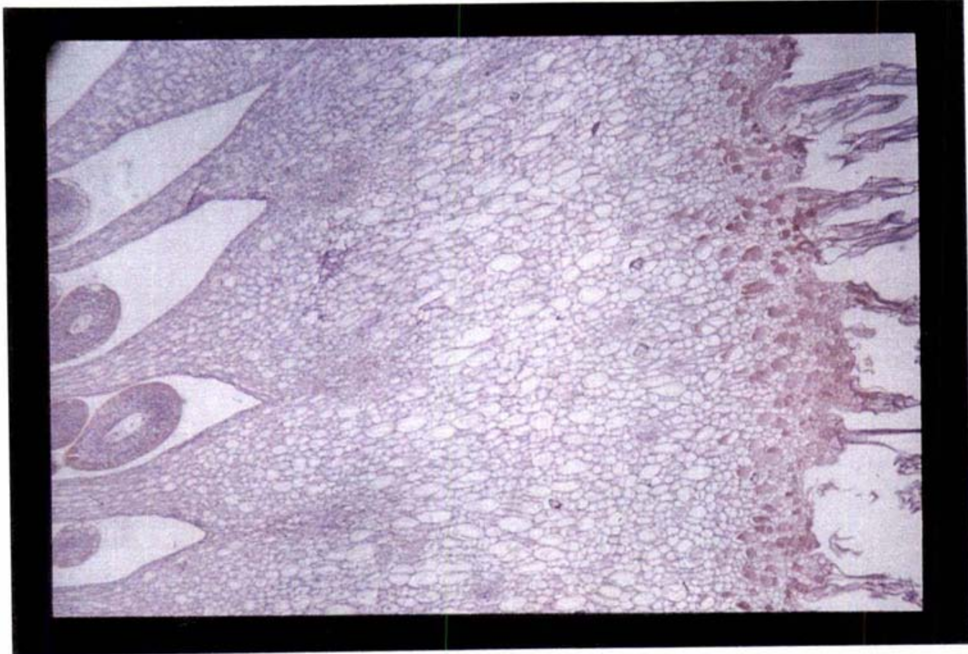

Plate 1.1 Transverse section of a 'Hayward' kiwifruit seven days after anthesis showing undifferentiated inner and outer pericarp tissue. Dorsal carpellary vascular bundles can be seen to the right of the locules. Magnification 30x.

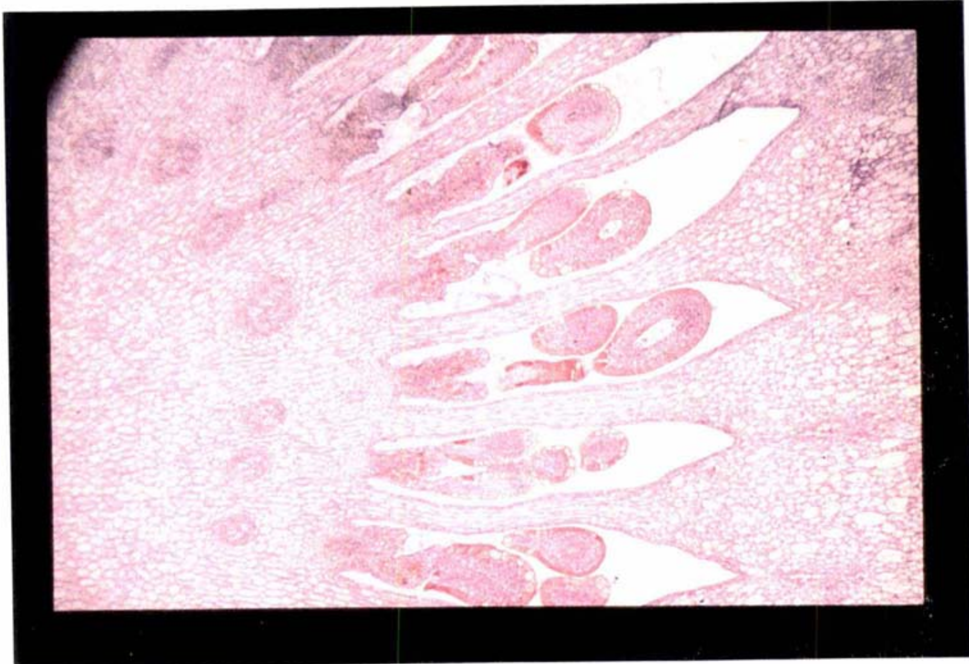

Plate 1.2 Transverse section of a 'Hayward' kiwifruit seven days after anthesis showing core tissue, developing seeds in locules and undifferentiated inner pericarp tissue. Ventral carpellary vascular bundles can be seen to the left of the locules. Magnification 30x.

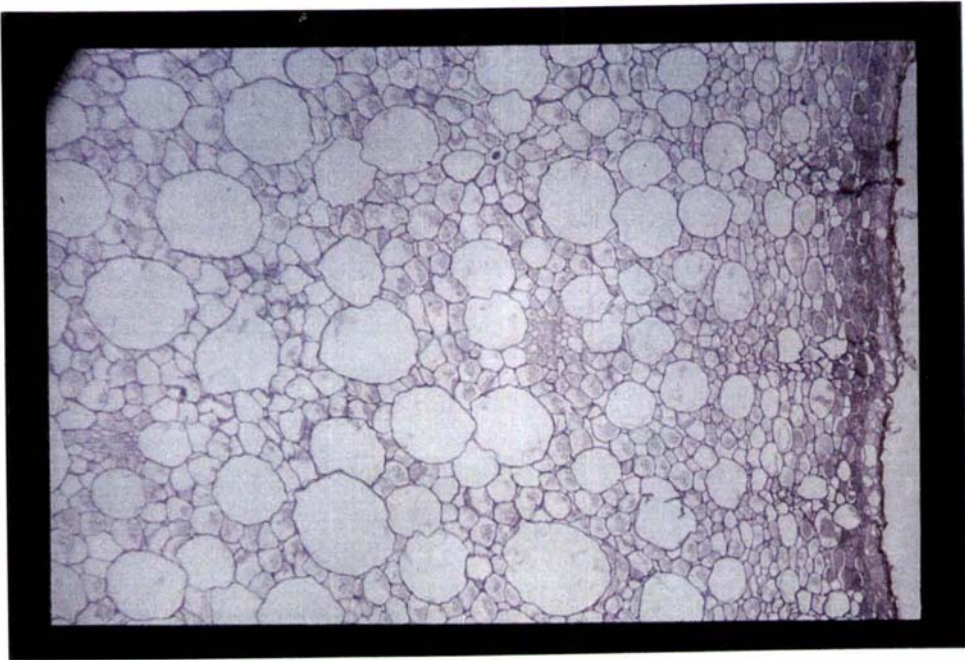

Plate 1.3 Outer pericarp transverse section from 'Hayward' kiwifruit at maturity. Large 'juice' cells are dispersed throughout the outer pericarp among small, starch containing parenchyma cells. Magnification 30x.

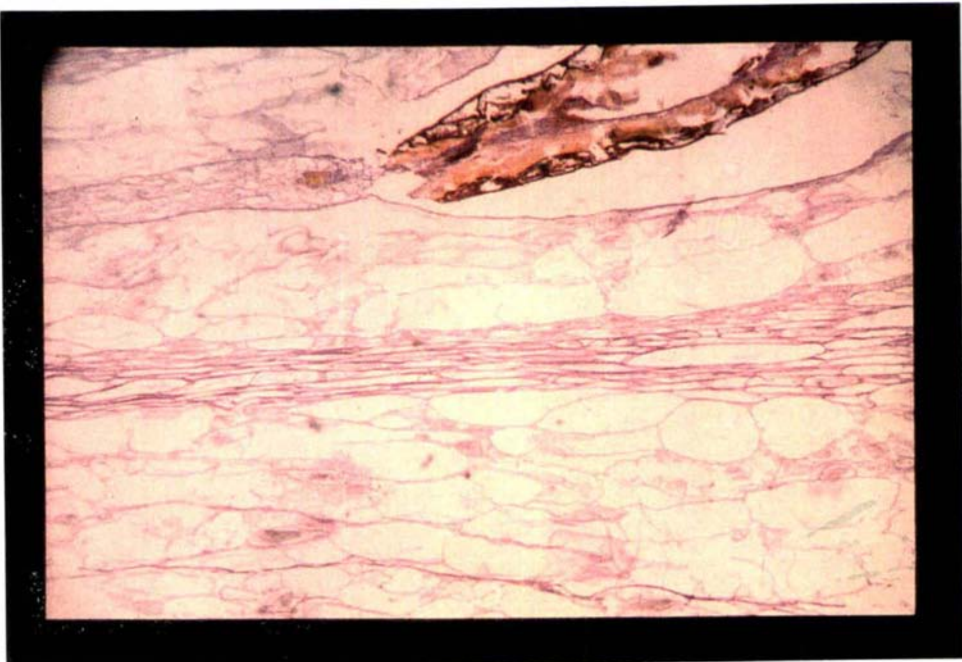

Plate 1.4 Inner pericarp transverse section from 'Hayward' kiwifruit at maturity. Long thin cells elongated perpindicular to the epidermis form the locule wall (centre) which runs between large placental cells which surround seeds (top right). Magnification 30x.

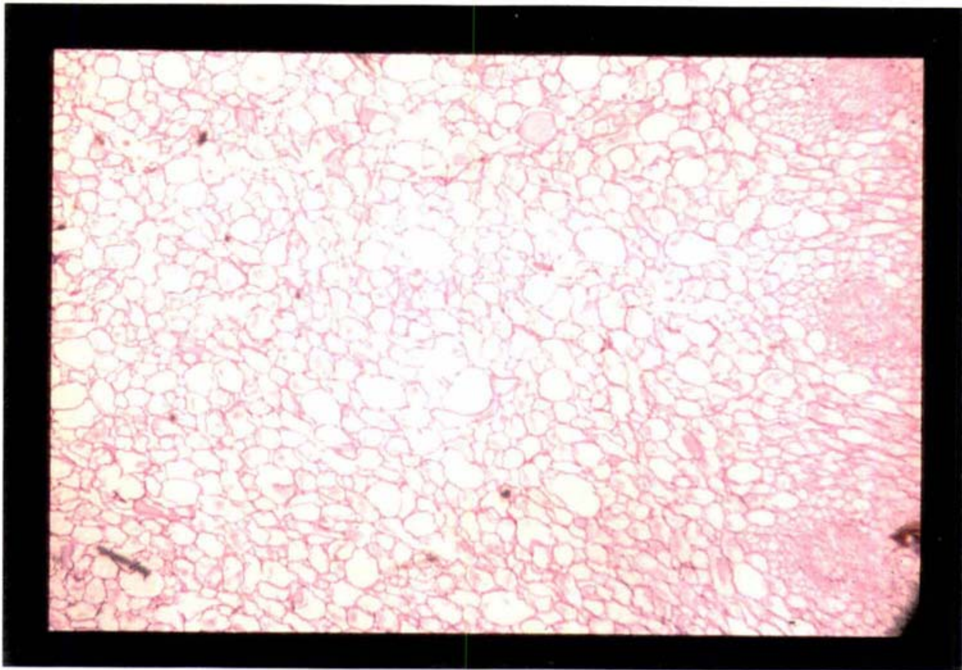

Plate 1.5 Core transverse section from 'Hayward' kiwifruit at maturity. Core tissue is composed of isodiametric starch containing parenchyma cells and the core is bordered by a ring of ventral carpellary vascular bundles (left). Magnification 30x.

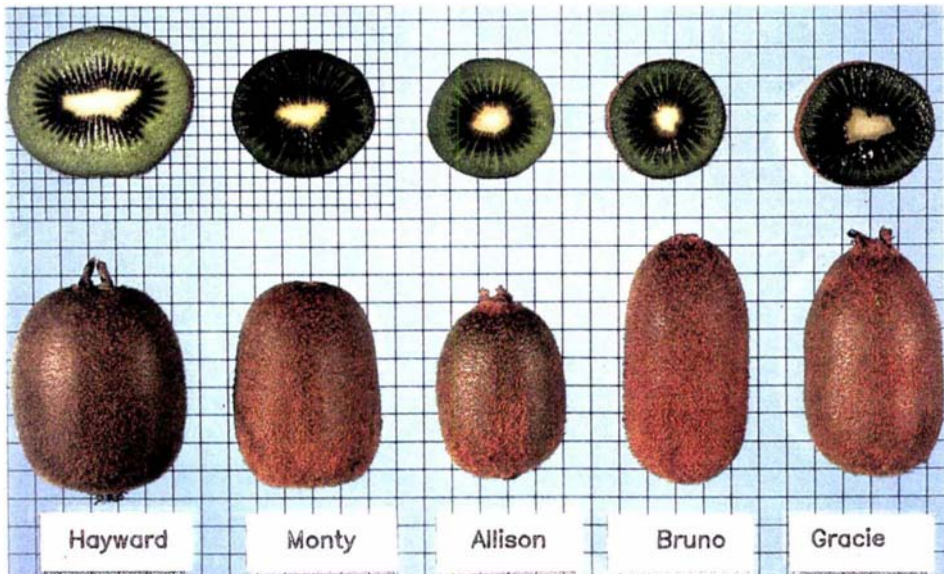


Plate 1.6 Five named cultivars of Actinidia deliciosa. Reproduced from Beever and Hopkirk (1990).

## 1.2 Fruit Sink Strength Characteristics

### 1.2.1 Definition of sink and source strength

A sink is defined as a plant organ which is a net importer, while a source is defined as a plant organ which is a net exporter (Warren Wilson, 1972). In this thesis, source-sink terminology refers to sources and sinks for carbohydrates. As sinks such as reproductive structures (flowers and fruit) and storage organs (e.g. tubers, rhizomes), usually form the yield forming parts of plants, they are of primary economic, as well as physiological importance. Usually the most important sources are the leaves of a plant which produce photosynthetically fixed carbohydrate, although storage organs will at some stages of development switch from accumulation of assimilates (sink) to become a net exporter of the stored assimilates (source). The most important source-sink relationships are the interactions between leaves and the variety of sinks present on a plant competing for exported assimilates.

Once produced by source organs (mainly leaves), carbohydrates may be initially partitioned between use by the source leaf and transport out to other plant organs. Carbohydrates available for transport must then be allocated between the many competing sinks on the plant. Competing sinks include roots, expanding shoot tips, expanding leaves, buds, flowers, fruits and stems. Although productivity is likely to be improved by increasing the allocation of photo-assimilate to harvestable sinks (Turner, 1986), net accumulation of carbohydrate will be either source or sink limited depending on the type of sink and stage of development (Patrick, 1988).

The ability of individual sink to accumulate assimilates is determined by an intrinsic property of the sink and this has been defined as sink strength. Sink strength was initially defined by (Warren Wilson, 1972):

**Sink strength = sink size x sink activity,**

where sink size is the dry weight of the sink and sink activity is the relative growth rate. However sink strength defined in this way has a number of shortfalls. Firstly, dry matter

values do not account for respiratory losses which may be as high as 50% of the total accumulated carbohydrates (Wareing and Patrick, 1975). Secondly, the equation formulated in this way does not consider the availability of carbohydrate which may be limited and therefore competition with other sinks will occur. Thus sink strength estimated from dry matter data is more aptly considered as competitive ability relative to other sinks (Wareing and Patrick, 1975), or apparent sink strength (Ho, 1988).

### 1.2.2 Competitive ability of a sink

At most times there will be a competitive situation between the sinks present on a plant. In the case of a fruiting plant, there will be both inter-fruit competition, as well as competition between fruit and other sink organs such as shoot apices, expanding leaves and the root system. The availability of mobile assimilates to a particular sink, will be determined by the competitive ability of individual sink organs (Ho, 1988). The competitive ability of various organs is consistent through a plant, but may change as a plant grows and develops (Ho and Hewett, 1986). For example in tomato, where extensive work has been done on sink hierarchy, the apex appears to be a stronger sink than an initiating inflorescence but as inflorescences develop into fruit, they become the dominant organ (Ho, 1988; Ho and Hewett, 1986). Based on the relative effect of defoliation on individual components of kiwifruit vines, Buwalda and Smith (1990b) suggested the ranking of competitive strength of sink organs present on a kiwifruit vine during the growing season is shoots > fruit > roots > return bloom. Root growth and return bloom in particular have been found to be more sensitive to a limitation in carbohydrate availability than fruit growth in kiwifruit (Buwalda and Smith, 1990; Snelgar and Manson, 1992a; Tombesi et al., 1993).

Proximity to the source of carbohydrate is important in determining competitive ability. Sinks which are close to a source appear to have an advantage in obtaining carbohydrate (Patrick, 1988; Wardlaw, 1990). However the actual distance that assimilate must be transported between source and sink is not usually the factor which limits sink accumulation, rather the presence of closer and/or stronger sinks which are able to divert assimilate (Wardlaw, 1990). Thus the competitive ability of sinks is particularly

important for sinks distant from the carbohydrate source (Cook and Evans, 1978). Kiwifruit are able to obtain carbohydrate from relatively distant sources on a vine. Lai et al. (1989b) found that fruit on lateral shoots with few leaves were able to easily obtain carbohydrate from neighbouring laterals. Even fruit from lateral shoots with no leaves were not found to be adversely affected compared with fruit from lateral shoots with many leaves (Lai et al., 1989b). When Buwalda and Smith (1990) severely defoliated the lateral shoots in the upper canopy (replacement cane zone) of kiwifruit vines, fruit weight was reduced in all parts of the vine, but to a greater extent within the region of defoliation. When defoliation was carried out in the tied down canes (fruiting zone), the growth of fruit in this zone was severely inhibited, while fruit weight in the replacement cane zone, which has a high leaf:fruit ratio, was not affected. The apparent contrast between the results of Lai et al. (1989b) and Buwalda and Smith (1990) demonstrate the importance of proximity to the source of carbohydrate when competing sources are present and carbohydrate is very limited.

### 1.2.3 Dominance or correlative inhibition

Competition between sinks may be amplified when the assimilate supply is limited (Ho, 1988). However competition for assimilates does not explain all of the observations associated with dry matter accumulation by competing sinks. For example dominance of one sink over another can be observed very early in fruit development when assimilate supply is unlikely to be limiting (Bohner and Bangerth, 1988b). In some cases competition is so severe that complete dominance of one sink over another occurs and the growth of some sinks is completely excluded (Bangerth, 1989). For example abscission of reproductive structures in the presence of other developing fruit commonly occurs in apple (Quinlan and Preston, 1971), bean (Tamas et al., 1979) and soybean (Heindl and Brun, 1984). Dominance phenomena are widespread, the most well known being the inhibition of growth of lateral buds by the presence of the apical bud which is known as apical dominance (Tamas, 1995). Dominance is also common between fruit (Bangerth, 1989) and may occur between fruit and vegetative sinks (Garcia-Martinez and Beltran, 1992), although a distinction between dominance and competition is often difficult to make (Bangerth, 1989). However some form of sink priority occurs in many

situations, often related to the time of initiation of development, with earlier initiated fruit often dominant over later initiated ones (Ho, 1992). For example in tomato, proximal fruit normally grow to a larger size than the distal fruits which normally set several days later unless pollination is synchronised (Bohner and Bangerth, 1988b). However if distal tomato fruit on a truss are induced to set prior to proximal fruit the dominance is reversed such that distal fruit are larger (Bangerth and Ho, 1984). The dominance of the earlier formed ovaries over later formed ones has been termed primigenic dominance to identify the dissimilarity to apical dominance which is dependent on the morphological position on the plant (Bangerth, 1989).

In addition to the timing of fruit set, the number of seeds formed may influence dominance relationships. In many fruit species, fruit with high seed numbers are often dominant over fruit which are poorly pollinated. For example, the growth of zucchini fruit with low seed numbers is inhibited by nearby fruit with high seed numbers (Stephenson et al., 1988). The presence of seeded persimmon fruit on the same tree increased the proportion of seedless fruit which abscised (Kitajima et al., 1992). Similarly, removal of seeds from older dominant bean pods was found to release both lateral buds (Tamas et al., 1981) and young fruits from dominance (Tamas et al., 1986).

Basipetal transport of auxin appears to be involved in inter-sink dominance relationships. Apical dominance can be maintained by replacing the apex with a source of auxin (Thimann and Skoog, 1934), while the application of auxin transport inhibitors below the apex releases lateral buds from dominance (Tucker, 1978). The rate of indole-3-acetic acid (IAA) diffusing from dominant organs has been found to be higher than from inhibited organs (Bangerth, 1989). Releasing dominance by removal of the dominant tomato fruit from a truss or the king apple fruitlet from a cluster results in increased rates of auxin transport from the previously inhibited organs (Gruber and Bangerth, 1990). Young bean pods were able to be released from dominance by the removal of seeds from dominant older pods, however dominance could be re-established by replacement of seeds with auxin (Tamas et al., 1986). Dominance relationships between zucchini fruit has been found to be partially related to the number of seeds in the fruit, with lower seeded fruits less able to exert dominance over high seeded fruits even if these developed later (Stephenson et al., 1988). Diffusion of auxin from tomato fruit has been shown to

be related to the presence or number of seeds (Sjut and Bangerth, 1984; Bangerth et al., 1989), which suggests that developing seeds may be largely responsible for auxin production and may therefore provide fruit with the ability to export auxin and establish/maintain correlative inhibition of competing sinks.

The mechanism by which IAA signals from dominant organs inhibits the growth of inhibited organs is not clear. Active IAA transport only occurs in a basipetal direction (Lomax et al., 1995), thus clearly it cannot move into apical buds to inhibit them (Bangerth, 1989). Bangerth (1989) has suggested that the IAA signal from dominant organs causes autoinhibition of polar IAA transport from inhibited organs. This hypothesis is based on the assumption that IAA transport is essential for the growth of a sink organ for reasons such as differentiation of vascular traces and assimilate transport (Bangerth, 1989). There are also indications that inhibition of auxin transport out of an organ causes reduced free IAA levels in the organ itself due to either increased metabolism or reduction in synthesis (F. Bangerth, personal communication 1996). Thus there could be a direct influence of auxin levels on cell division and expansion of sink cells or on metabolism and compartmentalisation of imported assimilates. In contrast, application of the polar auxin transport inhibitor (PATI) naphthylphthalamic acid (NPA) to cucumber ovaries stimulates parthenocarpic fruit development (Quebedeaux and Beyer, 1972; Kim et al., 1994). Similarly another PATI 2,3,5-triodobenzoic acid sprayed onto cucumber plants induced parthenocarpic development of parthenocarpic fruits which grew to a similar size to pollinated fruit (Cantliffe et al., 1972), while application of 25 ppm NPA to tomato plants has been found to stimulate fruit set and increase yield (Moore, 1957). The effects of auxin transport inhibitors on fruit set and development suggest that IAA export itself may not be essential for fruit growth.

Dominance relationships may be related to interactions with cytokinins. Application of cytokinins to axillary buds has been shown to release these from apical dominance (Sachs and Thimann, 1967; Boswell et al., 1981; Elfving, 1985). Correlations of endogenous cytokinin levels to the degree of correlative inhibition have been found in many situations. Protea stems afflicted with witches broom, a malformation which causes a loss of apical dominance, were found to have massively higher endogenous cytokinin levels in stems at the stage of maximum loss of inhibition, although levels declined fairly quickly

(Cutting, 1991). Endogenous cytokinin levels were found to increase in decapitated normal branching Cal John tomato whereas no such increase occurred in the non-branching tomato mutant Torosa-2 (Mapelli and Lombardi, 1982). It was concluded that insufficient quantities of cytokinin for lateral bud differentiation led to the strong apical dominance in Torosa-2 (Mapelli and Lombardi, 1982). However the relation of cytokinin to dominance may not be as simple as a deficiency of cytokinin in the tissue. Rose buds subject to correlative inhibition were found to contain higher levels of endogenous cytokinins than less inhibited buds (van-Staden et al., 1981). Although the mechanism of cytokinin release of apical dominance is not established, Tamas (1995) has suggested that cytokinin produced elsewhere may be directed towards sites of auxin transport. The role of cytokinins in dominance may be partly linked to their effect on cell division, as axillary buds show increased cell division activity when released from apical dominance by application of endogenous cytokinin (Usciati et al., 1972; Mauseth, 1976). Alternatively, cytokinins may stimulate synthesis and the transport of IAA out of organs which could either maintain or release apical dominance (Li and Bangerth, 1992).

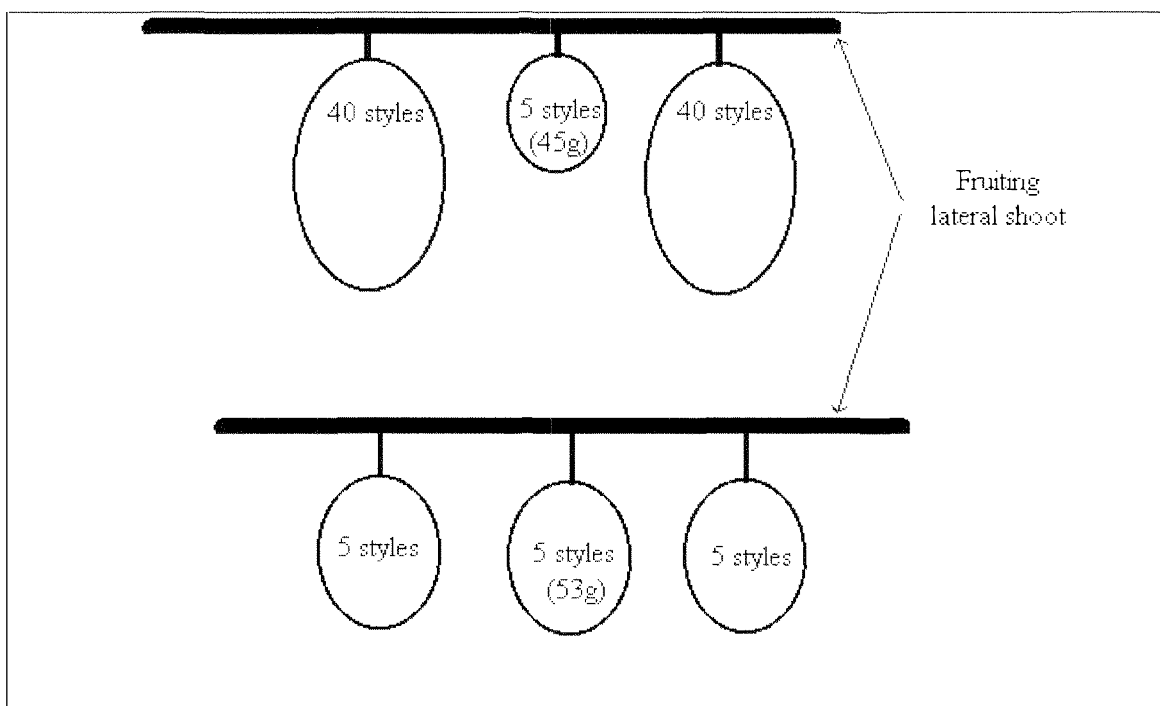


Figure 1.3 Diagrammatic illustration of the dominance exerted by high seeded kiwifruit over low seeded fruit found by Lai et al. (1990). Fruit seed content was reduced from 1500mg to below 750mg by reducing style numbers from 40 to five styles prior to pollination. When style manipulation was carried out on the centre fruit only, the size of this fruit was lower than when all three fruit were de-styled.

There are indications that kiwifruit may be subject to dominance related to the number of seeds in the fruit. The normal growth of kiwifruit is dependent on the presence of high numbers of viable seeds (see section 1.2.4). When seed numbers were manipulated prior to fruit set by controlled style excision, kiwifruit with low seed numbers which were in close proximity to fruit with high seed numbers were found to be smaller than expected (Figure 1.3) (Lai et al., 1990). This suggested that high seeded kiwifruit were able to exert an inhibitory effect on the growth of adjacent low seeded kiwifruit (Lai et al., 1990). even when ample supplies of carbohydrate were available

### 1.2.4 The role of seeds in kiwifruit growth

One of the major determinants of fruit size and sink strength in many fruits is the number of seeds which are formed. Positive relationships between the number or presence of seeds and fruit size have been found in fruit species as varied as strawberry (Nitsch, 1950), apple (Denne, 1963), tomato (Dempsey and Boynton, 1964), blackberry (Webb, 1971), blueberry (Moore et al., 1972), avocado (Blumenfeld and Gazit, 1974) and zucchini (Stephenson et al., 1988). Similarly, if seed distribution around the fruit is uneven, the fruit may become mishapen due to less growth in areas lacking seeds (Nitsch, 1950; Webb, 1971). However parthenocarpic fruit development can often be stimulated by application of growth regulators, in particular auxin or gibberellin (see section 1.2.6). Early studies made by (Nitsch, 1950) demonstrated that the auxins napthoxyacetic acid or indolebutyric acid applied in lanolin could substitute for the achenes in stimulating continued growth of strawberry receptacles. This followed work which had shown that the levels of auxin-like substances were very high in and immediately surrounding seeds in tomato (Gustafson, 1939). In some cases however, naturally parthenocarpic fruit will develop only when competing sinks such as pollinated fruits are not present (Goldwin and Schwabe, 1975), or if developing leaves are removed from the plant (Carbonell and Garcia-Martinez, 1980). This suggests that seed produced growth regulators may be essential for determining the relative sink strength and/or the dominance of an organ when other strong sinks are also present.

In kiwifruit, very high seed numbers are required for the growth of fruit to export size. For example a 100g 'Hayward' fruit usually contains between 1000 and 1200 seeds (Hopping and Hacking, 1983). Transfer of pollen to female flowers is generally accepted to be conducted mainly by insect vector although wind pollination does contribute to some extent (Costa et al., 1993; Craig et al., 1988). Eight hives of honey-bees per hectare are routinely placed in commercial orchards at the time of flowering for the sole purpose of providing pollination (Palmer-Jones and Clinch, 1975; Matheson, 1991). However occasional inconsistent results with pollination by honey-bees, particularly when poor weather is experienced during the pollination period, have led to the development of artificial pollination systems which can be used either in place of, or alongside honeybee pollination (Hopping, 1982; Hopping and Jerram, 1980; Stevenson, 1990).

Hopping (1976a) first demonstrated a positive relationship between fruit seed number and fruit weight in three *A. deliciosa* cultivars. The relationship fitted was curvilinear in shape, with an incremental increase in weight at low and high seed numbers, while at medium seed numbers fruit weight plateaued. This type of relationship was also clearly shown by Trustrum (1983) and Lai (1987) was able to fit a similar curve. Snelgar et al. (1992) failed to find evidence of the increase at high seed numbers, instead they found that the relationship was non-linear at low seed numbers, but above 300 seeds per fruit the relationship could be adequately described as linear. Grant and Ryugo (1984) also demonstrated a linear relationship for fruit from both shaded or exposed shoots, however the visual impression of data presented from shaded shoots suggested a curvilinear character, although there were few data points greater than 1000 seeds. Pyke and Alspach (1986) found that the relationship increased steeply at low seed numbers, but the rate of response to seed number decreased at higher seed numbers and a power relationship fitted the data adequately. As in other fruit species, mishapen fruit is associated with uneven pollination (Hopping, 1976a; Trustrum, 1983).

In a comparison of seed number and fruit weight of three *A. deliciosa* cultivars 'Hayward', 'Monty' and 'Standard', Hopping (1976a) found that at 800 seeds per fruit, fruit of all three cultivars attained a similar weight of around 60g. However at higher seed numbers 'Standard' and 'Hayward' fruit obtained a higher weight (Hopping,

1976a). No data was presented for 'Monty' fruit with higher seed number than this. As well pollinated 'Monty' fruit attain an average fruit weight of 65g (Zhang and Thorp, 1986), this suggests that the lower fruit weight in 'Monty' may be partially due to a lack of ovules available for seed formation. Zhang and Thorp (1986) reported seed numbers and fruit weight for nine cultivars of *A. deliciosa* and this has been summarised in Figure 2.1. There appears to be a consistent positive relationship between seed number and fruit weight in these cultivars (excluding 'Hayward'), suggesting that cultivar differences in fruit seed number may be responsible for the difference in fruit weight. However 'Hayward' shows a marked difference from the others, with a much higher fruit weight at comparable seed numbers. Thus it appears that 'Hayward' fruit in particular are larger than other *A. deliciosa* cultivars for reasons other than the potential for the fruit to form seeds.

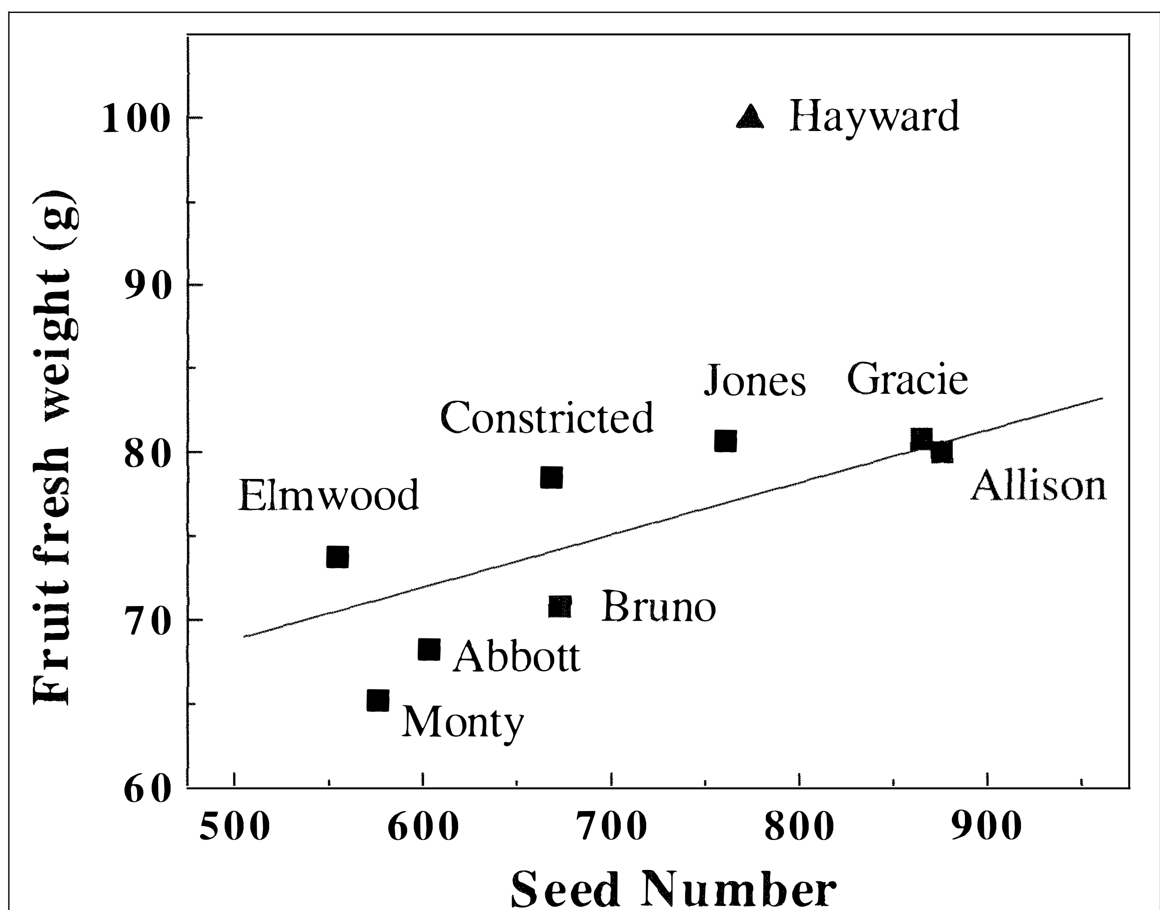


Figure 1.4 Average fruit seed numbers and fruit fresh weight of nine cultivars of *Actinidia deliciosa* reported by Zhang and Thorp (1986). A significant (P=0.021) positive linear regression was found for the eight cultivars excluding 'Hayward' of fruit weight versus seed number.

### 1.2.5 Fruit cell number and size

Fruits are entirely composed of cells, thus the fruit size is mainly a product of the total number of cells and the size (weight, volume) which they attain, although some of the total volume will be composed of intercellular spaces. Fruit growth is characterised by periods of cell division, cell expansion or periods where both occur simultaneously. Cell division occurs in fruits from as early as flower initiation well before visible ovaries are formed and often continues for a short time after pollination of the ovary. For example in tomato, cell division ceases at around 10 to 15 days after anthesis (Bohner and Bangerth, 1988b), while in avocado cell division can occur throughout the entire growth of the fruit (Schroeder, 1953). Conversely in 'Corinth' grape, cell division ceases prior to anthesis (Coombe, 1973).

Unlike most deciduous fruit crops in which flower buds are initiated in the season prior to emergence, initiation of kiwifruit flowers appears to occur around the time of bud-burst, approximately 10 weeks prior to flower opening (Polito and Grant, 1984; Brundell, 1975a), although evocation does appear to occur in the previous summer (Snelgar and Manson, 1992a; Davison, 1990). Thus kiwifruit may have a more limited time period over which pre-anthesis factors can affect fruit cell numbers. Post-anthesis growth of kiwifruit is usually described as a double sigmoid growth curve (Hopping, 1976b; Lai, 1987), although it has been suggested that it may be triple sigmoid (Pratt and Reid, 1974). The initial period of growth in kiwifruit approximates exponential growth and is characterised by a period of active cell division and cell expansion throughout the entire fruit (Hopping, 1976b; Woolley et al., 1992). Cell division activity slows to very low levels by about 30 to 40 days after anthesis, but continues slowly in the core (Hopping, 1976b; Woolley et al., 1992; Lewis, 1994). At around 50 to 60 days after anthesis, a 'lag' phase occurs at which time the fruit volume slows, indicating a slowing of cell expansion (Hopping, 1976b), although dry matter continues to increase at a steady rate (Lai, 1987). Following this, a second period of rapid growth occurs, associated with continued cell expansion, particularly in inner pericarp tissue, until growth slows as fruit reach maturity (Hopping, 1976b; Woolley et al., 1992).

The number of cells in the sink organ may be highly important in determining the size of a fruit, as this may provide a physical constraint on the ability of sink organs to accumulate imported assimilate and also to increase in volume (Ho, 1992). It has been suggested that the number of cells in a sink may provide a suitable measure of potential sink size (Ho, 1988). There are a number of reported correlations between fruit cell numbers and fruit size in the literature, particularly between different cultivars. For example, Denne (1963) found a strongly positive relationship between the fruit cell number and fruit weight in apple. Among some wheat varieties, the number of cells in the endosperm and the plastid number per cell determine the final grain dry weight (Gleadow et al., 1982). The pericarp of large fruit tomato mutants have significantly higher cell numbers than wild tomato fruits (Bohner and Bangerth, 1988b). Differences in fruit size between cultivars of strawberry (Cheng and Breen, 1992) and peach (Scorza et al., 1991) were found to be due to cell number differences.

Although cell number is probably important in providing the potential sink size, the size that the cells can achieve is also important in determining final fruit size. As Coombe (1976) has pointed out, increases in cell volume form the greatest proportion of fruit size increase for most fruits. In cucumber, fruit in which post-anthesis cell division was inhibited by a low initial assimilate supply, were able to recover later in development by an increased rate of cell expansion (Marcelli, 1993). Similarly, tomato fruit which were parthenocarpically induced by application of $GA_3$ had only 25% of the cell number of control (pollinated) fruit, but at maturity cells were up to 50% larger (Bunger-Kibler and Bangerth, 1982). Thus it appears that increased cell expansion may be able to compensate for decreased cell numbers in some cases.

In a number of fruits such as apple (Denne, 1963; Marguery and Sangwan, 1993), apricot (Jackson and Coombe, 1966) and kiwifruit (Lai, 1987), early flowers produce larger fruit than later flowers. This effect has been correlated to the larger size of ovaries from early flowers, which has been associated with higher cell numbers. For example in tomato, fruit cell number differences at anthesis were responsible for final fruit size differences between different clones (Bohner and Bangerth, 1988a). Although cell division after anthesis determines final cell number in tomato fruit, differences are accentuated by the cell number in ovaries at the time of anthesis (Bohner and Bangerth,

1988a; Bohner and Bangerth, 1988b). Differences in cell numbers of large and small fruited peach cultivars were established well prior to anthesis (Scorza et al., 1991). Similarly, kiwifruit flowers which arise early have larger ovaries at anthesis and have been shown to produce larger fruit (Lai et al., 1990; Cruz-Castillo et al., 1991; Smith et al., 1992b) Early kiwifruit ovaries have higher cell numbers in the core tissue of the fruit at fruit set (Cruz-Castillo, 1994). This suggests that in many fruit, including kiwifruit, cell division prior to anthesis plays a major role in determining fruit size.

Many fruits are viewed as being utilisation sinks during the cell division phase, with imported assimilates used for building new cellular constituents. Therefore it is possible that cell division could be affected by the availability of carbohydrate. Denne (1963) found that thinning of apples increased fruit cell number and fruit weight, which suggested that the increased in assimilate supply available to the fruit affected their competitive ability by stimulating cell division. Similarly, thinning of 'Sunset' apples at between 'pink bud' stage and three weeks after full bloom resulted in increased cell numbers, but cell size was not affected (Quinlan and Preston, 1968). Cucumber fruit exposed to limited assimilate supply by a high crop load had strongly decreased growth, due both to decreased cell numbers and cell size (Marcelli, 1993), which indicated an effect of assimilate supply on cell division. In an alternative approach, reduction of the availability of assimilates to tomato fruit by partial defoliation immediately following pollination, was shown to cause a large decrease in fruit cell number and fruit weight (Bohner and Bangerth, 1988b). The contribution of assimilate supply to cell division in fruit is worthy of further investigation.

Several authors have examined cell numbers and cell growth in transverse fruit sections of *A. deliciosa.* Hopping (1976b) examined the cultivar 'Monty' which is a smaller fruiting variety. Woolley et al. (1992) and Cruz-Castillo (1994) have examined cultivar 'Hayward'. These studies may be comparable as the major difference in the fruit shape of these two cultivars is in diameter of the fruit rather than length, although 'Hayward' fruits are also slightly longer (Zhang and Thorp, 1986). The main differences between these cultivars appear to be associated with cell number differences. 'Hayward' cell numbers were similar or higher than 'Monty' at anthesis in all three fruit tissues and by

harvest these differences were further accentuated (Table 1.1). These results also suggest that there is a higher level of cell division post-anthesis in 'Hayward', as cell numbers increased proportionally more in this cultivar. There appears to be no consistent difference in cell size between the two cultivars although the size of 'Hayward' core cells is initially larger then 'Monty' and final size of 'Monty' inner pericarp cells are larger than 'Hayward' (Table 1.2).

Table 1.1 Summary of cell number measurements made from transverse sections of *A. deliciosa* kiwifruit core, inner pericarp and outer pericarp tissue. Measurements were made in a straight line across the diameter of the core and across a radius of inner and outer pericarp tissues.

| Cultivar | Author | Mature weight | Core (half) | | Inner pericarp | | Outer pericarp | |
|---|---|---|---|---|---|---|---|---|
| | | (g) | initial | mature | initial | mature | initial | mature |
| Monty | Hopping (1976b)[1,3] | 65 | 14 | 21 | 23 | 36 | 27 | 44 |
| Hayward | Cruz-Castillo (1994)[1] | 96 | 19 | 55 | 23 | 55 | 37 | 65 |
| Hayward | Woolley et al. (1992)[2,3] | 90 | 35 | 40 | 30 | 55 | 30 | 55 |

[1] Initial measurements made at anthesis.
[2] Initial measurement made 10 days after anthesis.
[3] Values estimated visually from graphs.

A recent study of the cell size of *A. chinensis* has been made by Clayton (1996) using image analysis to calculate cell cross-sectional area of cells on transverse sections. Comparing cell sizes between 'Hayward' and *A. chinensis* kiwifruit, Clayton (1996) reported that the mean size of 'Hayward' outer pericarp cells were between 16 to 19 times larger, while core cells were approximately four times larger than in *A. chinensis.* Although the very large difference in the size of cells could account for fruit size differences between the two species, this conclusion would suggest that cell numbers in *A. chinensis* were considerably higher than in 'Hayward', as 'Hayward' fruit were only twice the size of the *A. chinensis* fruit.

Table 1.2 Summary of cell diameter (µm) measurements in transverse sections of *A. deliciosa* kiwifruit core, inner pericarp and outer pericarp tissue. Values are average cell width in a straight line across the diameter of the core and across a radius of inner and outer pericarp tissues.

| Cultivar | Author | Core | | Inner pericarp | | Outer pericarp | |
|---|---|---|---|---|---|---|---|
| | | anthesis | mature | anthesis | mature | anthesis | mature |
| Monty | Hopping (1976b)[1,3,4] | 40 | 115 | 50 | 300 | 40 | 105 |
| Hayward | Cruz-Castillo (1994)[1] | 26 | 74 | 46 | 212 | 24 | 103 |
| Hayward | Woolley et al. (1992)[2,3] | 60 | 70 | 85 | 220 | 60 | 115 |

[1] Initial measurements made at anthesis.
[2] Initial measurement made 10 days after anthesis.
[3] Values estimated visually from graphs.
[4] Cell size graph axis assumed to be µm x 10.

### 1.2.6 Involvement of plant growth regulators in fruit sink strength

Many aspects of the intrinsic sink strength of fruit are probably related to endogenous plant growth regulating substances. In particular, the development of viable seeds which are thought to be a major source of growth regulating substances (Crane, 1969; Nitsch, 1970; Goldwin and Schwabe, 1975), is usually crucial to stimulate fruit growth or prevent abscission (Stephenson et al., 1988). In some fruit pollination alone can provide the necessary stimulus for the commencement of growth (Gustafson, 1937) while in others, development may occur without any stimulus (George et al., 1984; Gil et al., 1973). However in most fruit which would normally form seeds, parthenocarpic development is only able to be stimulated by the application of either a single growth regulator or combinations of growth regulators to the ovary (Schwabe, 1981). The different requirements for growth regulators to stimulate or induce parthenocarpic development may indicate that these growth regulators are sub-optimum at the time of fruit set. Alternatively, fruit able to be parthenocarpically induced may require a pulse of growth regulator to start a developmental switch, which then starts autonomous fruit development thereafter (Bangerth and Schroder, 1994). In many fruit development of nucellis or endosperm tissue in the ovule occurs in parthenocarpic development and it is possible that these provide a source of hormones necessary for continued fruit

development (Nitsch, 1970 and references therein). However ovule development in parthenocarpic fruit is by no means ubiquitous (Goldwin, 1978) and fruit development can sometimes continue when seeds are removed (Abbott, 1959) which suggests that in these fruit, tissues other than the ovules can synthesise hormones and/or external sources are obtained.

### Auxin

The functions of auxins which could potentially influence sink strength include the promotion of cell elongation (Evans, 1985) and cell division (Gamburg, 1982), induction of phloem elements (Aloni, 1995), correlative inhibition of competing sinks (Bangerth, 1989), regulation of key enzymes involved in sugar metabolism such as acid invertase (Morris and Arthur, 1986) and enhanced mobilisation of assimilate (Patrick and Stearns, 1987).

Pollen is often a rich source of auxin and in some species pollination alone can stimulate fruit set (Gustafson, 1937). Application of auxin has been found to stimulate parthenocarpic development in the fruit of about 20% of horticultural crops, mostly those containing a large number of ovules per ovary (Naylor, 1984). For example, application of the synthetic auxin napthaleneacetic acid (NAA) as a spray or in lanolin paste to unpollinated strawberries which had the achenes removed, allowed 100% fruit set and normal development of the fruit (Mudge et al., 1981). Chlorophenoxyacetic acid (4-CPA), a synthetic auxin applied at 0.1% in lanolin paste to tomato ovaries resulted in development at a similar growth rate to pollinated fruits (Sjut and Bangerth, 1982). Inhibitors of auxin transport such as triiodobenzoic acid (TIBA) and NPA, have been found to induce parthenocarpic fruit development in cucumber (Cantliffe et al., 1972; Quebedeaux and Beyer, 1974) which is associated with auxin accumulation within the ovary (Kim et al., 1994).

Auxin concentrations are higher in seed than in the surrounding fruit tissue of many fruit (Gustafson, 1939), which suggests that seeds may be a source of auxin which then diffuses into fruit. However moderate levels of auxins are found to be present in both naturally occurring and hormone induced parthenocarpic fruit (Wang et al., 1993; Garcia-Papi and Garcia-Martinez, 1984; Sjut and Bangerth, 1982). Thus in fruit where

parthenocarpic development occurs, the post-anthesis auxin level is obviously not dependent on viable seed development.

In shoot tissues, auxins are transported basipetally, i.e. from morphologically apical to basal regions, in an energy requiring manner in living tissue (Kaufman et al., 1995). The auxin directed transport of photo-assimilates has been demonstrated in a variety of species (Patrick, 1976), although inhibition of auxin transport by inhibitors does not necessarily lead to reduced attraction of photo-assimilate (Gamburg, 1982). In addition, auxin transport appears to play a major role in dominance relationships (see section 1.2.3).

Cytokinins

Cytokinins have been defined as substances which in combination with auxin, stimulates cell division in plants (Skoog et al., 1965). Naturally occurring cytokinins are all $N^6$-substituted adenine derivatives (Shaw, 1994), although other substitutions are found at the 2, 7 and 9 positions on the adenine ring (Jameson, 1994). Structure-function relationships of cytokinins have been studied in bioassay systems by several authors (Skoog et al., 1967; Shaw et al., 1971). In general the structure of the side chain has been found to be critical for determining activity and cytokinins with unsaturated side chains such as zeatin and isopentenyl adenine show the highest activity. Addition of any side groups such as ribosides, ribotides and glucosides tends to reduce the cytokinin activity, particularly O-glucosides which are attached to the side chain. Plant roots appear to be a major biosynthetic source of cytokinins (Letham, 1994) and it is possible that root derived xylem cytokinins are a source of cytokinins for fruit (Varga and Bruinsma, 1974). However seeds also appear to be a biosynthetic source of cytokinins and fruit may be independent of xylem cytokinins (Letham, 1994).

The possible influences of cytokinins on sink strength include the regulation of cell division (Letham and Williams, 1969), induction of vascular tissue (Aloni, 1995), hormone directed transport of assimilates (Clifford et al., 1986) and maintenance of correlative inhibition (Karanov et al., 1992). Cytokinins have been shown to be very active in promoting cell division in culture (Miller et al., 1955) and there is evidence that

cytokinins regulate cell division in intact plants. Peaks of endogenous cytokinin concentration occur during the post-anthesis cell division of several fruit. For example high concentrations of cytokinins have been found in the early stages of apple (Letham and Williams, 1969) and tomato (Abdel-Rahman et al., 1975; Mapelli, 1981; Bohner and Bangerth, 1988b) fruit growth, which correlates to a period of high cell division activity. In addition, application of exogenous cytokinins can stimulate cell division activity in some cases. For example the application of cytokinin to apical buds on the long day plant *Sinapsis alba* grown under short day conditions induced mitotic activity similar to that able to be induced by a long day stimulus (Bernier et al., 1977). Tomato flower ovaries targeted for abortion in poor light conditions were found to have arrested cell division measured as mitotic activity and DNA synthesis (Kinet et al., 1985). However application of benzyladenine to the flowers caused resumption of cell division activity (Kinet et al., 1985), although complete development required exogenous gibberellin as well as cytokinin (Kinet et al., 1978). Benzyladenine applied to empire apple trees as a fruit thinning treatment during the time of active cell division, was found to result in increased fruit cell numbers compared with fruit from both control (un-thinned) trees and trees thinned with NAA, carbaryl or daminozide (Wismer et al., 1995).

Following identification of a cytokinin active phenylurea compound in coconut milk (Shantz and Steward, 1955), a range of synthetic phenylurea compounds with very high cytokinin activity have been synthesised, including the compound N-(2-chloro-4-pyridyl-N'-phenylurea (CPPU) (Takahashi et al., 1978). CPPU is an extremely effective stimulant of parthenocarpic development in kiwifruit (Iwahori et al., 1988; Lewis et al., 1996a), cucumber (Kim et al., 1994), apple (Bangerth and Schroder, 1994) and watermelon (Hayata et al., 1995). CPPU is also very effective in increasing fruit size by post-anthesis application to fruit such as grape (Nickell, 1986), avocado (Kohne and Schutte, 1991), kiwifruit (Lawes et al., 1992) and apple (Tartarini et al., 1993). Phenylurea cytokinins are structurally dis-similar to adenine based cytokinins and it has been suggested that phenylurea cytokinins may induce cytokinin activity by interacting with naturally occurring adenine cytokinins, perhaps by preventing their metabolism (Thomas and Katterman, 1986). However Shudo (1994) has pointed out that some similarities in structural conformation and structure-function relationships do exist. If

CPPU is functioning as a cytokinin in it's own right, it's effectiveness in stimulating fruit growth may be due to reduced metabolism compared with natural cytokinins which are readily conjugated into inactive forms (Jameson, 1994).

Abscisic acid

Abscisic acid (ABA) has usually been associated with the promotion of dormancy (Karssen et al., 1983; Finkelstein et al., 1985) and for its role in regulation of stomatal resistance and plant water stress (Wright, 1969; Tal and Nevo, 1973; Mansfield and McAinsh, 1994). However ABA may also have a role in controlling assimilate unloading in sinks (Tietz et al., 1981; Clifford et al., 1986) and could therefore be important for sink strength. ABA concentrations have been found to decrease from high concentrations at the early period of fruit development, but concentrations often rise considerably in the later stages of development (Kojima et al., 1993; Kondo and Gemma, 1993; Sagee and Erner, 1991; Beruter, 1983). In lemon (Aung et al., 1991) and cherry (Kondo and Gemma, 1993) fruits and in many seeds (Ross and McWha, 1990) the ABA content increases later in development, coinciding with the peak period of assimilate storage. Exogenously applied ABA also improves assimilate partitioning in some cases. For example the application of 1000 ppm ABA to cherry fruits 36 days after full bloom, was found to enhance the total sugar contents of the fruits (Kondo and Gemma, 1993). The application of ABA to barley grains two weeks after anthesis increased the mobilisation of recently fixed photo-assimilates to the grains when endogenous levels were comparatively low (Tietz et al., 1981). However at three weeks after anthesis, endogenous ABA levels increased five-fold and exogenously applied ABA did not increase uptake of photo-assimilate by the grains, while at high concentrations uptake of photo-assimilate was inhibited (Tietz et al., 1981).

The mechanism by which ABA influences sink strength may be by controlling uptake or compartmentalisation of imported sugars. Inclusion of ABA in the incubation solution of strawberry fruit explants and cortex disks increased $^{14}$C-sucrose accumulation by 45 and 37%, respectively, above control values (Archbold, 1988). In addition, the uptake of sorbitol by vacuoles from immature apple fruit flesh has been found to be enhanced by ABA (Yamaki and Asakura, 1991). Removal of the calyx lobes of persimmon fruit at two stages of development caused inhibition of normal growth of the fruit and a decrease

in ABA concentrations in the fruit, which were associated with an increase in the ratio of sucrose to hexoses and thus a decreased source-sink gradient of sucrose (Yonemori et al., 1995). Washing pea seed coats with a low concentration ($10^{-11}$ M) of ABA from which embryos had been removed, was found to stimulate unloading of radio-labelled photo-assimilate from the seed coat within 10 minutes of application (Ross et al., 1987). However washing with a high ABA concentration ($10^{-7}$ M) gave variable results with unloading being reduced, increased or not affected (Ross et al., 1987), thus it appears that there may an optimal dose response of ABA for unloading processes.

Plant roots are thought to be the major biosynthetic organs for ABA production (Cornish and Zeevart, 1985). Plant root systems have been shown to produce substantial ABA in response to soil drying and this is transported to leaves in the xylem stream (Zhang et al., 1990). However ABA in leaves may be redistributed within the plant towards sink organs. Setter et al. (1980b) demonstrated that ABA levels dramatically increased in soybean leaves following the application of a petiole girdle, suggesting that soybean pods may act as a sink for leaf ABA. In addition, radio-labelled ABA injected into soybean leaves was found to be exported in the phloem to both vegetative and reproductive sinks (Brenner et al., 1986). Further evidence of redirection of leaf ABA to sinks was provided by Clifford et al. (1990), who demonstrated that defoliation of dwarf beans led to a reduction in ABA concentration in seeds.

Gibberellins

Although gibberellins were not investigated at all in this thesis, their role in sink strength is probably very important and deserves mention. Gibberellins are well known to promote cell elongation (Kaufman and Jones, 1974) and they have also been shown to promote cell division in apical meristems (Liu and Loy, 1976). Thus they may also improve sink strength in an indirect manner, probably in combination with cytokinin and auxin. Gibberellins have also been shown to be involved with enhancing transport of assimilates by stimulating apoplastic unloading of phloem (Hayes and Patrick, 1985). Gibberellins are effective at inducing parthenocarpy on a very wide range of crops (Schwabe, 1981), including ovaries containing few ovules where auxins are generally ineffective (Naylor, 1984). A number of pip and stone fruit, grapes and some citrus can

be induced to set fruit in response to gibberellin sprays (Gil et al., 1972; Crane et al., 1960; Sabater and Rubery, 1987). Application of paclobutrazol (a gibberellin biosynthesis inhibitor) to 'Satsuma' mandarins which show high natural parthenocarpy, increases abscission of fruits unless the gibberellin $GA_3$ was applied at the same time (Stembridge and Gambrell, 1972). Exogenous $GA_3$ application substantially increases fruit retention of 'Valencia' orange (Turnbull, 1989) and 'Clementine' mandarin (Talon et al., 1992). A similar effect was noted in peach where post anthesis $GA_3$ application temporarily promoted fruit growth and delayed abscission of both seeded and gibberellin induced seedless fruit (Stembridge and Gambrell, 1972). All gibberellins except $GA_8$ have been found to be higher in parthenocarpic 'Satsuma' mandarins which have a higher fruit set than 'Clementine' mandarins which show a very low tendency to set fruit (Stembridge and Gambrell, 1972). Thus the ability to exhibit natural parthenocarpy in fruits may be associated with high endogenous levels of gibberellins in the fruit of different cultivars.

### 1.2.7 Plant growth regulators and kiwifruit growth

Attempts to replace the effect of seeds in kiwifruit with growth regulator application have met with only limited success. Application of auxin, cytokinin or gibberellin individually to poorly pollinated 'Monty' kiwifruit 21 to 35 days after flowering, failed to improve fruit size (Hopping, 1976a). However applications of a mixture of auxin and cytokinin or auxin, cytokinin and gibberellin markedly increases the size of both 'Monty' and 'Hayward' kiwifruit (Hopping, 1976a; Cruz-Castillo et al., 1993). Costa and Ryugo (1978) cited in Hopping (1990) were able to induce development of parthenocarpic fruit weighing up to 60g, with eight repeated applications of auxin+cytokinin+gibberellin, although fruit retention was dependent on continued application up until 115 days after flowering. Parthenocarpic fruit growth has also been stimulated after a single application of the synthetic cytokinin-like compound CPPU at the time of flowering (Iwahori et al., 1988; Lewis, 1994).

The post-anthesis application of CPPU to 'Hayward' kiwifruit provides a dramatic increase in fruit size 'Hayward' kiwifruit (Lawes et al., 1992; Patterson et al., 1993).

However when CPPU was applied in combination with gibberellin ($GA_3$) and auxin (2,4-D) fruit size was increased even further (Cruz-Castillo et al., 1992). In addition, the effect of CPPU on the growth of 'Hayward' kiwifruit has been found to be reduced in fruit with low seed numbers (Woolley et al., 1992). This suggests that at high seed numbers, kiwifruit growth is limited by a lack of cytokinin, while kiwifruit seeds may be a source of auxin and gibberellin which becomes limiting to fruit growth at low seed numbers.

*In-vitro* culture of kiwifruit tissue provides some evidence of growth hormone requirements for kiwifruit growth. Callus growth of kiwifruit inner pericarp tissue is not stimulated by the presence of seeds alone, but requires exogenous growth regulators (Trustrum, 1983; Cruz-Castillo, 1994). Although the presence of seeds usually enhanced inner pericarp callus growth, growth was able to be stimulated by auxin (2,4-D), or gibberellin ($GA_3$) but not cytokinin (BAP) in the presence or absence of seeds (Cruz-Castillo, 1994). If these results can be extrapolated to *in-vivo* fruit growth, they may suggest that the function of seeds in a kiwifruit may be in attracting growth regulators to the fruit which have been synthesised elsewhere in the plant. However these studies were carried out well after the period of normal fruit cell division had ceased and thus extrapolation of the results, particularly those associated with seed effects, cannot be made with confidence.

There have been relatively few studies of endogenous growth regulators in kiwifruit. Bioassays of carpellary tissue of kiwifruit for cytokinin like compounds revealed that young fruit were relatively rich in zeatin-riboside and zeatin (Okuse and Ryugo, 1981). The level of zeatin-riboside like compounds disappeared as the fruit developed, although they reappeared in mature fruits (Okuse and Ryugo, 1981). A similar pattern has been found for total cytokinin concentration, however the peak zeatin concentration was found to occur at 11 days after anthesis (Lewis et al., 1996b). Total amount of cytokinins in kiwifruit were not found to be related to seed number, although levels and the total amount of zeatin were reduced in unpollinated fruits (Lewis et al., 1996a). Investigation of other endogenous plant growth regulators present in kiwifruit is limited. Tao et al. (1994) claimed that IAA and ZR levels measured by ELISA in 'Hayward' kiwifruit

peaked seven days after flowering. Smith et al. (1995) measured ABA by ELISA and found that the ABA concentration in skin of 'Hayward' kiwifruit declined rapidly during the first seven weeks after anthesis and remained at a constant low level thereafter. No trends were found in the concentrations of ABA in pericarp and core tissue, however total ABA per fruit increased throughout development (Smith et al., 1995). Smith et al. (1995) suggested that total ABA in the fruit may be related to carbon import, as fruit from the upper canopy were larger and had a higher total ABA per fruit.

## 1.3 Source-Sink Relationships

As kiwifruit is a perennial crop, the relationship between source leaves and the various sink organs not only affects fruit production in the current season, but also the long term performance of vines. Annually, considerable biomass is lost from kiwifruit vines in fruit, prunings and root turnover, as well as in respiration of annual and perennial components. Source-sink relationships in kiwifruit are likely to be influenced by the total amount of photosynthetically fixed carbon over the growing season, the number and strength of fruit sinks present on the vine and the location of sources relative to sinks.

### 1.3.1 Source of carbohydrates - photosynthesis

The light response of kiwifruit leaves have been described by parabolic curves (Lai, 1987; Chartzoulakis et al., 1993b), rectangular hyperbola (Laing, 1985) and non-rectangular hyperbola (Buwalda et al., 1991). Although there is some evidence that photo-inhibition of kiwifruit leaves may occur at high light intensities (Greer and Laing, 1988), parabolic curves probably overemphasise this effect and a hyperbolic relationship appears to be more appropriate. Non-shaded, field grown 'Hayward' kiwifruit leaves commonly have a light saturated net photosynthetic rates ($P_{max}$) of between 10 and 15 $\mu mol.CO_2\ m^{-2}\ s^{-1}$ (Grant and Ryugo, 1984; Lai, 1987; Buwalda et al., 1991) and are light saturated at between 500 and 800 $\mu mol.PAR\ m^{-2}\ s^{-1}$ (Grant and Ryugo, 1984; Buwalda et al., 1991; Chartzoulakis et al., 1993b). In a comparison of 'Hayward', 'Bruno', 'Monty' and 'Abbott' cultivars, 'Bruno' leaves appear to have a lower $P_{max}$ than the other *A. deliciosa* cultivars (Chartzoulakis et al., 1993b), thus it is possible that some differences in fruit growth between cultivars may be due to differences in photosynthetic rates. However improvements in crop yields due to an improvement in maximum photosynthetic rates are difficult to demonstrate, as the photosynthetic rate is regulated by many endogenous and environmental factors (Gifford and Evans, 1981). The only report of photosynthetic rates of other *Actinidia* species is for *A. arguta* which were reported to have a maximum photosynthetic rate of 6.96 $\mu mol\ m^{-2}\ s^{-1}$ (Shim et al., 1985). The low $P_{max}$ for *A. arguta* may be due to low crop loads (Ferguson, 1991) compared

with *A. deliciosa* (Thorp et al., 1990; Cooper and Marshall, 1992), as the presence of a large sink is often required to maintain a high photosynthetic rate (Neales and Incoll, 1968; Geiger, 1976). Although Dick (1987) and Lai et al. (1989a) have found no evidence that high fruit sink demand results in increased photosynthetic rate in kiwifruit, there are indications that kiwifruit leaf photosynthetic rate is decreased at very low fruit sink demand (see section 1.3.4).

The exact mechanism by which photosynthesis is regulated by sinks is not known, although the suggestion that it may be due to feedback inhibition of photosynthesis by end-product accumulation in leaves was made as early as 1868 (Boussingault, 1868) cited in Goldschmidt and Huber (1992). This agrees with lowered photosynthetic rates and accumulation of carbohydrate observed on leaves in girdled plant parts. Although photosynthesis is not always affected by girdling, this may be related to the source:sink ratio present. Neales and Incoll (1968) proposed that satisfactory proof of the feedback hypothesis would depend on:

a) demonstration of a negative correlation between the photosynthesis rate and assimilate level in the leaf and;
b) elucidation of the biochemical mechanism involved.

The hypothesis has not been fully proven as yet (Neales and Incoll, 1968; Geiger, 1976; Sharkey, 1990; Goldschmidt and Huber, 1992). A negative correlation between leaf carbohydrates and photosynthetic rate has not been demonstrated in several species (Geiger, 1976), however there is evidence that the inhibition may be due to production of specific forms of carbohydrate by leaves (Sharkey, 1990). Roper et al. (1988) found that there was no difference in photosynthetic rate of sweet cherry trees which had no fruit and a normal crop load, despite increased non-structural carbohydrate levels in leaves and fruit. However sweet cherry leaves contain almost no starch, instead sorbitol is the major non-structural carbohydrate. Leaves of spinach show little photosynthetic inhibition by source-sink manipulation (Goldschmidt and Huber, 1992; Krapp and Stitt, 1995), but their leaves were shown to predominantly accumulate sucrose, which suggests that sucrose is unlikely to be the end-product causing inhibition (Goldschmidt and Huber, 1992). Several hexose sugars which

are synthesised inside the chloroplast have been shown to be biochemical inhibitors, either by competing for binding sites on the ribulose-bisphosphate-carboxylase-oxygenase enzyme or by exhausting the limited orthophosphate resource (Guinn and Mauney, 1980). It has also been suggested that the accumulation of starch interferes with photosynthesis, possibly by increasing the pathlength for $CO_2$ diffusion in the mesophyll (Nafziger and Koller, 1976), or physical damage to chloroplasts by starch grains (Schaffer et al., 1986). Inhibition by starch cannot be the case in all situations, as inhibition of the photosynthetic rate occurred in petiole girdled leaves of a starchless tobacco mutant (Goldschmidt and Huber, 1992). Accumulation of ABA in leaves of girdled plants (Loveys and Kriedemann, 1974; Setter et al., 1980b) may be associated with a direct inhibition of photosynthesis (Farquhar and Sharkey, 1982) or indirectly by increasing stomatal resistance (Setter et al., 1980a).

The photosynthetic rate of kiwifruit leaves has been found to be dependent on growing conditions. For example, $P_{max}$ was found to range from between 11 $\mu$mol $m^{-2}$ $s^{-1}$ for kiwifruit leaves grown at low temperature to 19 $\mu$mol $m^{-2}$ $s^{-1}$ for leaves grown at 25°C (Laing, 1985). Kiwifruit leaves which develop in shaded positions in the canopy have a lower $P_{max}$ (Dick, 1987). Kiwifruit leaf photosynthetic rate is also affected by water stress (Chartzoulakis et al., 1993a) and availability of nutrients (De Jong et al., 1984). In addition, $P_{max}$ of kiwifruit leaves displays a parabolic relationship over time, peaking after four months in leaves which emerge within two months of bud-burst, or after two months in leaves emerging later in the season (Buwalda et al., 1991).

### 1.3.2 Crop load

Fruit size in 'Hayward' kiwifruit has been found to be highly correlated with crop load (Cooper and Marshall, 1992; Richardson and McAneney, 1990; Burge et al., 1987). Several authors have found that the cultivar 'Hayward' has low fruit or flower numbers especially when compared with other cultivars or other *Actinidia* species (Ferguson et al., 1990; Wang et al., 1994; Brundell, 1975b). In particular, Brundell (1975b) found that 'Hayward' had a significantly lower percentage of floral shoots and lower total flower numbers per shoot than 'Monty', 'Bruno' and 'Abbott'/'Allison' [now thought to be a

single cultivar (Zhang and Thorp, 1986)]. Much of the difference in flower numbers was due to increased numbers of lateral flowers, however all other cultivars had higher numbers of inflorescence axils per shoot than 'Hayward', but this was only statistically significant for 'Bruno' (Brundell, 1975b). Thus some of the difference in fruit size of the different kiwifruit cultivars and species may be due to crop loads which are normally held by vines. Thorp et al. (1990) compared the fruit yields of seven *A. deliciosa* cultivars including 'Hayward'. 'Hayward' had one of the lowest total fruit yields of all cultivars. We have converted the data of Thorp et al. (1990) to an estimated average fruit size and crop load and this is presented here (Figure 1.5). This provides some evidence that differences in fruit size between *A. deliciosa* cultivars may be due to crop load. 'Gracie' and 'Hayward' were characterised in this data with low crop load (approximately half that of the other five cultivars) and had higher average grade size and lower vine yield. These types of relationships have been demonstrated for 'Hayward' vines with varying crop load (Cooper and Marshall, 1992). Unfortunately there is no reported literature illustrating crop load effects for any cultivar or *Actinidia* species other than 'Hayward'.

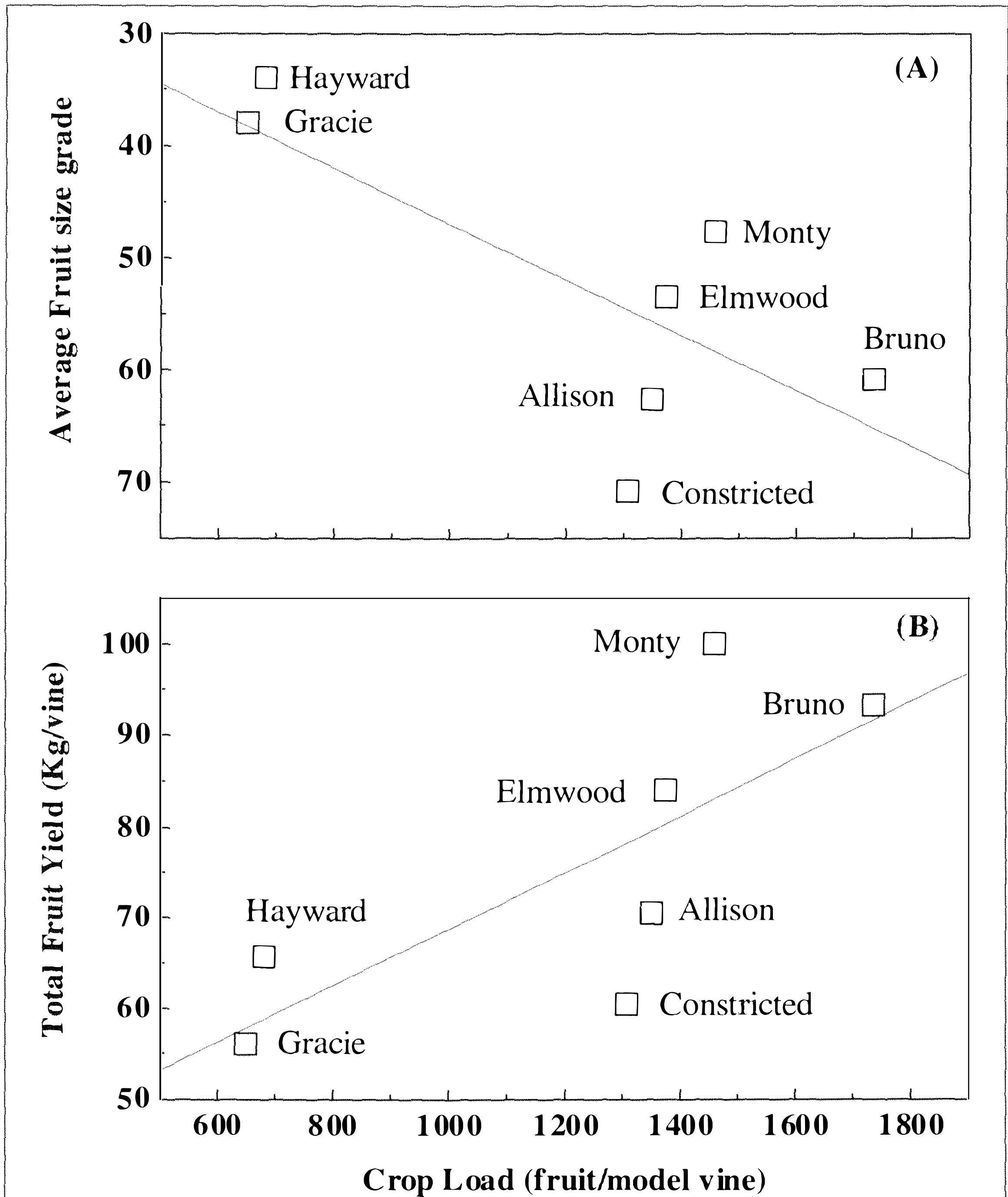


Figure 1.5 Plots of total yield and average fruit size grade versus crop load for seven pistillate cultivars of kiwifruit using data from Thorp et al. (1990). All data had been adjusted to a 'model' vine with a 6m central leader, to facilitate comparison between vines of different size. Crop load and average fruit size grade were estimated from data presented. (a) $r^2$=0.62; P =0.053. (b) $r^2$=0.56; P=0.054.

### 1.3.3 Manipulation of source-sink relationships by girdling

Girdling is an accepted horticultural practice with a wide range of applications and is thought to have been practised for many centuries. Girdling is also known as cincturing or ring-barking and has been defined as the removal of a complete cylinder, either narrow or wide, of all tissues external to the secondary xylem (Noel, 1970). Scoring is a related technique, in which a single narrow cut is made through the bark completely encircling the stem (Hoying, 1993). Occasionally girdling techniques are extended to penetrate or remove some of the underlying xylem, in which case they are termed notch-girdling, notching, chip-girdling or nicking (Noel, 1970; Hoying, 1993). Noel (1970) has reviewed the effects of girdling on tree growth in general, although the review concentrated on the physical effects and application to timber production, rather than physiological changes and the effect on fruit production.

There is a wide variety of reported beneficial effects of girdling on fruit production including:

- Increased flower number in kiwifruit (Snelgar and Manson, 1992a), olive (Eris and Barut, 1993), orange (Agusti et al., 1992) and lychee (Menzel and Simpson, 1987).
- Improved percentage fruit set in grape (Tafazoli, 1977), apple (Priestley, 1976) and avocado (Adato, 1979).
- Lower post-anthesis fruit drop in orange (Monselise et al., 1972) and grape (Dabas et al., 1980).
- Increased fruit size in kiwifruit (Woolley et al., 1992; Snelgar et al., 1986), citrus (Cohen, 1984; Hochberg et al., 1977), peach (Powell and Howell, 1985), grape (Orth et al., 1989), mango (Bhattacharyya and Mazumdar, 1990), fig (El-Kassas et al., 1988), pecan (Marquard, 1987) and olive (Lavee et al., 1983).
- Earlier fruit maturity in peach, nectarine (Fernandez-Escobar et al., 1987) and citrus (Yamanishi, 1995).
- Reduced vegetative growth in grape (Orth et al., 1989), apple (Autio and Greene, 1994) and peach (Dann et al., 1984).

- Reduced severity of alternate bearing in mango (Rath and Das, 1979) and olive (Lavee et al., 1983).
- Altered fruit quality characteristics in avocado (Adato, 1979), peach (North et al., 1988) and grape (Sharma and Jindal, 1982).

The result of girdling appears to be highly dependent on several variables, in particular the time the girdle is applied, location on the tree, crop load and species. Thus in a review of literature, apparent contradictions may appear. For example fruit size is decreased in some reports due to a higher crop load as a result of increased flower number, improved set or lower fruit drop.

When girdles are applied during the growing season, fruit size is usually increased. In grapes, the technique has been widely used to increase the size of table grapes and provide full attractive bunches. Either trunks or fruit canes are girdled soon after fruit set, prior to the period of rapid berry enlargement (Weaver, 1976; Winkler, 1954). Hochberg et al. (1977) reported a 28.4% increase in berry size of 'Bien Donne' grapes which were trunk girdled just after fruit-set. Girdling of stonefruit has been used commercially, particularly with cultivars which tend to produce small fruit. Girdling of `Mayfire' nectarines trees prior to stage 2 fruit growth, increased fruit weight by 22.5% and more than doubled the percentage of fruits in the three largest grades (Day and De Jong, 1990). Similarly in peaches, girdling has been found to increase fruit size by 10 to 25% (Powell and Howell, 1985) and/or overall yields by up to 59% (Perez and Rodriguez, 1987). However the effect of girdling on fruit size is highly dependent on the source-sink ratio within girdled branches. Fishler et al. (1983) showed that the fruit size of grapefruits could be increased by providing increased leaf area to fruit ratio up to a saturating level of around 1.5 to 2 $m^2$ per fruit on girdled branches. However at low leaf area to fruit ratios, fruit size on girdled branches was decreased compared with control fruit (Fishler et al., 1983).

Girdling combined with leaf and fruit manipulation has been used as a physiological tool in kiwifruit (Snelgar et al., 1986; Lai et al., 1988). The use of girdling has been primarily to investigate the carbohydrate requirements of fruit growth, timing of flower evocation and source-sink relationships such as leaf distribution and the ability of fruit to compete for

carbohydrate. Davison (1980) also claimed that girdling could potentially be used to enhance early yields from young kiwifruit vines, by increasing flower numbers. On girdled lateral shoots, a leaf:fruit ratio of about two has been found to produce fruit of about the same size as those on non-girdled shoots, while at higher or lower leaf:fruit ratio, fruit size is increased or decreased respectively (Snelgar et al., 1986). However increasing or decreasing the leaf:fruit ratio on individual non-girdled shoots does not affect fruit size, as carbohydrate appears to be able to move readily into and out of laterals (Snelgar and Thorp, 1988; Lai et al., 1988). The response of kiwifruit to girdling and leaf:fruit ratio is summarised in table 1.3. Seager and Hewett (1995) applied girdles to kiwifruit lateral shoots at a leaf:fruit ratio of either one or five in early summer to establish the effect that carbohydrate concentration in fruit has on aspects of fruit quality. Soluble solids concentration, percentage dry matter, total sugars and starch concentrations were found to be higher at the high leaf:fruit ratio.

Table 1.3 Summary of the reported effects that girdling of lateral shoots has on kiwifruit weight (g) at leaf:fruit ratios of between one and five.

| Leaf:fruit ratio | (Snelgar et al., 1986): 1982 season | (Snelgar et al., 1986): 1983 season | (Lai et al., 1989a) | (Woolley et al., 1992) | (Seager and Hewett, 1995) |
|---|---|---|---|---|---|
| Intact (control) | 93 | 110 | 105 | 84 | - |
| one (girdled) | 70 | 74 | 78 | 57 | 57 |
| two (girdled) | 100 | 114 | 110 | - | - |
| three (girdled) | 126 | 140 | - | 115 | - |
| four (girdled) | 121 | - | - | - | - |
| five (girdled) | 122 | 172 | - | 128 | 102 |

The effect of a girdle applied in the previous season, has often been found to increase flower numbers in the following season. However girdling in the previous season does not always lead to an increase in flowering, and cultivars differences may determine the response in some

cases. For example, an 8 fold increase in flowering of 'Bengal' lychee was recorded following application of a girdle in the previous season, however there was no response of 'Tai so' lychee (Menzel and Paxton, 1986). The difference appeared to be because 'Tai so' had a high level of flowering on control trees (Menzel and Paxton, 1986).

Girdling of kiwifruit has also been found to result in increased flower numbers. Kiwifruit lateral shoots destined to become replacement canes which were girdled in mid or late summer had a higher percentage bud-burst and higher number of flowers per lateral shoot on those canes in the following spring (Snelgar and Manson, 1992a). However when girdled shoots were also defoliated, flowering was reduced (Snelgar and Manson, 1992a). The effect of girdling on flowering was consistent even if carried out in late summer after flower evocation is thought to occur, which led Snelgar and Manson (1992) to suggest that the flowering response may be due to increased carbohydrate levels in the shoot leading to enhanced flower initiation or reduced abortion of flowers the following year. Davison (1980) reported that trunk girdling increased flower numbers in young kiwifruit vines, and could potentially be used to enhance early yields.

Girdles applied at fruit set generally improve the percentage of fruits set and may allow increased retention of fruit which abscise in the period between anthesis and harvest (Monselise et al., 1972). In many fruit species, yield is limited by fruit number and girdling these may allow increased crop loads, thus increasing yield. As kiwifruit do not demonstrate fruit abscission after fruit set (Ferguson, 1984) except for fruit which fail to be pollinated (McKay, 1976) or are infected with blossom blight (Everett and Henshall, 1994), girdling of kiwifruit cannot improve fruit set.

### 1.3.4 Effects of girdling on tree physiology

The beneficial effects of girdling on fruit and flower production and retention are thought to be mainly due to a trapping of carbohydrate in the girdled unit and therefore altering the source-sink balance by eliminating competing sources outside the girdled unit. By placing a girdle on a branch or trunk, phloem transport of carbohydrates produced in the leaves is disrupted, thus carbohydrate flow out of the girdled unit is inhibited. Girdling does not

(normally) affect the xylem which transports water and nutrients from the roots, because girdling cuts are not (normally) made into the underlying wood. Thus water and nutrient translocation is not directly affected. Carbohydrates such as starch and soluble sugars have been demonstrated to occur at higher concentrations in girdled plant parts such as fruit, stems and leaves (Schaffer et al., 1987; Goldschmidt et al., 1985; Roper and Williams, 1989). Although there are instances where carbohydrate was not found to accumulate in response to a girdle (Dann et al., 1984), this may be due to the ability of sinks present in the girdled region to fully utilise all carbohydrate produced. For example Fishler et al. (1983) demonstrated that the starch content of twigs on girdled grapefruit branches was positively correlated to leaf area-to-fruit ratio on the branch. Thus the build up of carbohydrate was greater when there was a high source:sink ratio on the branch.

However parts of a plant which are isolated by the girdle from carbohydrate sources, in particular the root system, may be adversely affected by a reduction in the availability of carbohydrate. Carbohydrates partitioned to the root system are essential for respiration and continued root function (Lambers et al., 1996), as well as the promotion of return bloom in deciduous trees (Loescher et al., 1990). Thus a root system completely isolated from shoot produced carbohydrate cannot survive indefinitely, but would be expected to generally decline as stored carbohydrates are exhausted. In grape, root carbohydrate levels in vines which had been trunk girdled were found to be lower than in control vines two weeks after the girdle was applied (Roper and Williams, 1989). A decrease in the levels of starch in roots of one year old sour orange trees was found within one week after application of a trunk girdle (Wallerstein et al., 1978). Within three weeks, root starch levels had dropped to 50% of the levels present prior to girdling (Wallerstein et al., 1978). In citrus trees, there was a decrease in root activity of trunk girdled trees which in some cases led to tree death (Cohen, 1977). The potential effect of girdling on root function is illustrated by it's occasional use for intentional killing of trees (Noel, 1970).

Although the effect of girdling on kiwifruit roots has not been investigated, kiwifruit roots may be quite sensitive to carbohydrate shortages which could be induced by girdling. The peak period of root growth in kiwifruit vines does not occur until late in the season, when the vegetative and fruit growth rate is lower indicating that roots may be poor competitors for assimilates required for growth (Buwalda and Hutton, 1988). Partial defoliation of kiwifruit vines during the growing season was found to reduce root growth and return bloom to a

greater extent than fruit growth (Buwalda and Smith, 1990). The perennial components of a kiwifruit vine has been estimated to accumulate over 1000g of starch during the growing season and during winter and spring the starch content decreases by around the same amount, mostly due to depletion of starch in the roots (Smith et al., 1992a). Starch is the major storage carbohydrate in kiwifruit (Davison, 1990) and the concentration of carbohydrate in kiwifruit xylem sap increases just prior to bud-burst (Ferguson et al., 1983), thus it is likely that root stored carbohydrates are important for bud-burst in kiwifruit.

The relationship between the source:sink ratio on girdled branches and carbohydrate accumulation is not as simple as might be expected due to the apparent inhibition of net photosynthetic rates ($P_n$) on leaves within girdled plant parts. For example, girdling limbs of 'Red Delicious' apple trees reduced $P_n$ by 10% compared to non-girdled limbs on the same tree (Schechter et al., 1994). A reduction in $P_n$ of 30% was found in grape leaves 13 days after a girdle was applied when compared with control vines (Harrell and Williams, 1987). When individual leaves are girdled at the petiole, the reduction in $P_n$ is even more marked. Goldschmidt and Huber (1992) demonstrated reductions in $P_n$ of between 62 and 100% when leaves of soybean, cotton, cucumber, tomato, broad bean, sunflower, bean, or tobacco were petiole girdled using hot wax. An exception in this trend was found in spinach leaves, which showed only a small (16%) reduction (Goldschmidt and Huber, 1992), but this species has been found to be relatively insensitive to source-sink manipulation in other studies (Krapp and Stitt, 1995).

Placement of a girdle potentially isolates leaves from the sinks of the root system and trunk storage capacity, as well as fruit and expanding leaves in other parts of a tree. This suggests that the negative response of photosynthesis to girdling is probably due to feedback limitation caused by low sink demand (see section 1.3.1). The removal of strong sinks such as fruit (De Jong, 1986; Setter et al., 1980a) and developing leaves (Schaper and Chacko, 1993) has also been shown to reduce the $P_n$, while the presence of such sinks reduces carbohydrate levels in source leaves (Schaffer et al., 1987). Marquard (1987) found that net $P_n$ of pecan leaves was reduced from 20.1 to 6.5 mg $CO_2.dm^{-2}.hour$ by placing a girdle on fruit bearing shoots at a 12:1 leaf:fruit ratio and this was associated with a major increase in both starch and soluble sugars in shoots. On vegetative shoots, $P_n$ was reduced to as low as 0.6 mg $CO_2.dm^{-2}.hour$ (Marquard, 1987). Removal of soybean pods, or steam girdling of leaf

petioles resulted in a 70 or 90% reduction in the net rate of photosynthesis respectively. In both of these studies, the reduction in photosynthesis was associated with reduced stomatal conductance.

The effect of girdling and sink demand in kiwifruit is not clear. In one season no differences were found in net leaf photosynthesis between leaves on girdled and non-girdled lateral shoots of kiwifruit at leaf:fruit ratios ranging from one to three (Lai et al., 1989a). However in a different season, a leaf:fruit ratio of three or greater on girdled kiwifruit laterals resulted in reduced photosynthetic rate, although photosynthetic rate was not promoted by a leaf:fruit ratio of one (Woolley and Lai, 1990).

There have been many reports of changes in the plant hormone complement following the application of a girdle. These have been found on both a very localised basis near to the girdle itself (Dann et al., 1985) or at a point distant to the girdle (Weaver and Pool, 1965; Cutting and Lyne, 1993). Thus it is possible that some of the effects of girdling may be partially hormone mediated.

Dann et al. (1984) found that carbohydrate did not accumulate in limbs of peach trees which had been girdled, although the fruit growth rate was higher on girdled limbs. This led Dann et al. (1984) to suggest that the effects of girdling are initially due to the accumulation of growth regulators and longer term effects due to alterations in hormonal signals between roots and shoots. The nature of a hormonal signal from shoots to roots which is altered by the placement of a girdle has not been confirmed, although a hypothesis involving auxin is tenable. Auxin is transported in a basipetal direction in stems, i.e. auxin moves preferentially from apical to basal tissues (see section 1.2.6). Although transport of auxin has been reported to occur in xylem (Jacobs and Short, 1986), it is primarily transported by living tissue associated with the phloem (Kaufman et al., 1995). Removal of the bark by girdling, could therefore be expected to inhibit the basipetal movement of auxin towards the root system. Girdling can effectively release apical dominance, mimicking removal of the apical auxin source. For example, girdling of brussel sprout stems released buds below the girdle from apical dominance and they grew similarly to buds on decapitated plants (Thomas, 1983). Thus it appears likely that girdling inhibits the basipetal transport of auxin, which is necessary for apical dominance to continue (Thimann and Skoog, 1934). Girdling of peach trunks was found to decrease the concentration of IAA in the bark immediately beneath a girdle by 75%

(Dann et al., 1985). This was also associated with reduced cambial growth below the girdle and Dann et al. (1985) speculated that the reduction in basipetal IAA transport could be the signal mediating a change in root activity.

Skogerbo (1992) found reduced levels of zeatin riboside in xylem sap of trunk girdled apple trees. Cutting and Lyne (1993) found lower levels of both cytokinins and gibberellins in xylem sap of one year old shoots from girdled peach branches compared with shoots from control branches on the same tree. This suggests that the synthesis or allocation of gibberellins and cytokinins was altered between girdled and non-girdled branches. The reduced gibberellin and cytokinin concentrations were also associated with reduced growth of the shoots (Skogerbo, 1992; Cutting and Lyne, 1993). This suggests that reduced xylem sap gibberellin and cytokinins may be responsible for the general reduction in vegetative growth often reported to occur following girdling. In contrast, Wallerstein et al. (1973) found that a gibberellin-like substances accumulated above a girdle on orange branches and they concluded that this may be partly responsible for improved fruit set which occured.

Increased levels of ABA in leaves of girdled plants has been demonstrated by several authors. For example Loveys and Kriedemann (1974) found a 42% increase in the level of ABA in leaves following cincturing of grapevine stems and this was associated with a substantial increase in stomatal resistance, although there was no change in leaf water status associated with girdling. Setter et al. (1980b) found a 25-fold increase in leaf ABA levels 24 hours after a petiole steam girdle was applied to soybean. A previous study by the same authors showed that petiole girdling increased stomatal resistance and consequently photosynthetic rate, but did not alter leaf water potential or turgidity (Setter et al., 1980a). Thus it was concluded that the girdling treatment was not inducing a water stress related increase in ABA. Increase in leaf ABA levels has also been found after alteration of source sink ratio by removal of the reproductive sinks in grape (Loveys and Kriedemann, 1974), capsicum (Kriedeman et al., 1975) and soybean (Setter et al., 1980b).

## 1.4 Thesis Objectives

The objectives of this thesis were to investigate the contribution of sink strength to kiwifruit size in source limiting and source non-limiting conditions. These factors were investigated at the cell, organ and whole plant level. Source limitation was controlled by the use of girdling to isolate fruit sinks from alternate competing sinks for photosynthetically fixed carbohydrates. Sink strength was manipulated by genotype, seed formation after anthesis, inhibition of auxin transport and growth regulator application.

At the cell level, the objective was to determine the influence of cell division and cell expansion by the analysis of thin sections taken at different stages of fruit development. The main areas investigated were:

1. Is post-anthesis cell division influenced by assimilate availability (chapter three)?
2. Do seeds influence sink strength by controlling post-anthesis cell division or expansion (chapter six)?
3. Are genotypic differences in fruit size due to cell numbers or cell size (chapter six)?

At the organ level, the objective was to study factors which affect fruit size using measurement of growth rates during the growing season and fruit weight at maturity. The main areas investigated were:

1. Effects of shoot girdling and leaf:fruit ratio on fruit growth and size (chapter three).
2. The mechanism by which dominance is exerted in kiwifruit (chapter six).
3. Effects of exogenous growth regulators on fruit size of different kiwifruit genotypes (chapter six).
4. The necessity of auxin transport from kiwifruit for growth (chapter seven).
5. Interactions between CPPU and endogenous fruit hormones (chapter eight).

Investigations at the whole plant level resulted from an extension of girdling as an experimental technique, to its potential for increasing kiwifruit size commercially. The main areas investigated were:

1. Effects of cane girdling on fruit weight and yield from whole vines (chapter four).
2. Effects of cane girdling on return bloom (chapter four and five).
3. The response of root function, leaf photosynthesis, vegetative growth and xylem hormones to cane girdling (chapter five).

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# 2. General Materials and Methods

## 2.1 Sample Preparation for Hormone Analysis

### 2.1.1 Extraction and preliminary purification

Fruit tissue was harvested, immediately dissected into core, inner pericarp and outer pericarp tissue and snap frozen in liquid nitrogen. Fruit tissue was lyophilised, ground to a fine powder in a bench grinder (1 mm screen) and stored at -70°C until required. Sap was stored at -70°C in polypropylene tubes.

Up to 400 mg fruit tissue (dry weight) was weighed out on a balance (Mettler PM200) accurate to 0.1 mg. Tritiated abscisic acid ($^3$H-ABA, Amersham) and tritiated isopentenyl-adenosine ($^3$H-IPA dialcohol, see section 2.2.1) were added to each sample as internal standards. Bieleski solution (Bieleski, 1964) is often advocated for cytokinin extraction to avoid the conversion of nucleotide cytokinins to nucleosides (Morris, 1986). Phosphatase activity in 80% methanol extracts was measured by conversion of p-nitrophenol phosphate to p-nitrophenol, but was not found to be significantly different from Bieleski solution extracts. Therefore 80% methanol was used for routine extraction. Tissue was extracted in 100 times (v/w) of 80% methanol containing 10 mg/l butylated hydroxy toluene (anti-oxidant) for 24 hours at 1°C with continuous shaking. Extracts were rapidly filtered using a vacuum filtration apparatus fitted with Whatman no. 1 qualitative filter paper and then washed with a further 2 x 5ml methanol. Extracts were taken to near dryness, re-dissolved in 5ml 0.02M ammonium acetate and adjusted to pH 7 with 10% $NH_4OH$.

Before use, polyvinyl-polypyrolidine (PVPP) powder (Sigma P6755) was soaked in millipure water one day prior to use and fines were decanted five times to improve the flow rate. DEAE Sephadex A-25 (Pharmacia Biotech) was initially soaked in 0.1M ammonium acetate for two days prior to use. After use, sephadex was rejuvenated by

passing 2M NaCl in 0.1M NaOH through columns until the eluent was colourless, repeating with methanol and finally washing with 0.1M ammonium acetate. Rejuvenated Sephadex A-25 was stored in excess 0.1M ammonium acetate containing 200 $mg.l^{-1}$ $NaN_3$.

A 5ml bed volume PVPP column was connected in series to a 3ml bed volume Sephadex A-25 column and they were pre-conditioned with 10ml 0.1M ammonium acetate followed by 20ml 0.01M ammonium acetate. A $C_{18}$ Sep-pak pre-conditioned for cytokinin retention with 5ml methanol and 2 x 4ml 0.01M ammonium acetate was connected to the base of the PVPP and sephadex columns. Fruit extract or sap, adjusted to pH 7, was loaded onto the PVPP column and washed through with 20ml 0.01M ammonium acetate. The Sep-pak retaining cytokinins was removed, washed with 5ml $H_2O$ and cytokinins were eluted with 5ml 70% methanol. A second Sep-pak was connected to the bottom of the sephadex column, the PVPP column discarded and ABA eluted from the sephadex with 10ml 1M acetic acid. The second Sep-pak was removed, washed with 5ml $H_2O$ and eluted with 5ml 80% methanol. Both extracts were taken to dryness using a Savant AS290 automatic speedvac (speedvac).

Cytokinin extract was re-dissolved in 400 μl of the High performance liquid chromatography (HPLC) starting solvent (10% acetonitrile in TEA, see section 2.1.2), filtered though a 0.45μm filter (Gelman GHP acrodisc 13mm syringe filter with low hold-up volume). A further 100 μl of solvent was passed through the filter to minimise loss.

ABA extract was re-dissolved in 1ml TBS buffer (see section 2.3.2) and duplicate aliquots removed for radioactivity counting to determine recovery immediately prior to assay. Recovery of ABA usually exceeded 80%.

### 2.1.2 High performance liquid chromatography separation of cytokinins

High performance liquid chromatography (HPLC) was used to separate the different cytokinins for analysis. Separation was made on a 220 x 4.6 octadecyl silica (ODS)

column in line with a 3 x 4.6 ODS guard column (Applied Biosystems inc. Brownlee spheri-5).

Solvent delivery to the HPLC system was via a Waters 501 HPLC pump and a Waters 510 HPLC pump controlled by a Waters automated gradient controller. All solvents were HPLC grade and made up fresh prior to each run. TEA solvent was a 40mM acetic acid solution adjusted to pH 3.38 with re-distilled triethylamine. Both TEA and acetonitrile solvents were vacuum filtered through a 0.2μm nylon filter and dissolved gases were removed by sonication for 30 minutes. The retention times of cytokinin standards was established with a programmable multiwavelength detector (Waters 490E) at 268nm. Retention time of IPA dialcohol was established using a flow through radioactivity monitor (βRam, In/Us systems N.J., U.S.A.) with Inflow-3 liquid scintillant for homogenous counting cells at a ratio of 3ml scintillant to 1ml eluent, or by counting radioactivity of collected fractions on a liquid scintillation counter (Wallac 1409 series).

Prior to each HPLC run, retention times of cytokinins and dialcohols were checked. Retention times were found to be extremely consistent between runs.

Purified samples (500μl) were loaded via a Rheodyne injection port and separated with a solvent gradient (Table 2.1). After each injection, the column was returned to the starting conditions and allowed to re-equilibrate for 10 minutes. The column was cleaned by passing 100% acetonitrile through after every five samples.

Table 2.1 Solvent gradient used for separation of cytokinins on an octadecyl silica (ODS) column used in High performance liquid chromatography (HPLC.

| Time (minutes) | %Acetonitrile | %TEA | Curve |
|---|---|---|---|
| 0 | 10 | 90 | * |
| 30 | 40 | 60 | 6 |
| 33 | 40 | 60 | 7 |

One minute fractions were collected directly into 1.5ml RIA assay tubes in an ISCO 568 automated fraction collector. Fractions containing $^{3}$H-dialcohol internal standard

(fractions 22-24) were taken to dryness in the speedvac, re-dissolved in 200 μl methanol and counted for radioactivity. Other fractions were taken to dryness and assayed by radioimmunoassay (see section 2.2.4) using the appropriate antiserum. Typical results for retention times and assays are shown in Figure 2.1.

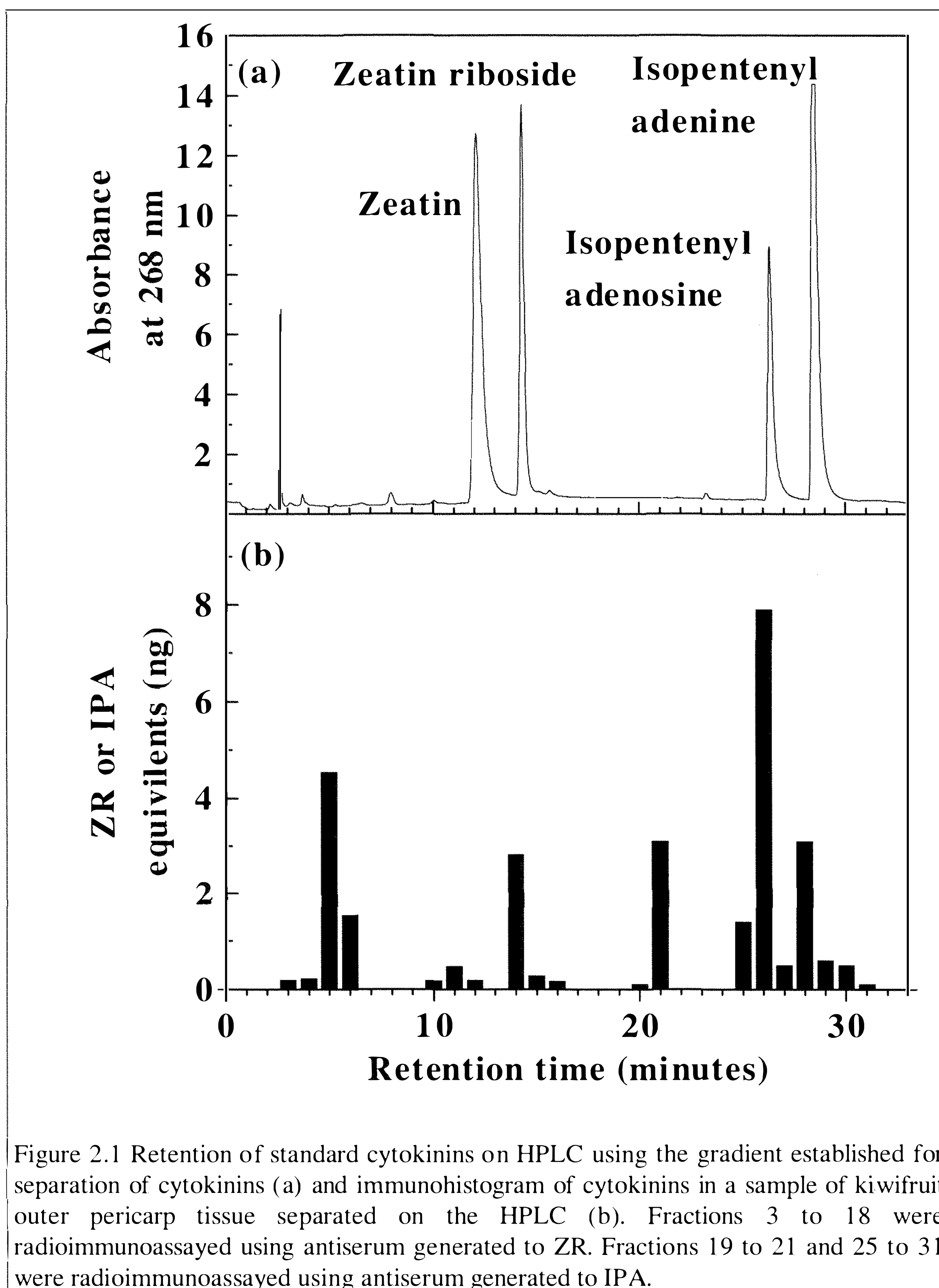


Figure 2.1 Retention of standard cytokinins on HPLC using the gradient established for separation of cytokinins (a) and immunohistogram of cytokinins in a sample of kiwifruit outer pericarp tissue separated on the HPLC (b). Fractions 3 to 18 were radioimmunoassayed using antiserum generated to ZR. Fractions 19 to 21 and 25 to 31 were radioimmunoassayed using antiserum generated to IPA.

## 2.2 Immunological Analysis of Cytokinins

Immunology is based on the ability of a pathogen to elicit an immune response in an animal. Upon infection with a pathogen, the immune system produces antibody proteins which bind to the pathogen and render it ineffective at causing damage. Collection of a blood sample from the infected animal yields serum containing antibodies to the pathogen, which are able to be used for a range of imunological based laboratory techniques.

Although immunology was developed primarily for use in medical science, its application in other fields has since been extensively developed, as it has become clear that animals can be immunised against a wide variety of substances (antigens) which may not normally be pathogenic (Galfre and Butcher, 1986). Antibodies thus formed can be used in quantitative or qualitative immunoassays because of their ability to bind to the antigen present in biological samples of interest, such as plant extracts.

A wide variety of immunoassays have been developed, mostly based on competitive binding of the sample antigen or a labelled antigen tracer. Radioimmunoasay (RIA) techniques were developed from the work of Yalow and Berson (1960) and are now widely used for analysis of plant growth regulators, including cytokinins (Weiler, 1980; MacDonald et al., 1981; Ernst, 1986). RIA is based on an equilibrium between unlabelled antigen and radioactively labelled antigen (tracer) for a saturable amount of antibody binding sites (Ernst, 1986). After equilibrium, bound and free tracer are separated and the radioactivity present (usually) in the bound form is measured. As the concentration of unlabelled antigen increases, competition with the tracer for limited binding sites will occur, leading to a decreased amount of radioactivity in the bound form. This allows a standard curve to be generated, enabling quantification of antigen concentration in samples.

The main requirements for RIA (Chard, 1990) are:

1. Purified antigen for use as standards.
2. Purified sample free of cross-reacting or interfering substances.

3. Radio-labelled tracer which behaves identically with the unlabelled antigen.
4. Antibodies which have high specificity (bind specifically to antigen), affinity (ability to bind antigen) and titre (concentration of binding sites).
5. An efficient and practical separation system.

### 2.2.1 Synthesis of $^3$H-cytokinin riboside dialcohol tracers

The method of MacDonald and Morris (1985) was used to prepare radio-labelled dialcohols for use in RIA. Although the riboside part of the cytokinin molecule is altered in the procedure, antibody specificity is usually directed to the opposite side of coupling to protein (Weiler, 1980). As coupling of cytokinins to protein for generation of antiserum is through the same position as alteration of the dialcohol molecule (carbon three and four of the ribose group), cross reactivity to the cytokinin dialcohol should be similar to that of the unaltered cytokinin riboside.

t-Zeatin-riboside (ZR) or Isopentenyl-adenosine (IPA) (15 μMol) were individually dissolved in 500 μl HPLC grade methanol and millipure $H_2O$ (500ul). $NaIO_4$ (Sigma S1878) (30μMol) was added, vortex mixed briefly and placed at 4°C for 45 minutes to convert the riboside group to a dialdehyde. The cytokinin dialdehyde was immediately loaded onto a $C_{18}$ Sep-pak cartidge (Waters) which had been pre-conditioned with methanol (5ml) and $H_2O$ (5ml). Columns were washed with $H_2O$ (5ml) to remove excess periodate. Cytokinin dialdehydes were eluted from the columns with methanol (6ml), dried under a stream of oxygen free nitrogen gas and re-dissolved in 100 μl methanol. A 20 μl sample was placed in a polypropylene micro-centrifuge tube and 1M NaHCO3 (100 μl, pH=9.3) added.

Tritiated sodium borohydride (1Ci, specific activity 50Ci/mMol obtained from Amersham) was dissolved in NaOH (100 μl 0.5M) in a vented fume cupboard. 25μl was immediately added to each dialdehyde preparation and left to react for 30 minutes to form a tritiated dialcohol. Glacial acetic acid was added very slowly to degrade excess borohydride until bubbling had ceased. Each putative dialcohol was purified on a pre-conditioned sep-pak column as for the dialdehyde.

Samples of the putative dialcohols diluted 2000:1 with 20% acetonitrile in TEA were injected onto a $C_{18}$ HPLC column running under isocratic conditions (20% acetonitrile, 80% TEA) connected to a βram radioactivity monitor (see section 2.1.2). A single radioactive peak was found to elute for each cytokinin preparation and these were considered likely to be cytokinin dialcohols. The retention time for each putative $^{3}H$-dialcohol was lower than for standard cytokinin ribosides and similar to that expected for the dialcohol, but in each case the radioactive peak corresponded to a single UV peak.

Specific activity was estimated for each of the dialcohols using a standard curve of UV absorbance for each cytokinin to estimate the amount of cytokinin dialcohol and by counting radioactivity on the scintillation counter. IPA was estimated at 47 Ci/mMol and ZR was estimated at 37 Ci/mMol.

### 2.2.2 Preparation of cytokinin-protein conjugates

Low molecular weight molecules such as cytokinins will not normally induce an immunogenic response and thus need to be conjugated to a larger molecule such as a protein prior to immunisation. When this is done, antibodies specific to both the protein and the small molecule (hapten) are generated.

ZR and IPA were linked to bovine serum albumen (BSA) using the periodate oxidation method of Erlanger and Beiser (1964) modified by MacDonald et al. (1981). ZR (Sigma Z0375) or IPA (Sigma D7257) (22mg) were dissolved in 5ml millipure water. $NaIO_4$ (27 mg) was added and the mixture incubated for four hours at 4°C. BSA (60 mg of fraction V, Sigma A3350) was added and allowed to dissolve without shaking. The pH was adjusted to 9.3 with $K_2CO_3$ solution (5% w/v) and the solution was incubated at 4°C for three hours. Ethylene glycol (25µl, Sigma E9129) was added to destroy excess periodate and the pH was checked and readjusted to 9.3 if required before a further 2 hour incubation at 4°C. $NaBH_4$ (35mg) was added, the vial covered and placed to incubate at 4°C for 26 hours. Excess borohydride was eliminated by adjusting to the pH to 5.5 with

1M formic acid, slowly to avoid excess foaming and then readjusting the pH to 8.5 with 5% $K_2CO_3$.

The cytokinin-BSA conjugate formed was placed into wide dialysis tubing, double knotted and dialysed repeatedly against 0.05M phosphate buffered saline (PBS) pH 8.5 for four days with three changes per day, 20ml vs. 20 litres with constant stirring at 4°C. The conjugates were stored frozen in PBS.

Binding ratios were estimated spectrophotometrically. Protein (BSA) concentration of conjugates was estimated using the biuret assay. Absorbance of conjugate at 269nm was measured against a reference cell containing a BSA solution of the same concentration. This gave the absorbance at 269nm due to the presence of cytokinin (bound to the protein). The concentration of cytokinin was then estimated using a standard curve of absorbance of IPA or ZR at 269 nm (Figure 2.2). The binding ratio was calculated as mol-cytokinin/mol-BSA (BSA, molecular weight=68 000 $g.mol^{-1}$) and was 15.6 for ZR and 16.5 for IPA (Figure 2.2), which is comparable to that cited in the literature (MacDonald et al., 1981; Ernst, 1986). BSA has 30-35 lysine residues available as binding sites (Harlow and Lane, 1988). Approximately one mol of hapten per 50 amino acid residues is a minimum binding ratio for the generation of antibodies to the hapten (Harlow and Lane, 1988). BSA contains 211 residues, thus a minimum binding ratio of 4.2 is required for suitable for generation of antiserum, which was exceeded by the conjugates prepared.

### 2.2.3 Generation of polyclonal antiserum.

The use of adjuvants to enhance immune responses is widespread. Adjuvants perform two functions. Firstly they form a deposit to protect antigens against rapid catabolism and secondly they act to stimulate the immune system non-specifically (Harlow and Lane, 1988). Freunds incomplete adjuvant (iFA) contains 85% mineral oil and 15% mannide mono-oleate emulsifier (Harlow and Lane, 1988). Freunds complete adjuvant (cFA) is formed when iFA is mixed with heat killed mycobacteria. Although cFA has been the adjuvant of choice for immunisation, use in recent years has declined due to

adverse side effects such as granulomas and hypersensitivity reactions (Broderson, 1989) and thus concern for animal welfare.

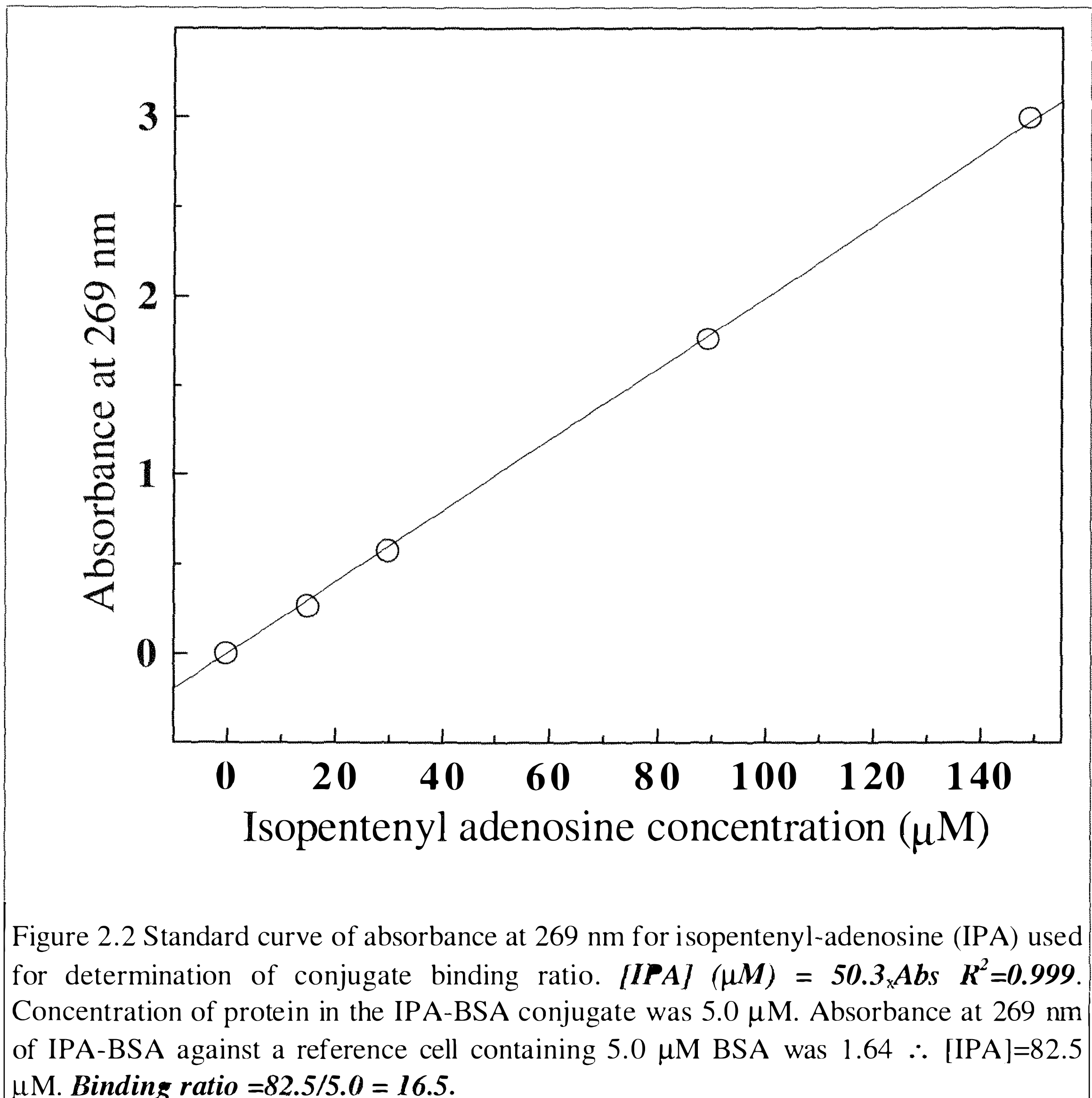


Figure 2.2 Standard curve of absorbance at 269 nm for isopentenyl-adenosine (IPA) used for determination of conjugate binding ratio. ***[IPA] (μM) = 50.3×Abs*** $R^2$***=0.999***. Concentration of protein in the IPA-BSA conjugate was 5.0 μM. Absorbance at 269 nm of IPA-BSA against a reference cell containing 5.0 μM BSA was 1.64 ∴ [IPA]=82.5 μM. ***Binding ratio =82.5/5.0 = 16.5.***

Ethics approval was granted from the Massey University animal ethics committee to immunise New Zealand white rabbits using conjugate plus iFA by subcutaneous and intramuscular injections.

Conjugate (1 mg) dissolved in 1ml PBS was mixed with 1ml iFA by passing the mixture repeatedly between two 3ml luer lock glass syringes connected by a needle with luers on each end. This was repeated until the mixture would form a non-dispersible droplet on a still water surface. Each conjugate was injected subcutaneously and intramuscularly (two

sites per rabbit) into rabbits held in individual cages at the Massey University small animal production unit. Booster injections were made at 3 and 6 weeks. Blood samples (5ml) were collected from the marginal ear vein. The blood was allowed to clot at room temperature for one hour and placed at 4°C overnight for the clot to solidify and shrink. Serum was drawn off and centrifuged at 4°C for 30 minutes at 15000xg. Serum was tested for immunogenicity in the radioimmunoassay described in section 3.2.5.

Serum titre was measured as the dilution which would bind 50% of $^3$H-cytokinin dialcohol in a standard RIA. The titre of all serums were very low (<100).

After new ethics approval had been granted, alternative injection schedules were trialed on new rabbits which included:

1. weekly boosters
2. boosters at wide intervals (eight weeks)
3. multiple (10) injection sites per rabbit.

Blood collection was always made at 7 to 10 days after the final booster. A minor improvement was noticed in serum titre, however titres were well below those cited by other authors (Ernst, 1986; Weiler, 1980) and were too low to be useful for processing of samples. Although some antigens such as large proteins and bacteria may elicit a strong immune response in the absence of adjuvants, small molecules (haptens) conjugated to a protein carrier may require significant non-specific stimulation of the immune system to generate a satisfactory response (Chard, 1990). On the basis of the poor response of anti-cytokinin titre using iFA, ethics approval was granted to use cFA for an initial injection, to be followed by boosters using iFA. Injections were made at 0, 2, 5 and 13 weeks and a 1ml blood sample was taken 7 days after the final booster. Serum titre was found to be significantly improved for both ZR (titre=4500) and IPA (titre=5500) (Figure 2.3). On the following day, blood (40ml) was collected from the marginal ear vein of each rabbit, processed as described above and serum stored in 100μl aliquots at -70°C.

Scatchard plots (Scatchard, 1949) were produced for ZR and IPA antiserum using the method of calculating bound to free ratio and bound antigen mass of Dotti and Castagnetti (1978) cited in Chard (1990):

$$\text{Bound to free ratio (B / F)} = \frac{\text{counts bound - NSB}}{\text{Total counts - (counts bound - NSB)}}$$

$$\text{Bound mass (B)} = (\text{mass standard} + \text{mass tracer}) x (\frac{\text{counts bound}}{\text{total counts}})$$

where NSB is the non-specific binding.

If the antiserum is homogenous and has a single affinity constant (K), the scatchard plot is linear and the slope of a scatchard plot provides an estimate of K for the equilibrium reaction between antigen (Ag) and antibody (Ab); Ag + Ab $\leftrightarrow$ AgAb, $K = \frac{[AgAb]}{[Ag]x[Ab]}$ (Chard, 1990). Scatchard plots for ZR and IPA were found to deviate from linearity with a right-hand skewness (Figure 2.4). However polyclonal antiserum usually contain several populations of antibodies to a given antigen, each with a different K value (Chard, 1990). In this case, the scatchard plot is a curve composed of a combination of lines from each specific antibody population. Affinity constants were calculated from the steepest and shallowest regions of the curve and demonstrated the presence of very high affinity antibodies in both antiserum (ZR: $1.1 \times 10^{11}$ to $2.3 \times 10^{12}$ $l.mol^{-1}$, IPA: $2.2 \times 10^{11}$ to $2.1 \times 10^{12}$ $l.mol^{-1}$).

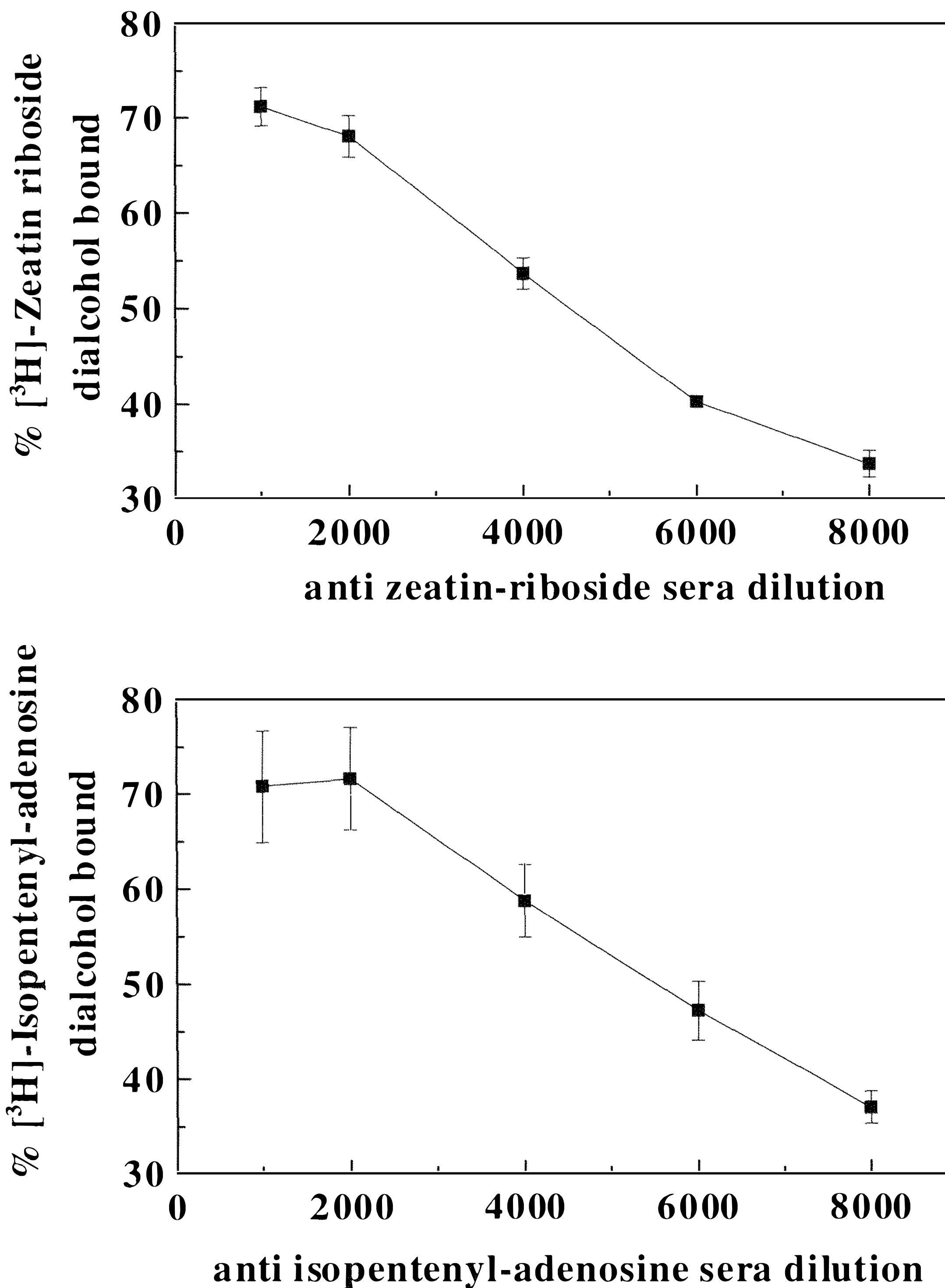


Figure 2.3 Antiserum dilution curves for zeatin-riboside and isopentenyl-adenosine in the absence of cytokinin. Data were generated using the standard radio-immunoassay technique.

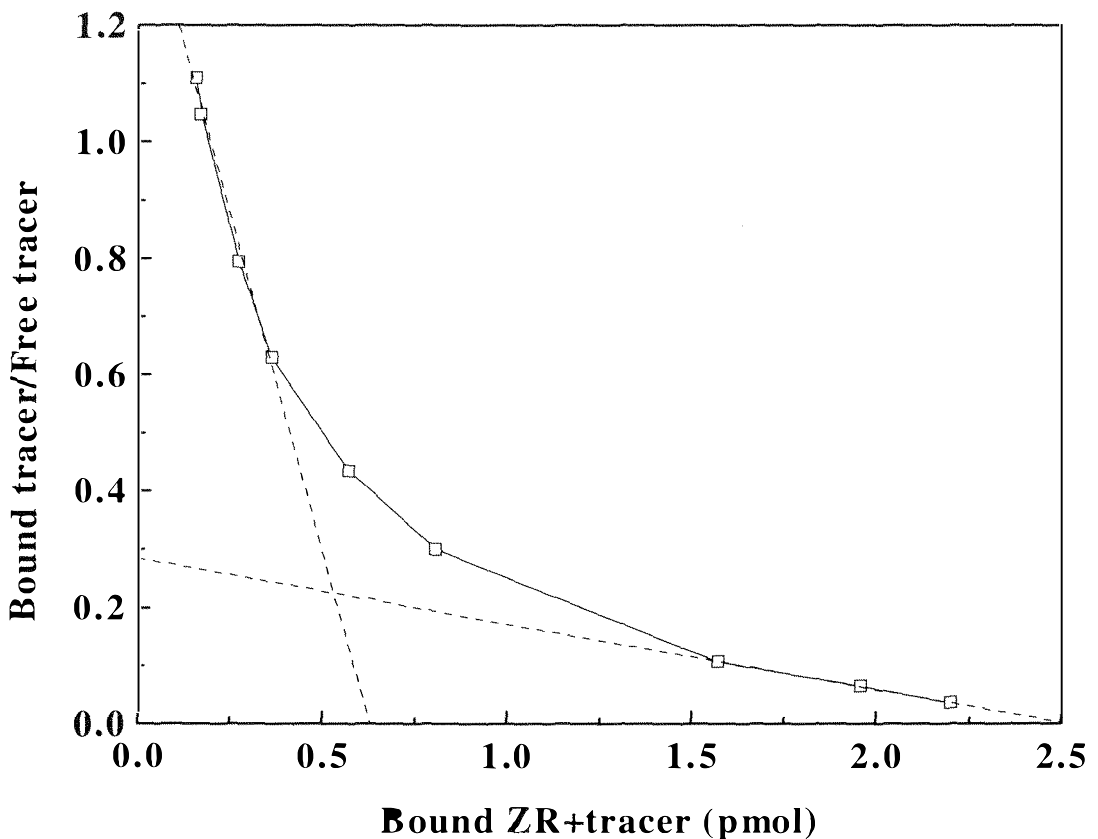


Figure 2.4 Scatchard plot (Scatchard, 1949) for antiserum raised against zeatin-riboside and used in radioimmunoassay. The slope of the curve gives an estimate of the affinity constant (K). Non-linearity is common with polyclonal antiserum due to a heterogeneous population of antibodies. The maximum and minimum slopes (- - -) were estimated for the range of the standard curve.

Cross reactivity of antiserum to naturally occurring and synthetic cytokinins was tested in RIA (Table 2.2) and these were found to be similar to those found in other laboratories [see Badenoch-Jones et al. (1987) for review]. Of the cytokinins tested for cross reactivity, only the corresponding free base for each cytokinin (zeatin and isopentenyl-adenine) showed appreciable cross reactivity (Table 2.2). Logit transformed standard curves for the free base cytokinins had parallel slopes to ribosyl cytokinins (Figure 2.8).

Table 2.2 Molar cross reactivity of anti-zeatin-riboside sera or anti-isopentenyl-adenosine sera. Cross reactivity were determined at the concentration of compound which bound 50% of the radioactivity bound in the presence of nil cytokinin and represents $\frac{\text{moles cytokinin equivalents detected}}{\text{moles compound added}}$.

| Compound | Zeatin-riboside antiserum | Isopentenyladenosine antiserum |
|---|---|---|
| trans-Zeatin-riboside | 100 | 1.1 |
| trans-Zeatin | 40 | 0.3 |
| cis-Zeatin | 0.6 | 0.8 |
| Dihydrozeatin | 1.0 | 0.2 |
| Dihydrozeatin-riboside | 1.3 | 0.2 |
| Isopentenyl-adenosine | 3.1 | 100 |
| Isopentenyl-adenine | 0.9 | 37.6 |
| 6-Furfurylaminopurine (Kinetin) | 0.5 | 0.9 |
| 6-Benzylaminopurine | 0.5 | 3.2 |
| Adenosine | 0 | 0 |
| Adenine | 0 | 0 |
| N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) | 0 | 0 |

### 2.2.4 Radioimmunoassay (RIA) of ZR and IPA

Polypropylene micro-centrifuge tube (1.5ml, Sarstedt D51588) were tested for their binding of $^3$H-cytokinin dialcohols and $^3$H-ABA. Absorption of $^3$H-tracers from a buffer solution, was found to be not significantly ($P<0.001$) above background counts and these tubes were used as RIA reaction tubes.

Phosphate buffered saline (PBS) was prepared by dissolving $KH_2PO_4$ 0.38g, $K_2HPO_4$ 1.3g, NaCl 8.78g in 1l millipure water and adjusted to pH 7.4. For preparation of 100% saturated $NH_4SO_4$, a super saturated solution was prepared by dissolving $NH_4SO_4$ to saturation in boiling water and allowing excess $NH_4SO_4$ to precipitate as the solution cooled to room temperature. 90% saturated $NH_4SO_4$ was then prepared by diluting 9 parts saturated $NH_4SO_4$ with 1 part $H_2O$ and adjusting pH to 7.4.

Tritiated dialcohol in methanol (20 000 disintegrations per minute (DPM), 100μl) was added to cytokinin sample or standard in an assay tube and taken to dryness in the speedvac. PBS (400μl) containing antiserum (diluted to bind 50% of $^3$H-dialcohol) and 5μl/ml new-born calf serum was added and the lid closed. The addition of the entire reaction mixture at once, rather than the standard method of separate addition of buffer and antiserum (Ernst, 1986) enabled fewer pipette operations and therefore reduced between tube variation, but did not affect the assay.

Tubes were allowed to pre-incubate at room temperature for 20 minutes to allow the $^3$H-dialcohol and cytokinin to re-dissolve, after which tubes were vortexed briefly to mix and incubated for a further 40 minutes. Binding of $^3$H-tracer by antiserum reached equilibrium after 40 minutes and was insensitive to total incubation time between 40 and 150 minutes (Figure 2.5). A 60 minute total incubation period was used for routine assay for convenience and to allow for slight differences in timing between tubes. Retention of radioactivity by the tube was approximately 300 DPM or 1.5% of radioactivity added following the 20 minute pre-incubation period and was not influenced by total incubation time up to 150 minutes.

To precipitate the antibody and bound tracer, 500 µl of 90% saturated $NH_4SO_4$ was added to bring tubes to a final 50% $NH_4SO_4$ saturation. Tubes were vortex mixed and incubated at room temperature for a further 20 minutes. Tubes were then placed into a 24 place fixed angle micro-centrifuge (Heraeus Biofuge) with hinges facing out and centrifuged for 3 minutes at 13000 RPM (~10000 x g). The fixed angle causes the precipitate to be coated along the wall facing outwards (below hinge). Although temperature was not controlled in the micro-centrifuge, the rotor was well insulated from the spindle and consequently there was no appreciable rise in temperature. A syringe needle attached to a vacuum was slid down the wall of the tube opposite to the hinge to aspirate the entire supernatant. The pellet was re-dissolved in 200µl methanol, mixed with 1ml In-Flow 3 scintillation fluid (IN/US Systems Inc., Fla.) and measured for radioactive emission on the scintillation counter. Radioactive emission was automatically corrected for counting efficiency using the external standard method and was recorded as DPM. Duplicate measurements of DPM were taken for each tube.

Non-specific binding (NSB) was measured as the amount of tracer retained in the absence of antiserum. In the absence of antiserum, NSB of the tracer was usually less than 4% of the total tracer added. This was able to be reduced by approximately 0.5% by washing the tube with 50% saturated $NH_4SO_4$, but this was not considered necessary for routine assay. Up to 150 minutes after addition of buffers, NSB was insensitive to total incubation time (Figure 2.5). Perlstein (1987) points out the NSB does not always remain constant over the entire concentration range. However NSB was not found to be affected by the addition of cytokinin at concentrations present in the standard curve, although it was significantly lower (contrast, $p=0.0005$) when cytokinin was present in vast excess (10 000 ng). This was due to significantly lower (contrast, $P=0.0007$) permanent binding of the tracer to the tube in the presence of an excess of cytokinin. The inability of standard curve range cytokinins to inhibit tracer binding to the tube suggests that either the tracer itself, or that breakdown products of the tracer had a higher affinity for binding sites on the tube than cytokinin. As the tracer which had degraded (see next paragraph) showed increased levels of NSB, it is possible that tube-bound radioactivity consisted of breakdown products rather than dialcohol.

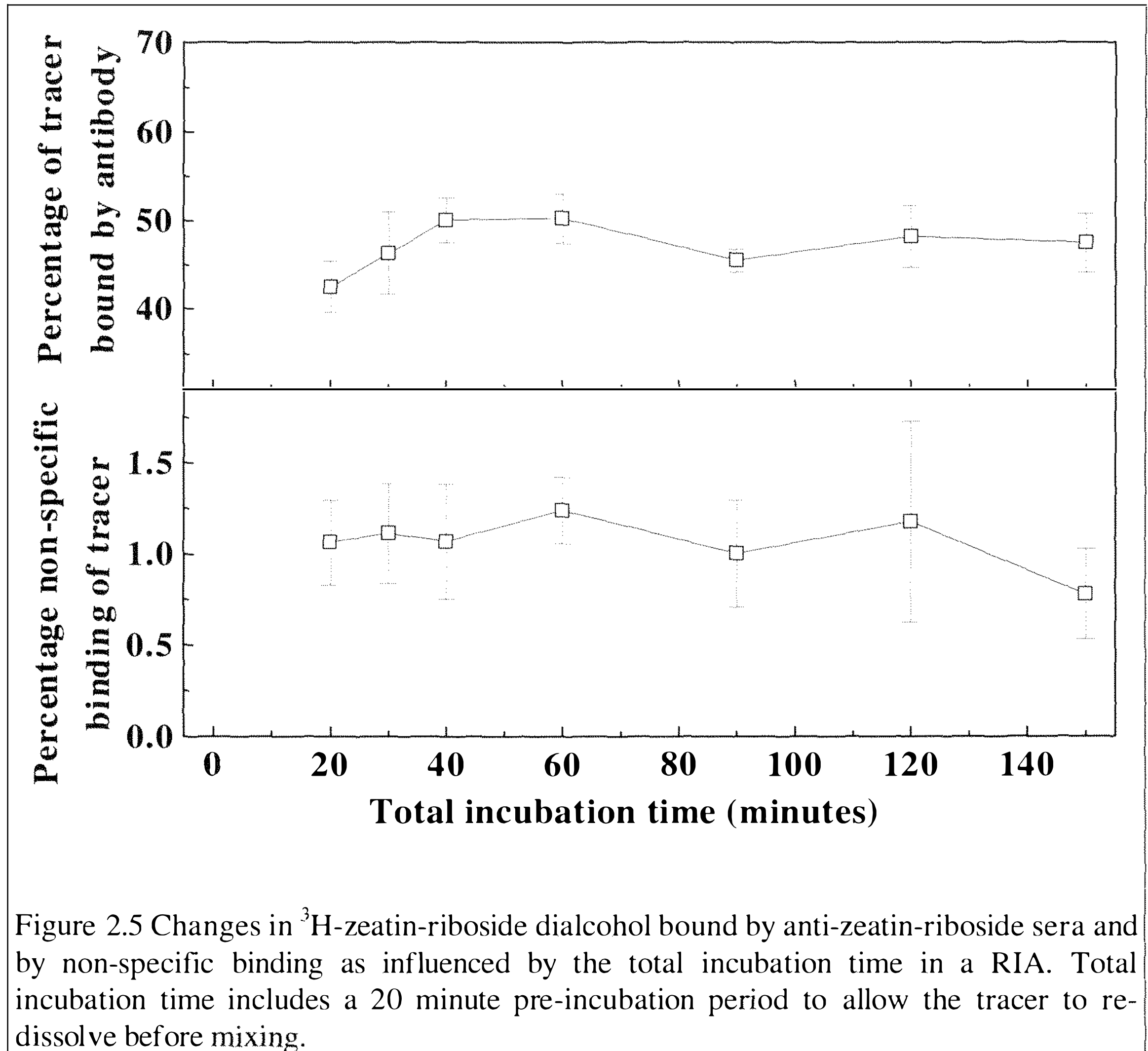


Figure 2.5 Changes in $^3$H-zeatin-riboside dialcohol bound by anti-zeatin-riboside sera and by non-specific binding as influenced by the total incubation time in a RIA. Total incubation time includes a 20 minute pre-incubation period to allow the tracer to re-dissolve before mixing.

Degradation of the $^3$H-dialcohol tracer occurred over time which appeared to result in increased levels of NSB in the assay. Degradation may have been caused by storage in a small volume of methanol (F. Bangerth, personal communication, 1996) or unintentional storage at room temperature for an unknown period of time. $^3$H-dialcohols were able to be re-purified on a c-18 sep-pak column.

As constituents in samples could affect pH and ionic strength and in order to optimise the RIA procedure, the effect of pH and ionic strength was tested under standard RIA conditions. Binding of tracer in the absence of cytokinin was significantly influenced by both the ionic strength and the pH of the buffer. The percentage binding was quite stable between pH 6.5 and 7.5 at the normal ionic strength (0.01M phosphate), but binding was lower at pH extremes of 5.5 and 8.0 (Figure 2.6). Although the highest binding was

attained at a low ionic strength (0.005M), this also provided weaker buffering capacity and it was reasoned that this may have been less suitable in the presence of samples. The amount of cytokinin estimated in a tube appeared to be slightly overestimated at pH 6.4 or 6.9 compared with pH 7.4 (Table 2.3), due to lower percentage binding of the tracer at the lower pH's. This is in contrast to Weiler (1980) who found that binding of ZR tracers to polyclonal antibodies raised against ZR-BSA conjugates were not pH dependent between pH 5.5 and pH 9.5. However binding of a number of antibody antigen complexes have been shown to be sensitive to pH in RIA (Chan, 1987; Chard, 1990). Samples for RIA were not found to reduce the pH of the assay buffer, so this was not considered important and buffer pH 7.4 was used for routine assay.

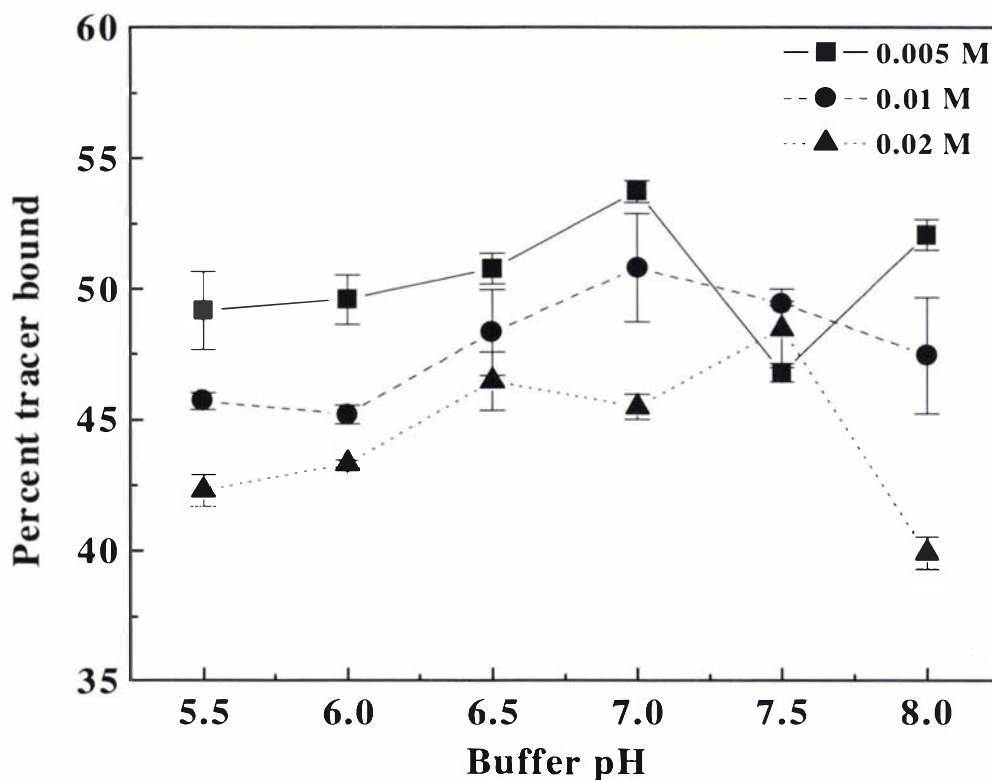


Figure 2.6 The effect of phosphate ionic strength and pH of PBS on percentage of tracer bound by IPA antiserum with no added ligand. NaCl concentration in buffer was constant for all tubes (0.15 M). (n=2).

Standard curves of cytokinin concentration were generated by the addition of a fixed volume of serially diluted cytokinin to the assay tube. Curves were able to be transformed to a straight line using the logit transformation (Figure 2.8). Both the IPA and ZR assays had a similar range of sensitivity, approximately 0.03 and 60 pmol, although the highest accuracy was obtained between 0.1 and 30 pmol. Standard curve tubes were included with all sample runs, to account for day to day variation in assay conditions such as pH and temperature which can potentially affect the equilibrium between antigen and antibody (Chan, 1987).

Table 2.3 The effect of pH on the estimation of cytokinin concentration in RIA standard curve tubes. Values shown are mean concentration of IPA and standard error in brackets estimated from the standard curve generated at pH 7.4 (n=2).

| Actual IPA concentration (ng/tube) | IPA concentration (ng/tube) estimated from standard curve | | |
|---|---|---|---|
| | pH 7.4 | pH 6.9 | pH 6.4 |
| 0 | 0 (0) | 0.03 (0.01) | 0.01 (0.01) |
| 0.1 | 0.10 (0.03) | 0.17 (0.03) | 0.11 (0.000) |
| 0.2 | 0.20 (0.001) | 0.23 (0.004) | 0.20 (0.007) |
| 0.5 | 0.61 (0.01) | 0.78 (0.03) | 0.75 (0.006) |
| 1 | 1.08 (0.06) | 1.39 (0.04) | 1.32 (0.003) |
| 5 | 4.27 (0.75) | 3.87 (0.20) | 4.29 (0.56) |

Standard error of the mean in parentheses.

### 2.2.5 Identification and quantification of putative cytokinins in sample extracts

Cytokinins in extracts of sap or fruit were putatively identified following RIA of fractions obtained from HPLC separation. Retention times of known standards of Z, ZR, IPA and 2iP were consistent between runs and these retention times corresponded to peaks of cross reacting fractions in the RIA (Figure 2.1). In addition, the putative cytokinins which were found to occur in kiwifruit tissue have also been identified using gas

chromatography-mass spectroscopy (GC-MS) (Lewis et al., 1996). As the RIA demonstrated high specificity for these cytokinins (Table 2.2), this strongly suggests that the compounds identified in the RIA were in fact Z, ZR, IPA and 2iP. An additional cross reacting compound occurred at a retention time of around five minutes, probably corresponding to zeatin-9-glucoside (Lewis, 1994), however the retention time of this peak was somewhat variable. A further cross reacting peak occurred at around 21 minutes, probably corresponding to isopentenyl-9-glucoside (Lewis, 1994), however retention of this compound overlapped with retention of the internal standard.

Putative cytokinins in appropriate HPLC fractions were quantified using standard curves for ZR and IPA (Figure 2.8). The concentration of ZR and IPA were quantified directly from the appropriate standard curve. Standard curves for Z and 2iP were parallel with those for the respective riboside cytokinins (Figure 2.8), which allowed estimates of ZR- and IPA-equivalents to be converted to Z and 2iP using a single value for cross reactivity (Table 2.2).

### 2.2.6 Assay validation and the influence of non-specific binding

Non-specific interference by compounds present in xylem sap or fruit tissue was tested in samples which had only been purified by passing through a sep-pak cartridge. This was done by the standard method of adding antigen to samples at different dilutions (Pengelly, 1986; Jones, 1987). Initially, it appeared that samples contained significant levels of interfering compounds, as curves generated were not parallel with the standard curve. Further purification by use of PVPP, Sephadex A-25 and C-18 sep-pak columns as described in section 2.1.1 did not initially result in satisfactory validation.

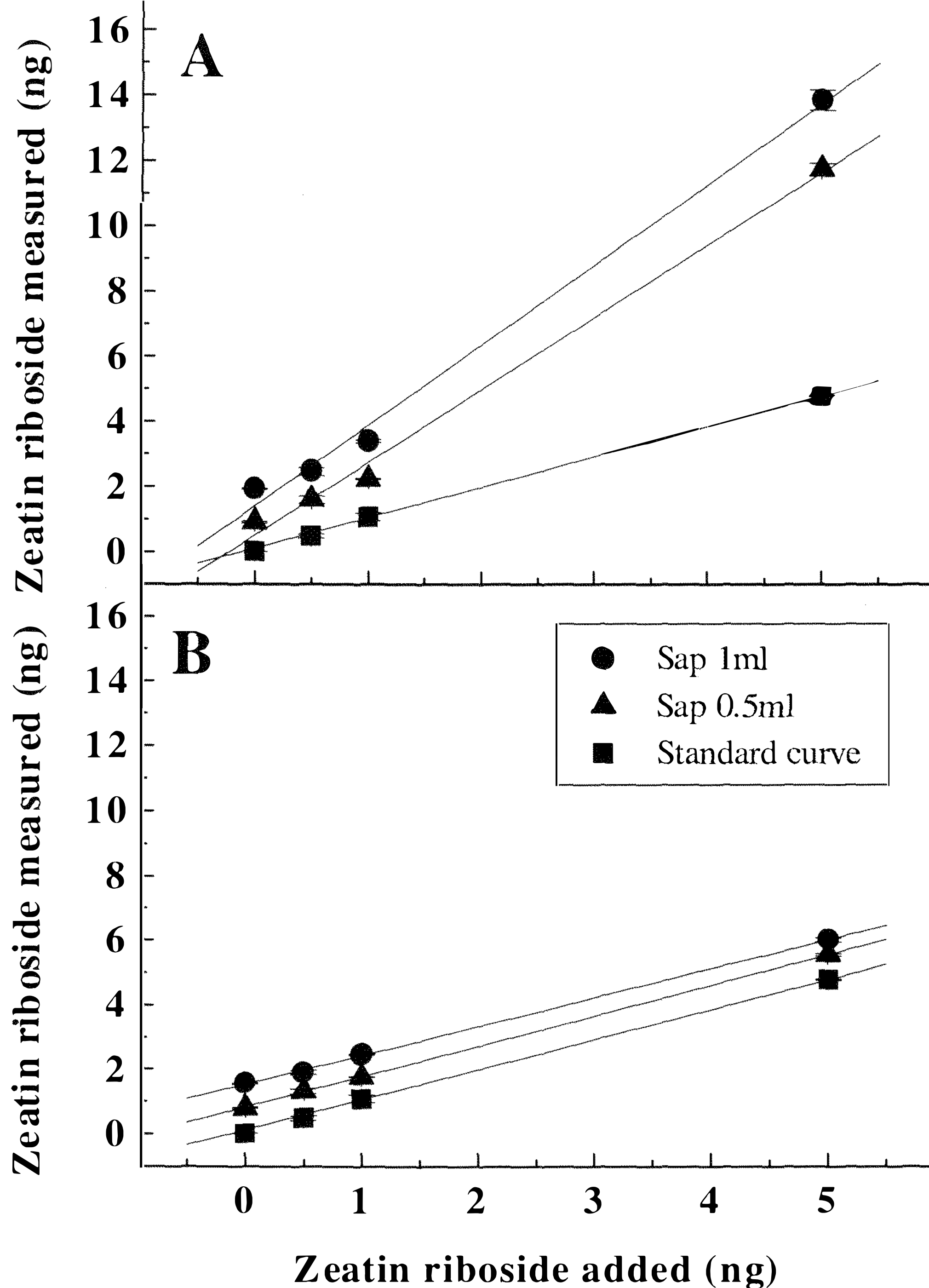


Figure 2.7 Validation curve for zeatin-riboside in xylem sap of kiwifruit using NSB measured for standard curve (A), or using different NSB measured for sample and for standard (B). Sample NSB= 228 DPM, standard NSB= 690 DPM. Absence of interference is indicated by parallel slopes of each sap concentration with the standard curves.

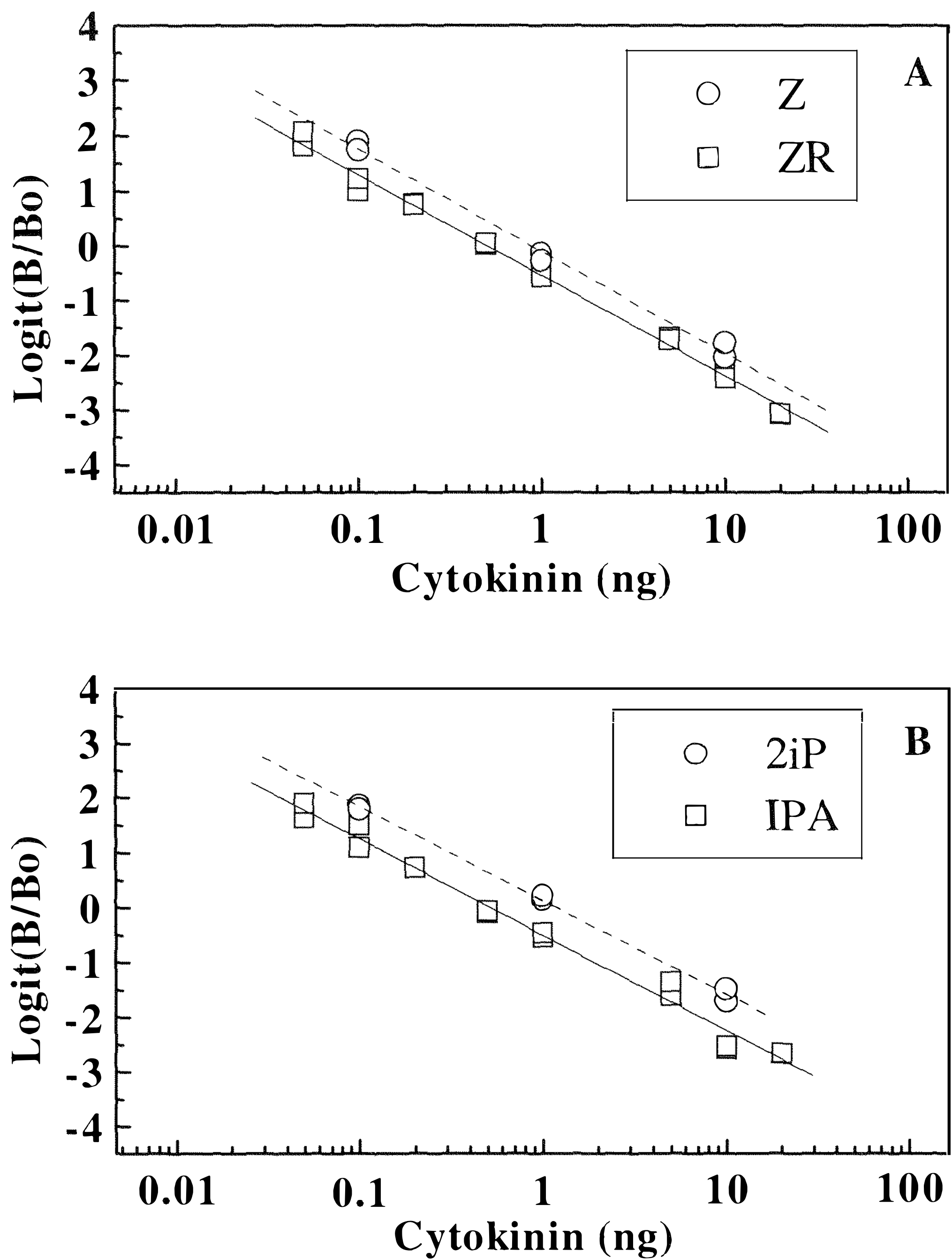


Figure 2.8 Logit transformation of RIA standard curves based on polyclonal antiserum to zeatin-riboside (A) or isopentenyl-adenosine (B). $Logit(B/Bo) = \ln(\frac{B/Bo}{(1-B/Bo)})$, where B=tracer bound in the presence of sample or standard and Bo=tracer bound in the absence of cytokinin. Z=zeatin, ZR=zeatin-riboside, 2iP=isopentenyl-adenine, IPA=isopentenyl-adenosine.

Table 2.4 Intercept and slopes of cytokinin validations for xylem sap. Sap samples were partially purified by passing them through PVPP, sephadex A-25 and c-18 (sep-pak) columns. To obtain parallel lines, it was necessary to correct samples and standards for a different level of measurable non-specific binding.

| Sap equivalent | Zeatin-riboside antibody | | Isopentenyl-adenosine antibody | |
|---|---|---|---|---|
| | Intercept | Slope | Intercept | Slope |
| Nil | 0.13 | 0.929 | 0.08 | 0.83 |
| 0.5ml/tube | 0.82 | 0.944 | 0.739 | 0.93 |
| 1ml/tube | 1.53 | 0.895 | 1.807 | 0.999 |

It was discovered that NSB in the presence of sample was only approximately 1 to 2% of total tracer added, whereas when sample was not present NSB was usually between 3-4% of tracer added. This suggested that antibody specific-binding of tracer could have been under-estimated in the presence of sample and this would lead to the over-estimation of cytokinin concentration in sample tubes. A further series of validations were tested in both sap and fruit samples. In this series, standard curve DPM were corrected for normal NSB, while sample DPM were corrected for NSB in the presence of sample to ensure that an accurate measure of antibody specific binding was obtained in each case. Following this correction, logit transformation was performed and cytokinin concentration in samples taken off the standard curves as normal. This resulted in adequate validation of fruit and sap cytokinin concentrations (Table 2.4, Figure 2.7).

A possible explanation for the lower NSB in samples, is that compounds other than the antigen present in samples, could compete with the radioactive tracer for NSB sites on either the tube, the calf serum or both. However HPLC fractions which would be expected to be highly pure, also over-estimated cytokinin concentration and could be corrected similarly to less purified samples. No satisfactory conclusion as to the reason for non-equivalence of NSB was reached, although the correction for differential NSB allowed satisfactory validation of cytokinin levels in samples.

A lack of precision in the estimation of NSB obviously causes inaccuracy in the estimation of antibody specific binding in an immunoassay. Although this may affect precision of estimates at high antigen concentration, good standard curves are able to be obtained using a range of arbitrary estimates of NSB. It is clear from the results we have obtained that a single estimate of NSB may not be sufficient in all situations.

In theory, the NSB tube should contain everything in other tubes including sample, except the antibody (Feldkamp and Smith, 1987). However literature related to RIA usually only mentions the requirement for the omission of antiserum in NSB estimation (Weiler, 1986; Chard, 1990). Additionally, although NSB measured in the presence of sample would relate directly to samples, it does not relate to the standard curve, as standard curve tubes cannot have sample present.

We have not found any other reference in the literature to a difference in NSB measured between samples and standards, or which suggest using both methods to check that they are comparable. There are many references to the presence of interfering compounds in crude samples and the subsequent elimination of interference upon further sample purification (Jones, 1987; Pengelly, 1986; Neuman and Smit, 1990). In addition there may be many instances where the inability to validate an assay has led to abandonment and non-publication of results. Although most of these situations are unlikely to be related to inaccurate estimation of antibody-specific binding as described here, it is possible that some may have mistaken this for the presence of interference.

## 2.3 Immunological Analysis of Abscisic Acid (ABA)

### 2.3.1 Synthesis of ABA-BSA conjugate for use in indirect immunoassay

The method of Weiler (1980) was used with minor modifications. To 45mg (±)cis-trans-abscisic acid (Sigma A1012) dissolved in 15ml methanol was added 145mg tyrosine hydrazide (TH) (Sigma T3135). Oxygen free nitrogen gas was bubbled through to purge oxygen from the solution and headspace. A balloon filled with $O_2$ free $N_2$ was placed over the vial, the vial wrapped in aluminium foil and incubated at 50°C for 5 days. Purging as above was repeated daily. Methanol was evaporated and the residue re-dissolved in 0.5ml $H_2O$. A small sample was loaded onto a semi-prep HPLC column (Econosphere $C_{18}$, internal diameter 10mm, length 250mm) and the absorbance (280 nm) trace compared with standard elution times for TH and ABA (Table 2.6) using a linear gradient of increasing acetonitrile (Table 2.5).

Table 2.5 Solvent gradient for the separation of ABA-TH from un-reacted tyrosine hydrazide on a preparative $C_{18}$ HPLC column. Flow rate was 5ml/min. Curve 6 is a constant rate of increase over the time period. TEA is 40mM acetic acid in water adjusted to pH 3.38 with triethylamine.

| Time (minutes) | %Acetonitrile | %TEA | Curve |
|---|---|---|---|
| 0 | 10 | 90 | * |
| 10 | 30 | 70 | 6 |
| 35 | 50 | 50 | 6 |

No attempt was made to identify the unknown products. Unknown peaks 1 and 2 were assumed to be the syn- and anti- isomers of ABA-TH referred to by Weiler (1980). As the conjugate was not required for production of anti-sera, no further attempt was made to separate these isomers. The remainder of the preparation was injected using the same

protocol and eluent was collected from 17 to 20 minutes. The collected fraction was taken to dryness and re-dissolved in 500μl methanol.

Table 2.6 Retention times of compounds present in a conjugate preparation of ABA and tyrosine hydrazide on a semi-preparative $C_{18}$ HPLC column.

| Chemical | Start | Stop |
|---|---|---|
| TH | 8:28 | 14:00 |
| Unknown 1 | 17:20 | 18:34 |
| Unknown 2 | 18:34 | 19:50 |
| ABA | 20:15 | 22:33 |
| Unknown 3 | 23.30 | 26.00 |
| Unknown 4 | 27.50 | 31.30 |

P-aminohippuric acid (pAH) (Sigma A3759) (200 mg) was dissolved in 120ml water, 200mg BSA (Sigma A3350) was added and the pH adjusted to 8.0 with 0.1M NaOH. The mixture was warmed slightly until the BSA completely dissolved. 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide-HCL (EDC) (200 mg, Sigma E1769) was added, the pH adjusted to 6.4 with 2M HCL and the solution stirred for 6 hours at room temperature on a well insulated magnetic stirrer. A further 100 mg EDC was added and stirring continued for a further 14 hours. The BSA-pAH conjugate formed was dialysed against $H_2O$ (20 l, 12 changes) at 4°C and lyophilised.

To 25mg lyophilised BSA-pAH was added 5ml $H_2O$, the mixture cooled on ice and 0.5ml $NaNO_2$ in water (120 mg/ml) was added dropwise. The mixture was allowed to incubate for five minutes and 0.5ml ammonium sulphamate (Sigma A4630) in water (60 mg/ml) was added dropwise. The ABA-TH in methanol was added to 0.1M borate buffer pH 9.0 (10ml). The protein solution was added dropwise and the solution turned a deep gold orange colour. The solution was gently stirred for 30 minutes and immediately dialysed against $H_2O$ (20 litre, 12 changes) at 4°C. The ABA-BSA conjugate was

lyophilised and then re-dissolved in 3.6ml of 0.05M $NaHCO_3$ (pH 9.6). Aliquots of 30 µl were stored at -20°C.

### 2.3.2 Indirect enzyme linked immunosorbent assay (ELISA) for ABA

Indirect ELISA was used to assay samples for ABA using the method of Walker-Simmons (1987).

A tris buffered saline buffer (TBS) was prepared by dissolving 6.1g Tris, 0.2g $MgCl_2$, 8.8g NaCl in one litre of water and adjusting the pH to 7.8. On the day of use, a washing buffer (TBST-BSA) was prepared by adding 0.5ml/l Tween-20 and 1 mg/ml BSA (fraction V, Sigma A3350) to TBS buffer.

Nunc 'maxisorp' 96 well immuno plates were used for ELISA. An Eppendorf 4780 multi-pette was used for delivery of all bulk reagents to plates due to the speed and high precision obtained between aliquots. Monoclonal antibody to +ABA (Mab) was purchased from Idetec Inc., Calif. Mab was mixed with TBS containing 2 mg/ml BSA to give a concentration of 33.3 µg/ml MAb and aliquots of 1ml were stored at -20°C. Immediately prior to use, one aliquot was thawed and diluted with 10ml TBS containing 2 mg/ml BSA. Standards of (±)cis-trans-abscisic acid were used, however as the antibody only recognises +ABA, the results had to be corrected by a factor of 50%.

Conjugate (30µl) was diluted to 20ml with $NaHCO_3$ buffer and 200µl aliquots were delivered to each well of a new ELISA plate, The plate was wrapped in aluminium foil and incubated at 4°C overnight. Diluted Mab (350 µl) was delivered to polypropylene micro-centrifuge tubes containing 350µl of standard or sample in TBS. Standards used were in the range 0.5 to 500 pg/100µl +ABA, although the assay was most sensitive between 2.5 and 100 pg/100µl. At least two Bo (nil ABA) and one NSB (nil Mab) tubes were included per plate. Tubes were vortexed briefly to mix, covered in aluminium foil and incubated at 4°C overnight.

On the following morning, the conjugate was emptied from plate wells by inverting. Each well was rinsed with 200 µl TBST-BSA three times, with the second and third rinses left

in wells for five minutes to block non-specific binding sites. Aliquots (200μl) of Mab+sample/standard was added to triplicate wells and the plate incubated in the dark at 20°C for 2.5 hours. Plates were drained and rinsed as above. Rabbit anti-mouse alkaline phosphatase conjugate (Sigma A3350, 200 μl) diluted 1:1000 with TBS was added to each well and the plate incubated as above for two hours. Plates were drained and rinsed as above. P-nitrophenyl phosphate (Sigma 104-105) was dissolved in 0.05M $NaHCO_3$ pH 9.6 at 1 mg/ml and 200μl aliquots were added to each well.

Plates were read in a microplate reader (Dynatech MR5000). Readings of absorbance at 410nm were taken at regular intervals, until absorbance of the Bo wells was approximately 1.0 (usually between 1 to 1.5 hours). Results were converted to ABA concentrations by using absorbance values converted to logit[B/Bo] and plotted against $\log_{10}$ [ABA].

$$Logit[\frac{B}{Bo}] = \ln\left(\frac{B/Bo}{(1 - B/Bo)}\right)$$

Results were validated using recovery plots (Jones, 1987; Walker-Simmons, 1987) which involves determination of ABA concentration determined in samples at several dilutions spiked with different concentrations of standard ABA (Figure 2.9). The monoclonal antibody has very high specificity for +ABA (Mertens et al., 1983) and results obtained in a similar indirect ELISA for ABA have been validated by GC-MS (Norman et al., 1986) cited in Walker-Simmons (1987). This strongly suggests that the ELISA procedure described here allows specific quantification of +ABA.

Table 2.7 A validation of a sample of kiwifruit outer pericarp in the indirect ELISA for abscisic acid showing the intercept (Sample ABA pg/100 µl) and the slope due to addition of standard ABA. In the absence of interference, the slope should be close to one.

| Dilution | Intercept | Slope |
|---|---|---|
| 1/100 | 252.9 | 1.04 |
| 1/500 | 52 | 0.96 |
| 1/1000 | 33.5 | 0.97 |
| Nil Sample | 1.2 | 0.95 |

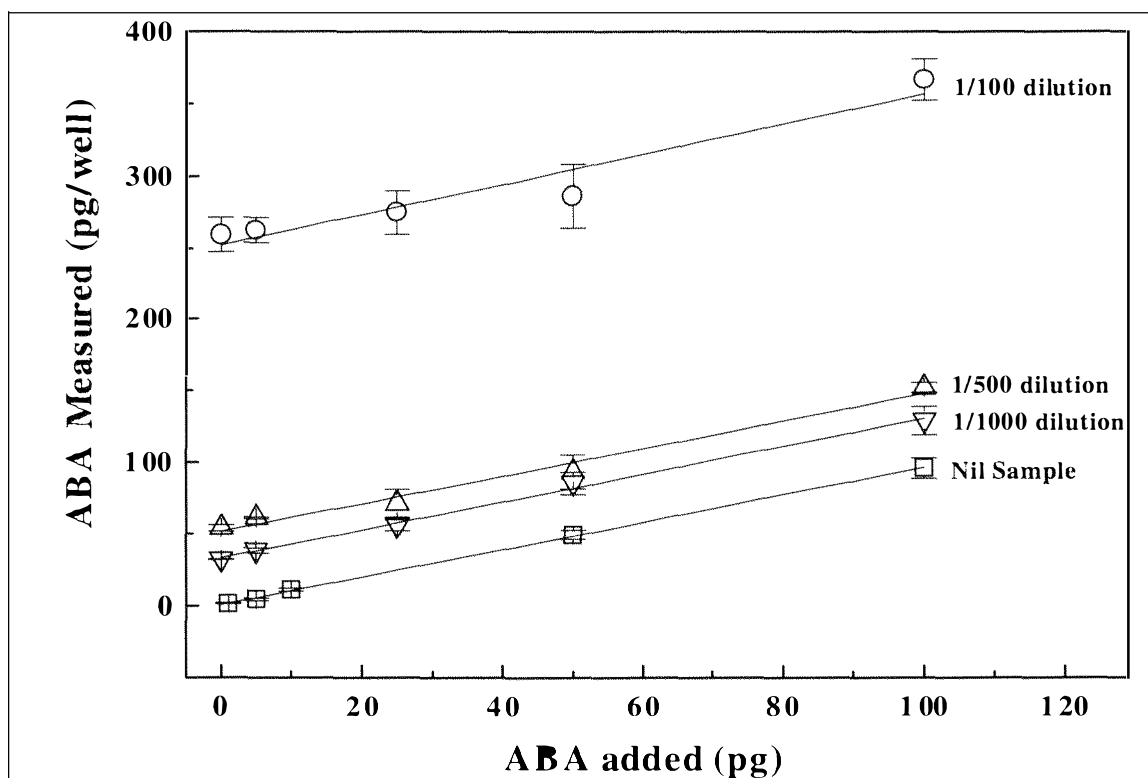


Figure 2.9 Example of the validation of a kiwifruit outer pericarp sample in an indirect ELISA for abscisic acid (ABA). The bottom line is standard ABA with no sample added. Assay validity is indicated by the parallel slopes of each dilution of the sample to the standard (nil sample) and the proportional decrease in ABA concentration with increasing dilution (Table 2.7). Values are means and standard error bars of ABA measured from three triplicate wells.

## 2.4 Mineral Analysis

Fruit for mineral analysis were dried and ground to a fine powder (1mm screen) in a bench grinder. Dried and ground fruit was held at 60°C for 24 hours and cooled in a dessicater prior to weighing and analysis.

Analysis of potassium, magnesium and calcium was by wet nitric acid digestion followed by atomic absorbtion spectroscopy. Concentrated nitric acid (4ml) was added to 100 mg of dried and ground fruit matter in glass digestion tubes and the mixture was refluxed at 150°C for at least four hours until it had cleared. At this stage the temperature was increased to 250°C until all acid was boiled off. Hydrochloric acid (0.2 M, 50ml) containing 250 ppm $Sr(NO_3)_2$ and 250 ppm CsCl was added to the residue. After appropriate dilution, samples were analysed on a GEC 904 atomic absorbtion spectrophotometer.

Analysis of nitrogen and phosphorus was by kjeldahl digestion followed by colorimetric autoanalysis (Twine and Williams, 1971; Technicon, 1973). Kjeldahl digestion solution was prepared by dissolving 100 g $K_2SO_4$ and 1g Selenium powder in one litre of concentrated sulphuric acid and heating until the solution cleared. Kjeldahl digestion solution (4ml) was added to 100mg fruit matter in a glass digestion tube and the mixture was heated to 350°C for at least four hours until it had cleared. The residue was diluted to 50ml with distilled water. After appropriate dilution, samples were analysed for atomic emission on a Technicon autoanalyser.

## 2.5 Manipulation and Estimation of Seed Numbers in Kiwifruit

### 2.5.1 Reduction of fruit seed number by style excission

Style number restriction has been used in the past to obtain fruit with experimentally induced low seed numbers (Hopping, 1976a; Lai et al., 1990). By removing the majority of styles prior to flower opening and thus pollination by honeybees, pollination of most ovules is prevented. However in several experiments, de-styling was found to be relatively inconsistent, with de-styling not resulting in low seed numbers in many fruit. An experiment was set up to ascertain if the procedure could be optimised to provide fruit with low seed numbers more consistently.

Six treatments of style reduction to two, three, four, five and eight styles or control were applied randomly to unopened kiwifruit flowers over a period of three days. Styles were removed close to the ovary with dissecting scissors on 18 flowers per treatment. An effort was made to ensure that the styles remaining were evenly distributed around the ovary so that the fruit would not be misshapen. Following treatment, flowers were immediately hand pollinated by rubbing a dehiscing male flower against the styles. Fruit were harvested at commercial maturity, weighed and all seeds extracted for counting and measurement of total seed weight.

Style manipulation followed by hand pollination successfully reduced seed numbers in kiwifruit to as low as 106 seeds when two styles were left intact (Table 2.8). Each style manipulation treatment had a significantly lower (contrast P=0.0001) mean seed number than the control treatment and as the number of styles left intact was increased from two through to eight, average seed numbers in the fruit increased from 252 up to 629. Variation of seed numbers was lower at the lower style numbers and appeared to increase at higher style numbers as a result of an increase in maximum seed number. Although data transformation was able to stabilise the variance, non-transformed data have been presented to illustrate the potential for style manipulation treatments to successfully and consistently reduce seed numbers. When between four and eight styles were left intact, fruit were present in the dataset which would have been considered to be

well pollinated (Table 2.8). It appears that reduction in the number of style to two styles per fruit can be successful in providing a guaranteed low seed number, below that present in a well pollinated fruit.

Table 2.8 Average seed numbers (arithmetic means) in kiwifruit that had styles excised prior to hand pollination (n=18).

| Styles intact after de-styling procedure | Mean | Standard error | Minimum | Maximum |
|---|---|---|---|---|
| 2 | 252 | 101 | 106 | 425 |
| 3 | 372 | 180 | 101 | 693 |
| 4 | 363 | 225 | 112 | 822 |
| 5 | 588 | 187 | 306 | 906 |
| 8 | 629 | 340 | 96 | 1067 |
| Control (not de-styled) | 1265 | 168 | 992 | 1545 |

### 2.5.2 Estimation of seed numbers in kiwifruit

A correlation has previously been obtained between seeds on 14 exposed surfaces of a kiwifruit cut into defined pieces and the total number of seeds in the fruit (D.J. Woolley, Personal communication, 1992). However the counting of seeds on 14 surfaces is relatively time consuming. In order to improve the procedure, a correlation was made between total seed number and seed number on six cut surfaces.

The fruit used were from the style manipulation experiment described in section 2.5.1. Fruit were sliced through the centre transversely and each half again cut through it's centre transversely (Figure 2.10). This gave six cut surfaces which could be easily and consistently obtained with different kiwifruits. The total number of seeds exposed on the six exposed surfaces were counted (a cut seed was counted as half a seed, so both halves together would contribute one count). Seeds were extracted, dried, the total number of

seeds was counted and a regression of total seed number against surface seed number made (Figure 2.11).

the counting of surface seed numbers was found to be simple, reproducible and fast which made this technique experimentally viable for estimating seed numbers of reasonably large numbers of fruit. By dividing fruit into three equal sections, longitudinal variation in distribution of seeds throughout a fruit was accounted for. This may be important especially in differentiating well pollinated fruit where seeds are distributed evenly throughout the entire fruit length and poorly pollinated fruits where there may be fewer seeds apparent at the end of the fruit distant from the styles. Seed weight was not obtained directly from surface counts, but was able to be estimated quickly by removal and drying of seeds present on the cut surfaces which appeared to be representative of the average seed size.

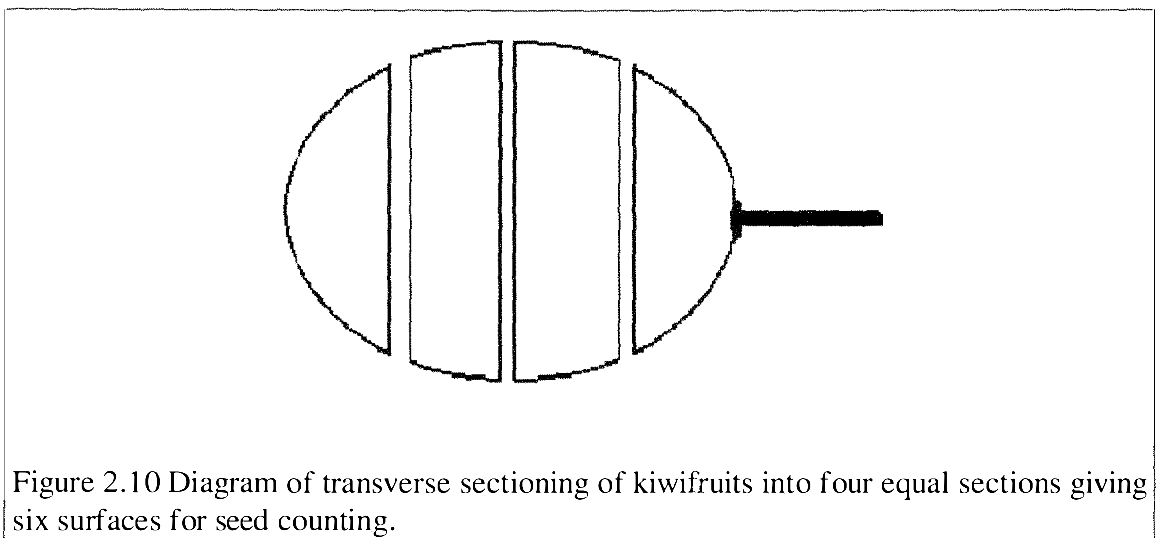

Figure 2.10 Diagram of transverse sectioning of kiwifruits into four equal sections giving six surfaces for seed counting.

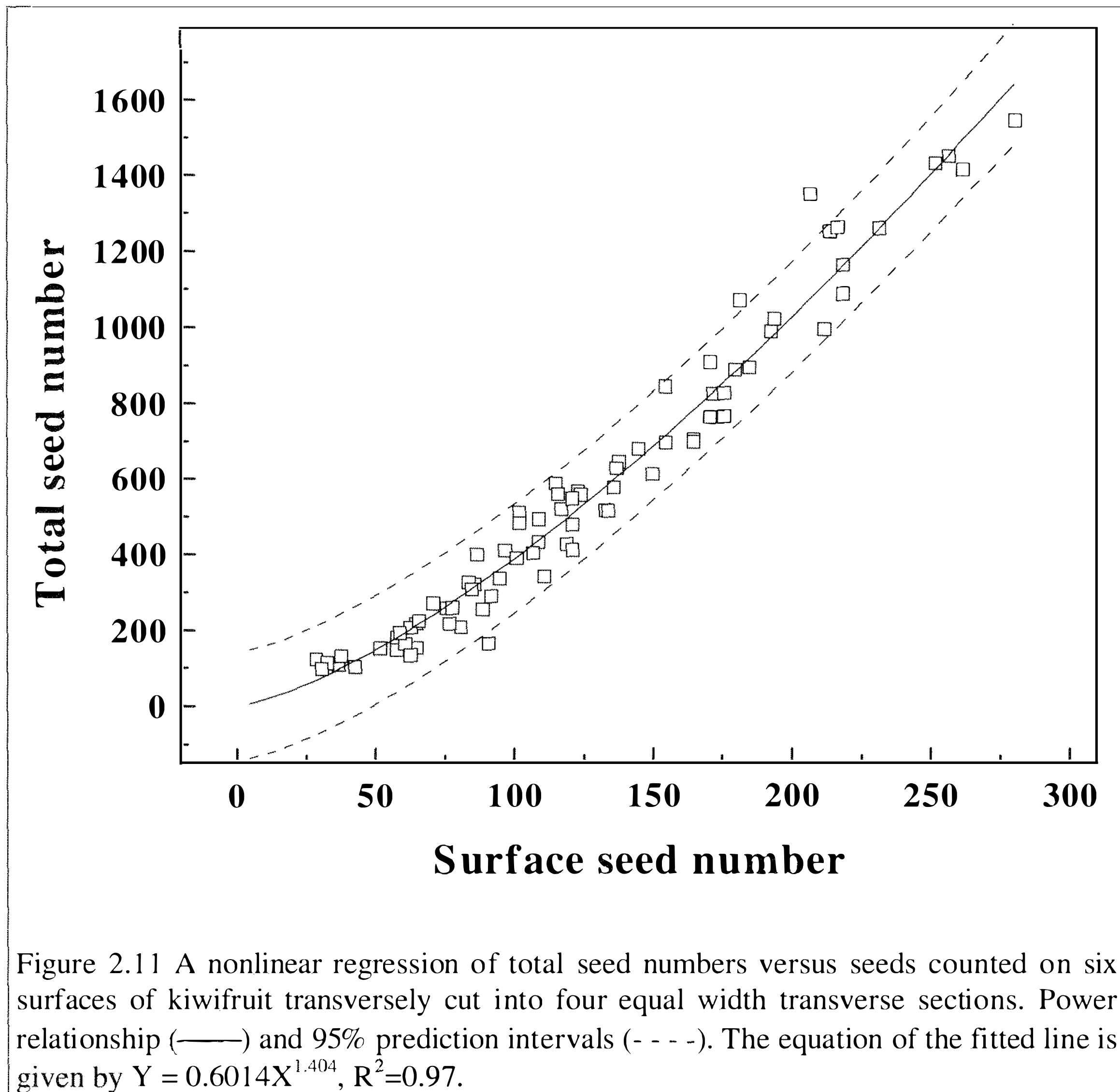


Figure 2.11 A nonlinear regression of total seed numbers versus seeds counted on six surfaces of kiwifruit transversely cut into four equal width transverse sections. Power relationship (——) and 95% prediction intervals (- - - -). The equation of the fitted line is given by $Y = 0.6014X^{1.404}$, $R^2$=0.97.

## 2.6 Estimation of Fruit Weight with Callipers

Kiwifruit were measured for three dimensions as described below and in figure 2.12:

- Length from the top to the base of the fruit (L)
- Maximum equatorial diameter ($D_{max}$)
- Minimum equatorial diameter ($D_{min}$).

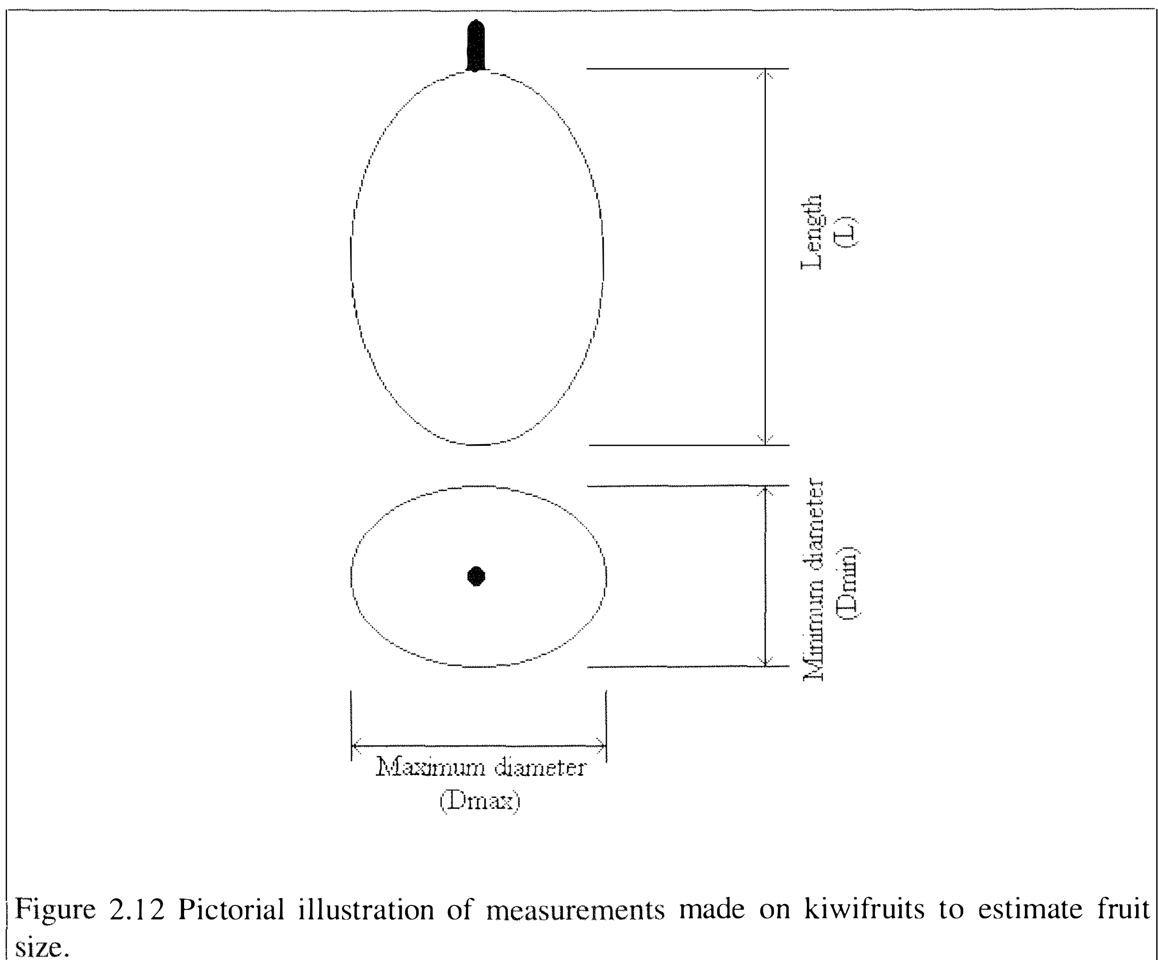


Figure 2.12 Pictorial illustration of measurements made on kiwifruits to estimate fruit size.

In order to obtain a correlation between fruit dimensions and fruit weight over a growing season, 40 fruit were harvested randomly from ten kiwifruit vines at 2, 3, 4, 5, 6, 7, 8, 9, 11, 15, 17 and 22 weeks after full bloom. Measurements were made with digital callipers (Mitutoyo, Digimatic) and fruit were weighed on a digital balance (Mettler 360).

Green et al. (1990) demonstrated a strong linear relationship between fruit fresh weight and fruit length cubed which could be used to predict fruit weight within 1g. Snelgar et al. (1992) found that a power curve $Fwt=0.454x(L \times D_{max} \times D_{min})^{1.05}$ usefully described the relationship. Although the relationship appears almost linear (Figure 2.13), it is likely that the relationship between fruit dimensions and fruit weight is curvilinear rather than linear as fruit density increases at a relatively constant rate from around 0.95 $g/cm^3$ at 20 days after full bloom (DAFB) to around 1.05 $g/cm^3$ at harvest (Lai, 1987).

Fruit fresh weight was plotted against $L \times D_{max} \times D_{min}$. A linear relationship gave a reasonable fit to the data, however there was evidence, from a residuals plot, of a curvilinear relationship. A power curve [$Fwt=0.411x(L \times D_{max} \times D_{min})^{1.07}$ $R^2$=99.2%] similar to that obtained by Snelgar et al. (1992) and a quadratic curve [$Fwt=0.528x(L \times D_{max} \times D_{min}) + 0.00031x(L \times D_{max} \times D_{min})^2$ $R^2$=99.2%] were fitted to the data. Although both were found to give a high correlation, visual observation of a residuals plot suggested the quadratic described the data better when fruit were very small. The quadratic relationship obtained (Figure 2.13) was used in non-destructive determination of fruit fresh weight, allowing fruit weight to be estimated without removal from the vine. For fruit being measured at repeated intervals non-destructively, soft tape was placed across the calliper jaws to avoid any damage to fruit which might have affected growth.

Fresh weight was also plotted against the cube of individual fruit dimensions L, $D_{max}$ or $D_{min}$ (figure 3.7.3). A linear regression adequately described the relationships for all three dimensions. The relationship between $L^3$ and fresh weight ($Fwt=0.356L^3$) was very similar to that obtained by Green et al. (1990) who found the relationship $weight=0.38L^3$. However of the individual dimensions, $L^3$ gave the worst fit. These results suggest that any one dimension could potentially be used for the prediction of fruit weight but the use of a fruit diameter appears to give higher accuracy than fruit length. However this was surprising as growth of poorly pollinated fruits tend to be disproportionately inhibited in the longitudinal direction (see chapter six), which would suggest that measurement of fruit length may be more appropriate to detect poorly pollinated fruit. The data collected here may not have included poorly pollinated fruit.

Where large numbers of fruit are being measured, measurement of one dimension could provide a reasonable measure of fruit weight, while still being experimentally viable and time efficient. When direct data transfer to computer is not available, the measurement and transfer of three numbers to a data sheet while measuring one fruit requires either exceptional concentration, or double/triple handling of the fruit. A reduction to even two dimensions per fruit would be likely to reduce transcription errors and/or potentially double the speed of data collection. However the individual relationships were not as strong as when all three dimensions were used together. In addition different experimental treatments, vines, locations or times could yield fruit which differ significantly in fruit shape, thus introducing additional error into the relationship. Thus where high accuracy of estimation is required or different fruit shapes are anticipated measurement of all three dimensions is recommended.

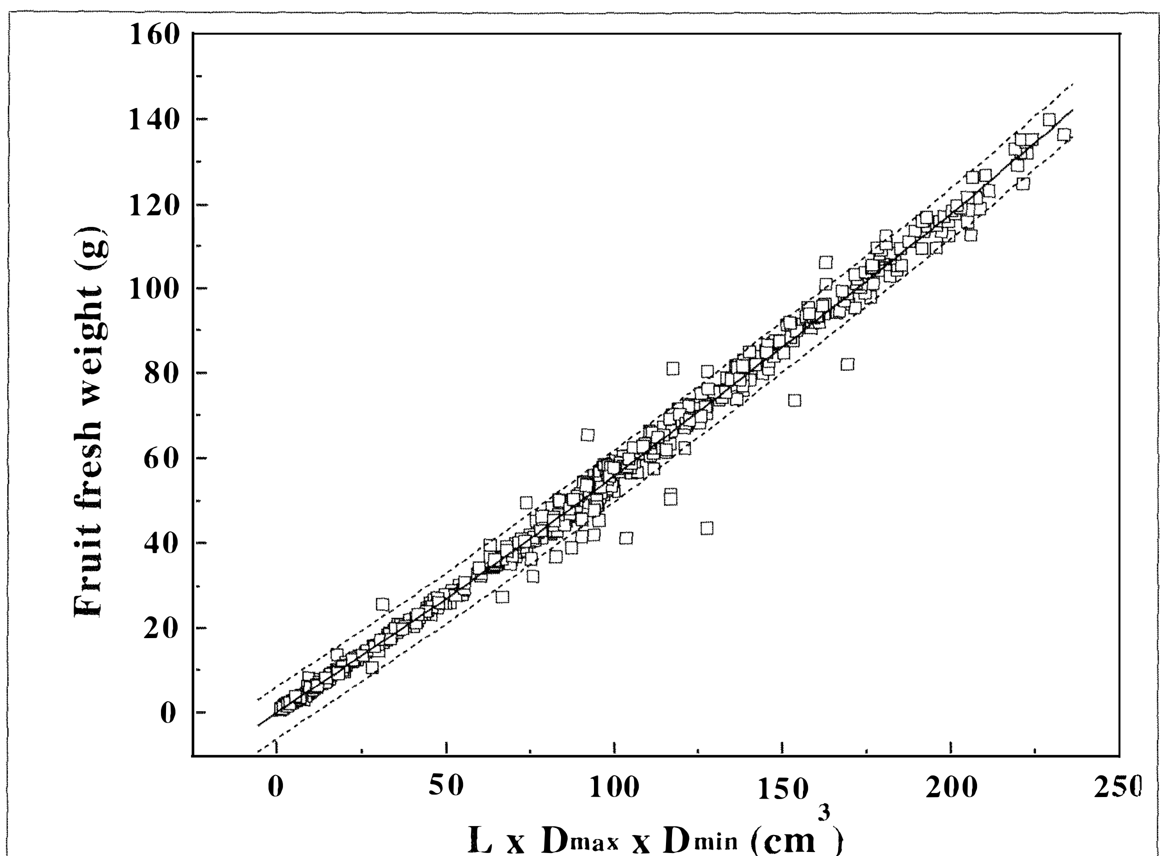


Figure 2.13 A quadratic curve (—) and 95% prediction intervals (- - -) for the relationship between kiwifruit fresh weight and calliper measurements of fruit dimensions. Measurements were made on fruit harvested over the period 14 to 150 days after full bloom. Fruit weight=$0.528(L \times D_{max} \times D_{min}) + 0.00031(L \times D_{max} \times D_{min})^2$, $R^2$=0.992.

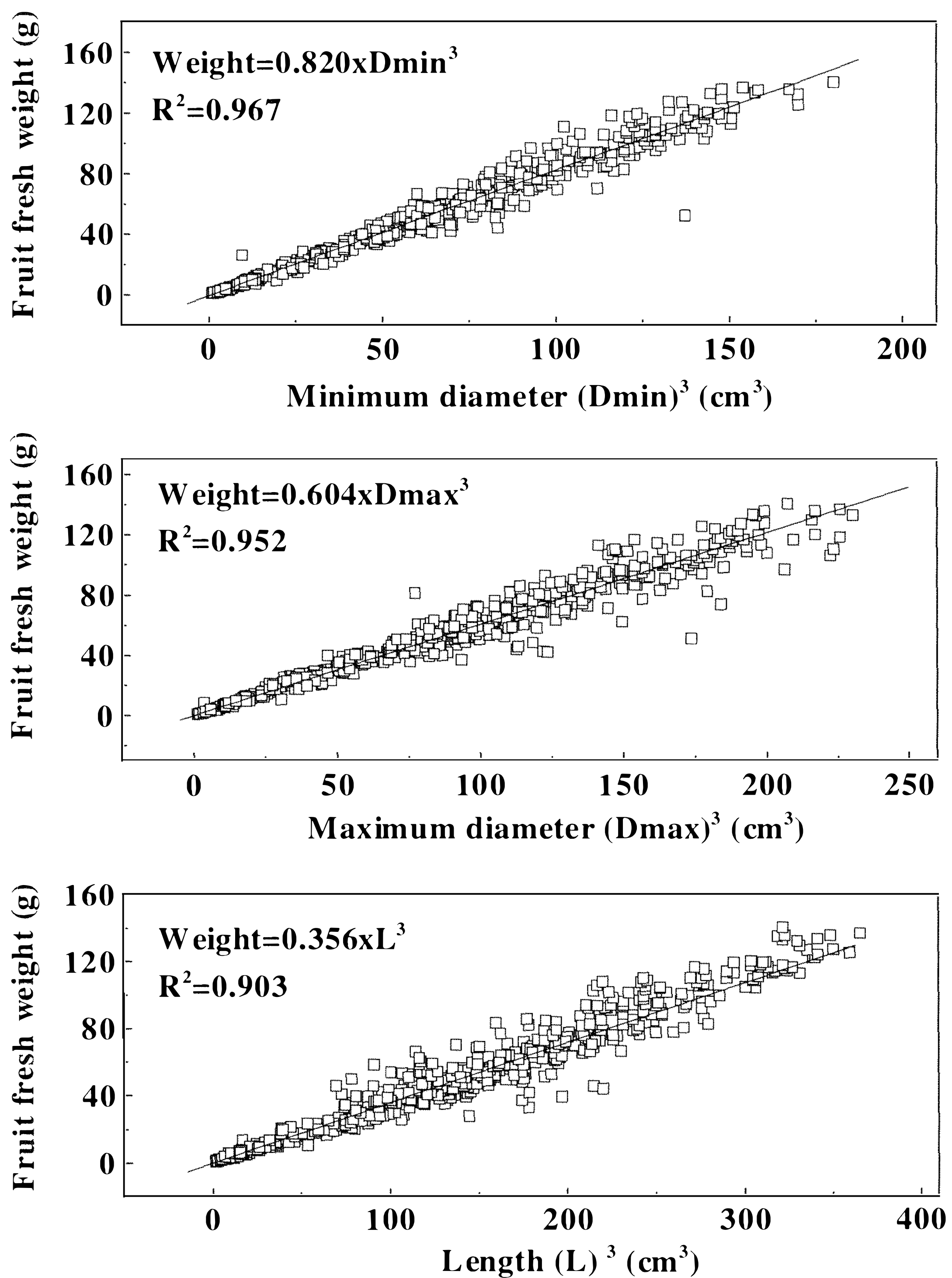


Figure 2.14 Linear relationships between the cube of individual fruit dimensions and fruit fresh weight for kiwifruit harvested between 14 and 150 days after full bloom.

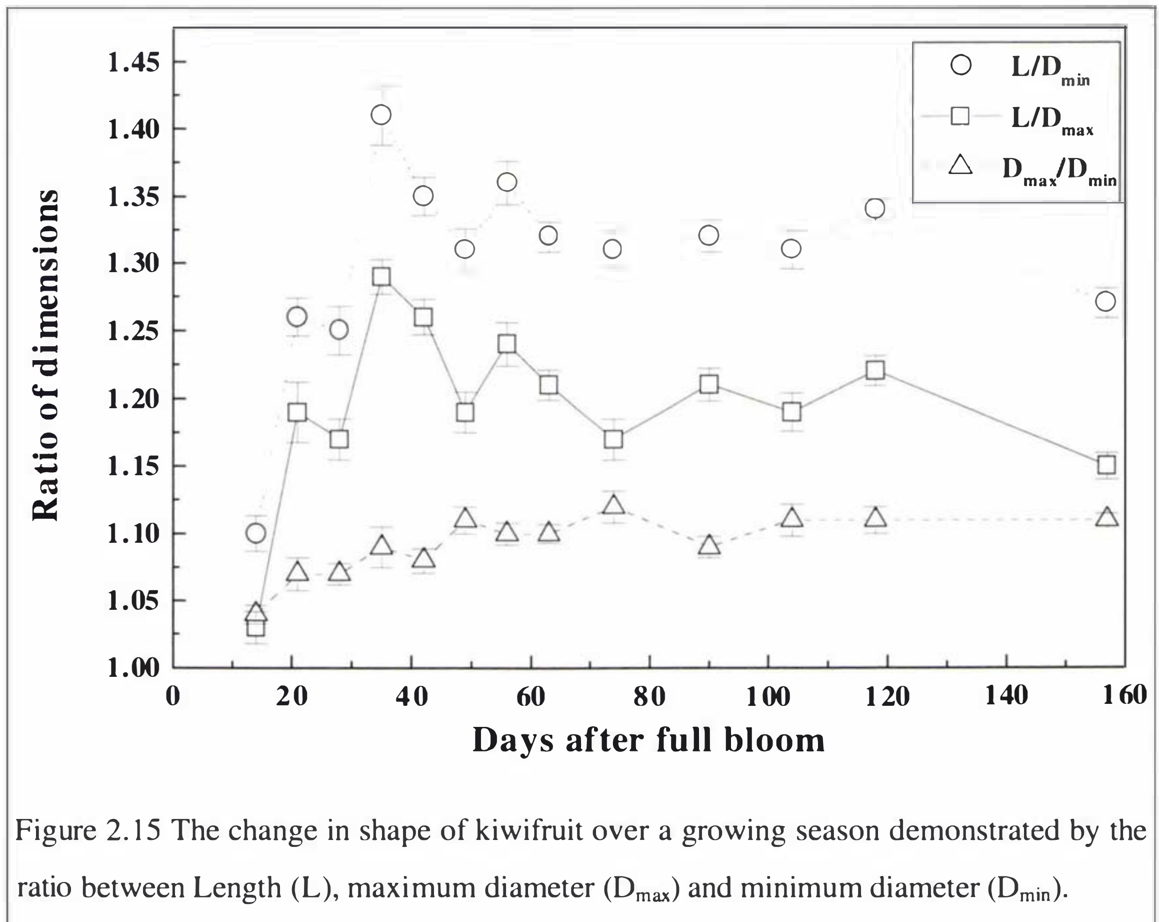


Figure 2.15 The change in shape of kiwifruit over a growing season demonstrated by the ratio between Length (L), maximum diameter ($D_{max}$) and minimum diameter ($D_{min}$).

Up until about 60 DAFB, there was a relatively constant linear relationship between fruit dry weight and ($L \times D_{max} \times D_{min}$). However over the following weeks, the relationship appeared to change, although it remained linear at each harvest date. As can be seen in Figure 2.16, the fruit percentage dry weight changes significantly over the growing season. At 21 DAFB, percent dry weight was around 11%. However by 42 DAFB it had dropped to around 7.5%. From 63 DAFB the percentage dry weight began to increase until 118 DAFB when it reached a plateau of 14%. The initial increase in percentage dry weight corresponds to the lag in fruit fresh weight increase which occurs in kiwifruit growth.

The data could be used to predict fruit dry weight from calliper measurements made during the season, if the predicted fresh weight is corrected for percentage dry matter. However if experimental treatments have an effect on percentage dry matter, this would render the calculation of little value.

There are marked changes in fruit shape over the growing season of a kiwifruit (Figure 2.15). Between 14 and 35 DAFB fruit length increases relatively quickly compared with either diameter. However from 35 DAFB until harvest, the ratio between fruit length and diameters gradually decreases, although the ratios of length to diameter at harvest are still higher than at the beginning of the season. There was very little difference between the minimum and maximaum equatorial diameters at the beginning of the season, however the ratio between these diameters steadily increased until approximately 49 DAFB after which the ratio remained at around 1.1 (Figure 2.15).

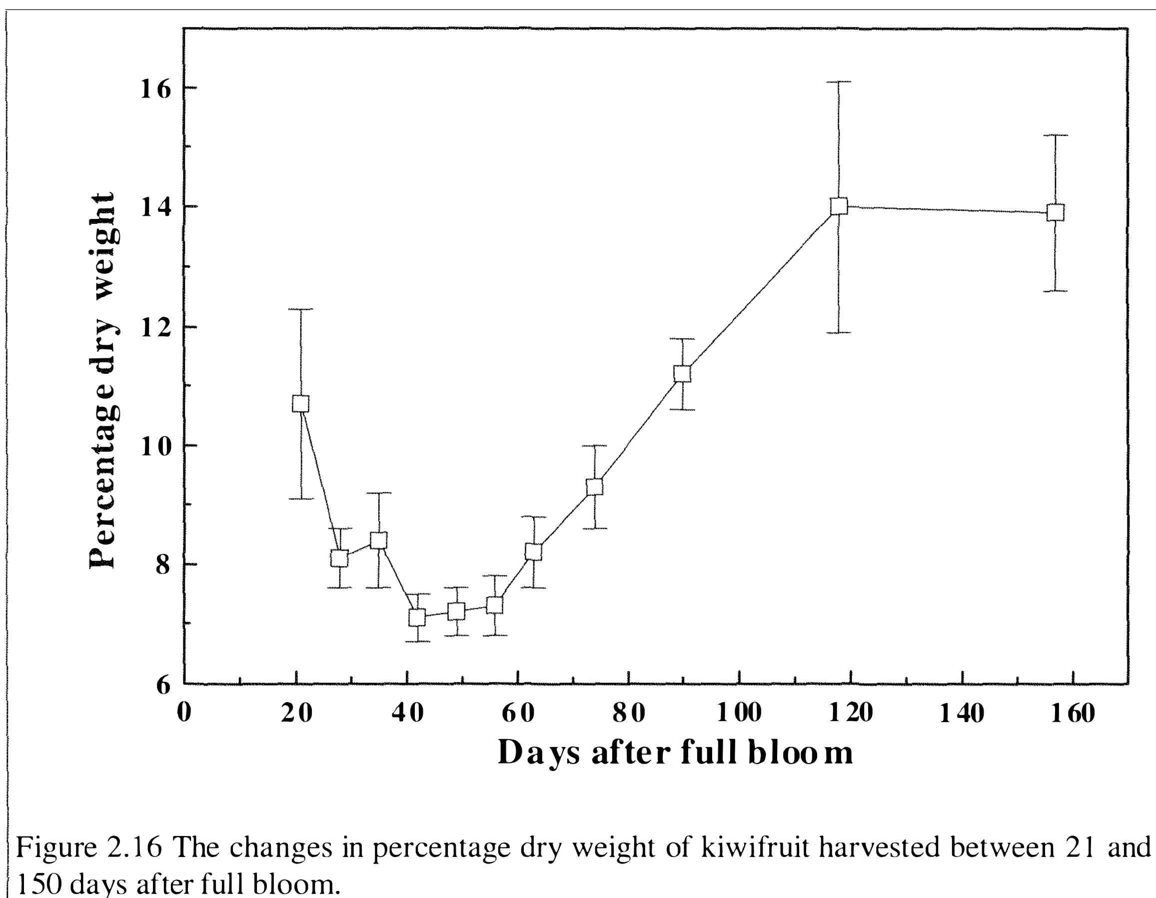


Figure 2.16 The changes in percentage dry weight of kiwifruit harvested between 21 and 150 days after full bloom.

## 2.7 Estimation of Cell Number and Size in Kiwifruit Sections

A kiwifruit in transverse section (Figure 2.17) can be separated into several components tissues, each of which is made up of cells of distinct and different sizes. Hopping (1976b) described kiwifruit as being made up of three distinct parts; core, inner pericarp and outer pericarp. The central core is made up of a relatively homogeneous population of large parenchyma type cells and is conveniently separated from the inner pericarp by clusters of vascular bundles which are associated with each locule (ventral carpellary vascular bundles (Schmid, 1978) see chapter three, plate 3.4). Inner pericarp tissue which is associated with the seeds and locules can be conveniently separated from outer pericarp by a second ring of vascular bundle clusters (dorsal carpellary vascular bundles (Schmid, 1978), see chapter three, plate 3.2). Inner pericarp cells are distinctly elongated in the radial plane particularly within the locule walls (see chapter three, plate 3.3). Outer pericarp cells are composed of a heterogeneous population of two distinct cell types (see chapter three, plate 3.1). Large ovoid cells are interspersed among small starch containing cells (Patterson et al., 1993).

In longitudinal section (Figure 2.18), kiwifruit can be separated into the core, inner pericarp and outer pericarp tissue as for transverse sections. However at the proximal and distal end of the fruit, distinction between the tissues becomes less clear, as the tissues lose their differentiation. For longitudinal sections used in this study, the sections were taken from the same point as transverse sections, i.e. at the point of minimum equatorial diameter, approximately half way up the fruit.

### 2.7.1 Fresh sections

Fresh sections were used in some experimental work due to the speed, ease and low cost of preparation. Fresh sections had the disadvantages of lower quality than wax imbedded sections, restriction to fresh material and inability to be stored for inspection at later dates. Transverse thin sections (approximately 20µm thick) of each fruit at the point of

maximum circumference was made using a Cambridge rocking microtome with a $CO_2$ freezing attachment. Freshly cut sections were transferred from the microtome blade to a water bath, floated onto a microscope slide and covered with a glass cover strip. Sections were viewed immediately following preparation.

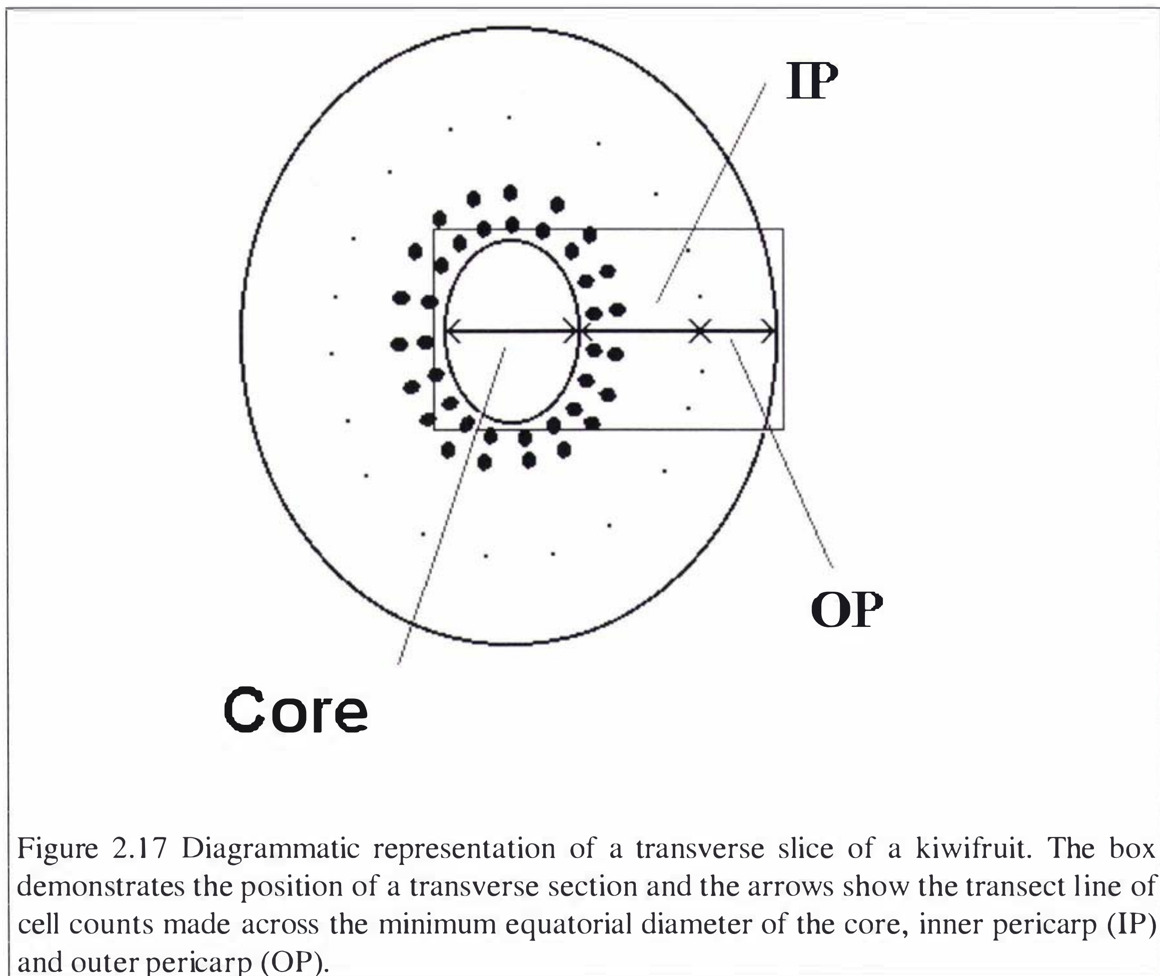


Figure 2.17 Diagrammatic representation of a transverse slice of a kiwifruit. The box demonstrates the position of a transverse section and the arrows show the transect line of cell counts made across the minimum equatorial diameter of the core, inner pericarp (IP) and outer pericarp (OP).

### 2.7.2 Permanent sections using wax imbedding technique

Permanent wax-imbedded sections were used for most experimental work, as these were able to be prepared and viewed at leisure and stored for re-inspection. Tissue slices for transverse or longitudinal sectioning were taken at the point of maximum diameter and were fixed in ethanol (60%)/formaldehyde/acetic acid 20:1:1 v:v:v. (Hopping, 1976b) prior to embedding. Tissue was wax imbedded in a Shanton Citadel 2000 programmable automatic tissue processor. Imbedding took place over a 16 hour period and involved a

progressive ethanol series up to 100% ethanol, two xylene baths and three molten paraffin wax baths. Air was removed from embedded slices by placing them in a vacuum oven for 5 hours at 60°C. Thin sections (7μm) of cold wax imbedded tissue were made with a Heitz 1512 rocking microtome. Sections were floated onto slides in a water bath and fixed to slides at 60°C overnight. Sections were de-waxed in xylene, rehydrated through an ethanol-water series, stained with Gills haemotoxylin and then rinsed in 'Scotts' tap-water. Finally sections were dehydrated through an alcohol series, cleared in xylene and permanently mounted in DPX.

### 2.7.3 Histology

Sections were viewed under an Olympus compound microscope fitted with an eye-piece micrometer.

In transverse sections, the number of cells in the core, inner pericarp and outer pericarp were counted along a straight line transecting the outer and inner pericarp on one side of the fruit and the entire diameter of the core (Figure 2.17). The mean cell diameter in each tissue was obtained by dividing the width of each tissue by the total number of cells counted across it. Core cell numbers presented were halved to represent a core radius.

In order to determine the possible influences of the two types of cell present in outer pericarp tissue, the number and mean width of outer pericarp 'large' and 'small' cells were counted across the radial line. Estimation of large cell number and width was achieved by counting the total number and the width of each large cell along the radial transect. By subtracting these two measures from total cell number and width, mean number and width of small cells could also be estimated. Large and small cells were not determined in fresh sections.

In longitudinal sections, cell lengths of cells were determined from four positions within the fruit at the point of minimum equatorial diameter (Figure 2.18).

1. The total length was measured (longitudinally) of 100 cells in a straight line at the mid-point of the core.

2. The total length was measured (longitudinally) of 50 cells parallel to the epidermis at the mid-point of the outer pericarp. Cells were counted as large or small cells. Counts were made at two randomly selected points in each fruit.

3. Average longitudinal cell length was estimated at a point 10 cells inside the epidermis. Extended counts were not found to be possible, so cell lengths were instead estimated by counting the number of cells in 1mm parallel to the epidermis at four randomly selected points.

4. Average longitudinal cell length was estimated just inside the outer edge of the inner pericarp as in 3.

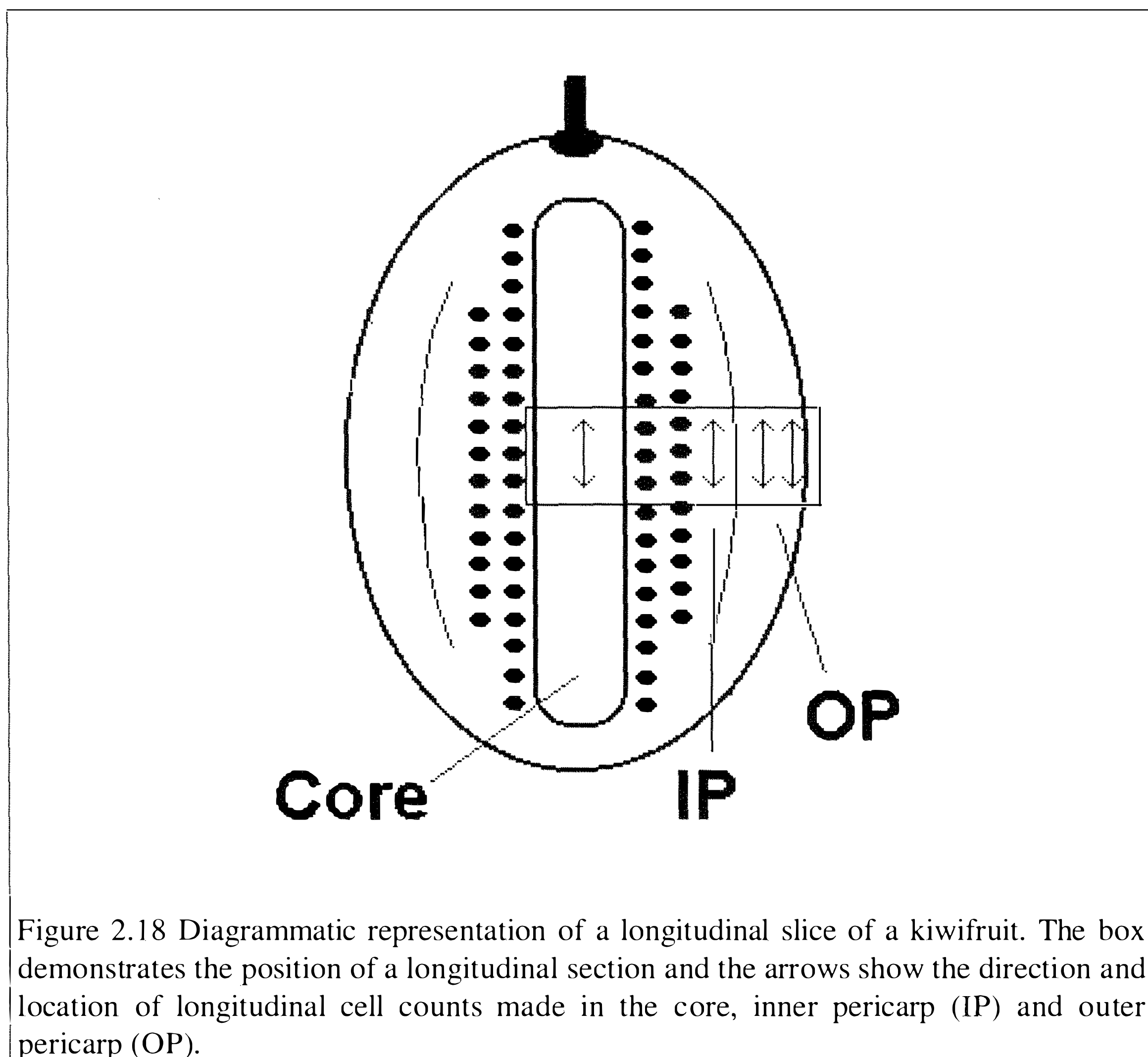


Figure 2.18 Diagrammatic representation of a longitudinal slice of a kiwifruit. The box demonstrates the position of a longitudinal section and the arrows show the direction and location of longitudinal cell counts made in the core, inner pericarp (IP) and outer pericarp (OP).

## 2.8 Data Handling and Statistical Analysis

### 2.8.1 Data handling

Where possible, data was collected directly onto computer e.g. using a portable laptop computer with input directly from a balance or digital callipers. This reduced the occurrence of transcription errors for such data. Datasets were checked for recording and transcription errors using three separate methods:

1. Each variable collected in a dataset was formatted to a fixed number of decimal places. Datasets were sorted by treatments and a visual scan was made of data in columns to identify any possible unusual data-points, which were manually checked for transcription errors from data-sheets.
2. Datasets were sorted individually in ascending sequence by each variable collected and by meaningful ratios such as percentage dry weight. Observations at the lowest or highest end were manually checked. Impossible or unlikely data such as kiwifruit weighing 600g were deleted from datasets. Scatterplots were often made of meaningful pairs of variables e.g. seed number and fruit weight and outliers were able to be identified and checked.
3. Inspection of residuals plots was made for each data set and outlying points were identified and checked. Identification of outliers was easily made using the interactive SAS Insight module.

### 2.8.2 Experimental design and analysis of variance

Most experimental designs utilised were randomised complete block designs. Individual kiwifruit vines or rows of vines were used as blocks, to remove random variation due to the position or prior history of the vines. In most cases, a lateral shoot was the minimum experimental unit to which treatments were applied. As there were normally two or more fruit present on a lateral shoot, the individual variables collected for each fruit on a shoot were always averaged because of the non-random selection of such fruit which could

otherwise lead to an incorrect estimation of the random variation for analysis of variance (Mead et al., 1993).

Analysis of variance was undertaken using the general linear models procedure (proc GLM) in the SAS system for statistical analysis available as SAS for windows release 6.10. Where data required transformation in order to conform to assumptions underlying ANOVA (Cochran, 1947), the method of Box and Cox (1964) summarised by Fernandez (1992) was used to estimate an appropriate transformation. For presentation, transformed means were back transformed to the original scale.

### 2.8.3 Multiple comparison testing

The ANOVA test statistic F was used to test model hypothesis that all treatment means were the same. If this hypothesis was rejected due to a significantly high value of F, an alternative hypothesis was accepted that at least one of the treatment population means differed from the rest. In order to compare treatment means, three different types of comparison were used.

1. Contrasts (SAS proc GLM, contrast statement) were used for pre-planned comparisons of individual means and to provide estimates of the probability that a pair of means are different for specific comparisons of interest. In addition to individual comparisons, this procedure also allowed comparison of groups of treatments with other groups, for example control treatment versus all other treatments. Estimates of the value of a combined treatment mean could be obtained using the estimate statement in proc GLM. A further use of contrasts was made in testing for polynomial trends between treatment levels with an orthogonal structure.

2. Fishers protected least significant difference (LSD) procedure was used for multiple comparisons between treatments from balanced datasets. The LSD procedure controls the comparisonwise type I error (falsely accepting the hypothesis that a pair of means are not different), but tends to inflate the overall or experimentwise error rate (falsely rejecting the hypothesis that a pair of means are not different) as the number of

comparisons increases (Ott, 1993). However if Fishers LSD is applied only after the F-test for treatment differences has been shown to be significant, it becomes a protected LSD and the experimentwise error rate is weakly controlled at a level approximately equal to the $\alpha$-level of the F-test (Ott, 1993). Fishers protected LSD has been observed to have the highest 'correct decision rate' in simulation studies made with ten commonly used multiple comparison procedures (Carmer and Swanson, 1973).

3. Where missing observations occurred, least-squares means otherwise described as population marginal means (PMM) (Searle et al., 1980) were used, as these provide a better estimate of the true population mean in the presence of missing values. Arithmetic means of data separated into cells (blocks, or treatments) with missing values give mis-leading results, as means are weighted towards cells with fewer missing data. PMM estimates an average of the population cell means for all cells using parameters of a linear model (Searle et al., 1980). The GLM procedure in SAS computes PMM using the lsmeans statement and can also provide a significance test that individual PMM's are different using a modified t-distribution.

### 2.8.4 Comparison of correlation coefficients for fitted lines

A comparison of correlation coefficients can be made by transforming the correlation coefficient (r) into a transformed value ($z_r$) (Edwards, 1976):

$$Zr = \frac{1}{2}[Log_e(1+r) - \log_e(1-r)]$$

$$\sigma_{zr} = \frac{1}{\sqrt{n-3}}$$

where r = correlation coefficient, n = sample size

The distribution of $z_r$ is approximately normal and has a standard error $\sigma_{zr}$ which is related simply to the sample size. To test the significance of the difference between two values of r, a Z test statistic of the difference between the two $z_r$ values can be obtained:

$$\sigma_{(zr1-zr2)} = \sqrt{\sigma^2_{zr1} + \sigma^2_{zr2}}$$

$$Z = \frac{Zr1 - Zr2}{\sigma_{(zr1-zr2)}}$$

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# 3. Lateral Shoot Girdling of Kiwifruit

## 3.1 Introduction

Once produced by photosynthetic sources (mainly leaves), carbohydrates may be initially partitioned between use by the source and transport out to other plant organs. The carbohydrates available for transport must then be allocated between the many competing sinks on the plant. These sinks include roots, expanding shoot tips, expanding leaves, buds, flowers, fruit and stems. The growth of sinks such as fruit is largely dependent on their intrinsic ability to obtain carbohydrate or sink strength (see chapter one, section 1.2).

Fruit weight is made up of three cellular parameters: cell number, cell size and cell density. Although the majority of cell division occurs prior to anthesis (Coombe, 1976), cell division usually continues post-anthesis, and determines the ultimate number of cells which are present in a fruit. It has been suggested that cell number may provide a suitable measure of sink size, and thus may provide the ultimate limitation on the size a sink may attain (Ho, 1988). A number of correlations have been made between kiwifruit cell number and fruit weight, which suggests that cell number is important for sink strength of kiwifruits. For example, application of the cytokinin-like substance CPPU to kiwifruit at the time of active cell division after anthesis has been shown to increase final fruit size (Iwahori et al., 1988; Lawes et al., 1992) and this was associated with significantly higher cell numbers (Woolley et al., 1992; Kurosaki and Mochizuki, 1990), although cell size is also increased in CPPU treated fruit (Woolley et al., 1992; Patterson et al., 1993). Kiwifruit which develop from early flowers are larger (Lai et al., 1990), and contain more cells (Cruz-Castillo, 1994) than those which develop from late flowers. Manipulation of cell numbers may therefore have the potential to improve the sink strength of kiwifruits, allowing increases in fruit size.

Fruit size of kiwifruit like many other crops is dependent on the crop load carried by vines (Cooper and Marshall, 1992; Richardson and McAneney, 1990). At high crop

loads, competition between fruit, or between fruit and other vine sinks for the limited supply of carbohydrate is increased, and fruit grow to a smaller size. It has been demonstrated by the girdling of fruiting lateral shoots that approximately two leaves supply the carbohydrate requirements of a kiwifruit (Snelgar et al., 1986). Normally, lateral shoots with a low leaf:fruit ratio can import enough carbohydrate to meet any carbohydrate shortage, while fruit on lateral shoots with a high leaf:fruit ratio are unable to take advantage of carbohydrate excess (Lai et al., 1988). However when low or high leaf:fruit ratios are imposed on girdled fruiting shoots, fruit size is markedly depressed or enhanced respectively (Snelgar et al., 1986; Lai et al., 1989a; Woolley et al., 1992). At high leaf:fruit ratios in girdled branches, carbohydrate is thought to accumulate in girdled branches, and fruit growth is generally stimulated (see section 1.3.4). Kiwifruit on girdled lateral shoots with a high leaf:fruit ratio have high dry matter, starch and soluble sugar concentrations (Seager and Hewett, 1995), which suggests that carbohydrate accumulates due to a lack of alternate sinks. However it is not known if carbohydrate levels act solely by influencing the partitioning of carbohydrate into fruits, or whether there may be some effect on cell division processes, which may increase the potential for fruit growth.

The aim of the experiments described in this chapter were to investigate girdling of kiwifruit lateral shoots as a means of altering the source-sink balance for fruit growth. Girdling effectively eliminates competing sinks and external carbohydrate sources which increases or decreases the availability of carbohydrate depending on the leaf:fruit ratio. The effects on fruit growth, dry weight, cell numbers and cell size of girdling and low or high leaf:fruit ratio were investigated in the experimental work described below.

## 3.2 Materials and Methods

### 3.2.1 Girdling of fruiting lateral shoots

The individual fruiting lateral shoots to be girdled were selected arising from a number of one year old canes on T-bar trained kiwifruit vines at the Massey University Fruit crops unit (MUFCU). Kiwifruit fruiting characteristics are affected by the type of lateral shoot (Lai et al., 1990), and by how well the lateral shoot is exposed to incoming radiation (Grant and Ryugo, 1984). Thus to eliminate these variables, the lateral shoots were always selected with uniformity in mind and strongly growing, indeterminate and well exposed lateral shoots were used. Thin lateral shoots were never used as these have low physical strength after girdling.

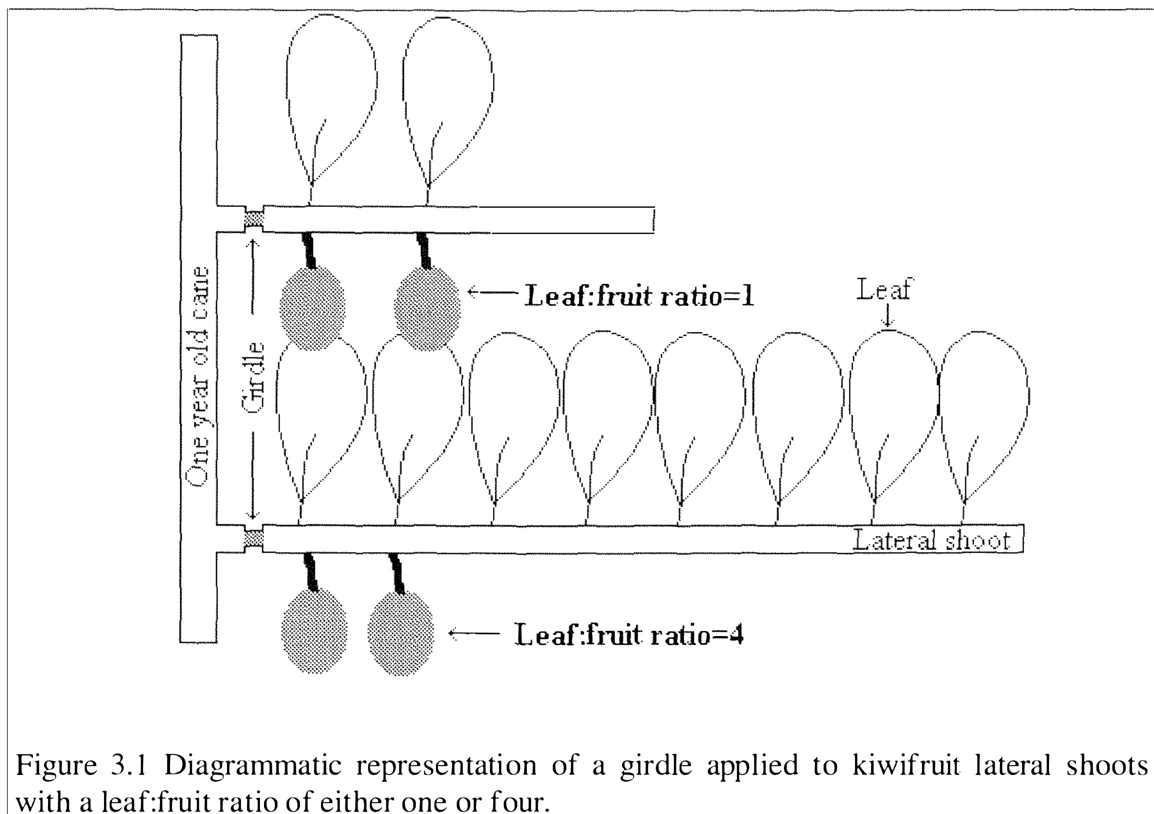


Figure 3.1 Diagrammatic representation of a girdle applied to kiwifruit lateral shoots with a leaf:fruit ratio of either one or four.

Girdling was performed by making two parallel cuts around the entire circumference of the base of the shoot with a double bladed knife, ensuring that the cut was made to the depth of bark and not into the wood. The bark was then peeled away and the girdle was

checked to ensure the complete absence of vascular connection. Girdles were 10 mm wide. Fruit numbers were reduced to two fruit per shoot and leaves were then thinned to give the required leaf:fruit ratio on the shoot, retaining only fully expanded, well exposed leaves. A leaf:fruit ratio of four consisted of eight leaves and two fruit, while a ratio of one consisted of two leaves and two fruit (Figure 3.1). Shoots were checked monthly between January and April for re-growth from axillary buds which was subsequently removed so that the source-sink ratio was not altered.

### 3.2.2 The effect of leaf:fruit ratio and time of girdling on fruit weight and percentage dry matter

Season one

Experimental design was a randomised complete block design with a factorial treatment structure. Seven levels of girdling date: 0, 7, 14, 28, 42, 56 days after full bloom (DAFB) and control (not girdled) were applied in combination with two levels of leaf:fruit ratio on a lateral shoot (one and four). Each treatment combination was replicated on two lateral shoots on each of six vines. Shoots were allocated randomly to treatment combinations by tagging at full bloom, and at this time all shoots were pruned to the appropriate leaf:fruit ratio. At the time of girdle application, a 10 mm girdle was applied near the base of the shoot as described in section 3.2.1.

Fruit were harvested at 160 DAFB, at which point other fruit sampled randomly in the same block were at commercial maturity (soluble solids=6.2%). Measurements were taken of fruit fresh weight, soluble solids and surface seed numbers (chapter two). Fruit dry weight was established by drying the entire sliced fruit to a constant weight in a ventilated oven at 70°C.

Season two

In order to confirm a trend in the data, the high leaf:fruit ratio treatments were repeated in the following season with the additional girdling times of 21 and 70 DAFB.

### 3.2.3 The effect of leaf:fruit ratio on cell division and expansion in kiwifruit

At seven DAFB, well exposed indeterminate fruiting lateral shoots were selected on two adjacent vines. Coloured tags signifying treatments were randomly placed on these lateral shoots, and leaf:fruit ratio was adjusted to either one or four. An effort was made at this time to ensure that fruitlets were even in size, and not misshapen. Girdles were placed at the base of each lateral shoot at seven, 28 or 56 DAFB as described in section 3.2.1.

One girdled shoot per vine was harvested from any one treatment at selected intervals throughout the season. Fruit fresh weight was measured, and a transverse slice was taken from the point of minimum equatorial diameter. Permanent transverse sections were made, and cell counts were performed across the core in the direction of the minimum and maximum equatorial diameter ($D_{min}$ and $D_{max}$ respectively), and in inner pericarp and outer pericarp in the direction of $D_{min}$ as described in chapter two.

## 3.3 Results

### 3.3.1 Fruit weight and dry matter accumulation

In season one, the leaf:fruit ratio within the shoot had no effect on any parameter unless a girdle was applied (Table 3.1). There were significant interactions between the time at which girdles were applied and the leaf:fruit ratio for all the fruit parameters measured except seed numbers (soluble solids P=0.026, percentage dry matter P=0.0025, fresh and dry weight p<0.0001). There were no significant main effects of treatment or date on fruit seed number. Soluble solids was strongly correlated to the percentage dry matter (Figure 3.3), and for this reason further discussion is confined to the percentage dry matter.

Table 3.1 The seed number, soluble solids, fresh weight, dry weight and percentage dry matter of fruit on shoots which were not girdled (control) at two leaf:fruit ratios in season one. Due to the presence of missing values, means shown are population marginal means[1] of up to 12 shoots per treatment.

| | Leaf:fruit ratio | | Contrast (P>F) |
|---|---|---|---|
| | one | four | (one vs. four) |
| Fresh weight (g) | 96 (34) | 97 (34) | 0.8103 |
| Dry weight (g) | 12.9 (0.6) | 12.9 (0.6) | 0.9857 |
| % dry matter | 13.4 (0.2) | 13.1 (0.2) | 0.2694 |
| Soluble solids ($^{o}$Brix) | 7.6 (0.4) | 6.4 (0.4) | 0.0794 |
| Seed number per fruit | 945 (78) | 917 (78) | 0.8337 |

[1] For description of population marginal means, see chapter two, section 2.8.3.
Standard error of mean in parentheses.

Low leaf:fruit ratio (one), season one

At a low leaf:fruit ratio, fresh weight, dry weight, and the percentage dry matter of fruit from girdled shoots was significantly lower (contrast, P<0.05) than controls at all

girdling dates (Figure 3.2). However, between the different dates when shoots were girdled, there was no evidence of any significant differences for any parameter, even between shoots which had been girdled as far apart as 56 days. Fresh weight averaged 61.9g across all girdling dates, while dry weight averaged 6.5g. Across all of the girdling dates, the percentage dry matter was reduced from 13.4% in control fruit, to 10.8% in fruit from girdled shoots (Table 3.2).

### High leaf:fruit ratio (four) in season one

The mean fresh weight of fruit from all girdled shoots at the high leaf:fruit ratio was higher than from control shoots. However only fruit from shoots girdled at full bloom (FB) or 28 DAFB were significantly heavier than control fruit (contrast, P=0.0157, 0.0460 respectively). Fruit from shoots girdled at 14 DAFB had a significantly lower dry weight (contrast, P=0.0083, 0.0255 respectively) than fruit from shoots which were girdled at FB or 28 DAFB (Figure 3.2), and were in fact not significantly different to fruit from non-girdled control shoots (contrast, P=0.2776). Furthermore, these fruit had a significantly lower percentage dry matter, than fruit from control shoots (contrast, P=0.003). In contrast, when fruit from 14 DAFB were excluded from the analysis, there was no evidence that the percentage dry matter of fruit from girdled shoots with a high leaf:fruit ratio were significantly different from control fruit (Table 3.2). Percentage soluble solids ($^{o}$Brix) followed a very similar pattern to the percentage dry matter (Figure 3.2), and there was a strong linear relationship between these two variables (Figure 3.3).

### High leaf:fruit ratio (four) in season two

In order to check the comparatively low fruit weight and dry matter content of fruit from shoots with a high leaf:fruit ratio girdled at 14 DAFB was real, this part of the experiment was repeated.

A similar trend to that found in the previous year was detected, although it was not as marked (Figure 3.4). The fresh and dry weight, and percentage dry matter of fruit on shoots girdled at 14 DAFB were lower than from shoots girdled at 7 or 21 DAFB, although differences were not statistically significant.

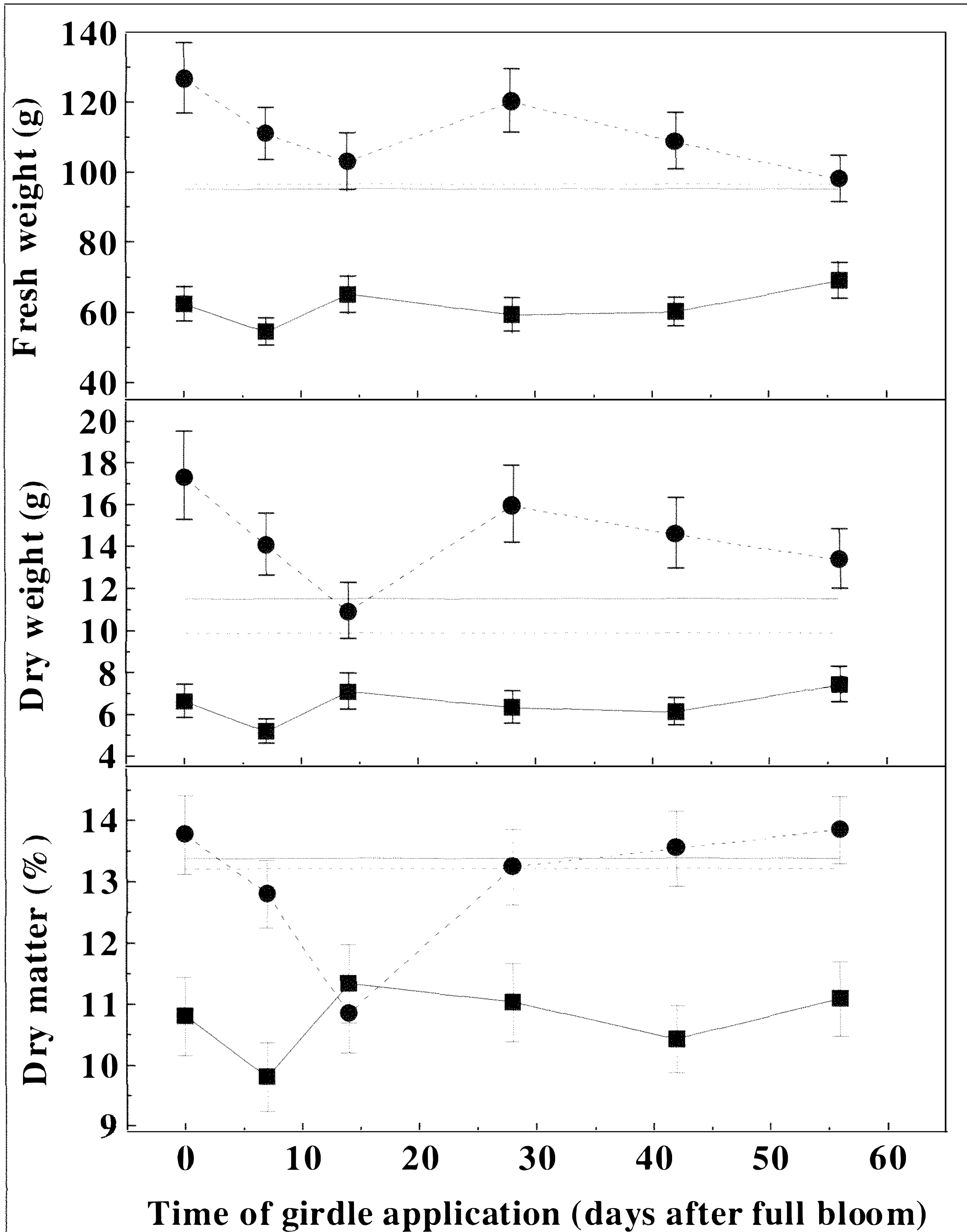


Figure 3.2 The fresh weight, dry weight and percentage dry matter of fruit from shoots pruned to a leaf:fruit ratio of one (—■—) or four (– –●– –) and girdled at six dates between full bloom and 56 days after full bloom in season one. Control (non-girdled shoots) are represented by horizontal lines. Fruit fresh and dry weight are back-transformed from $\log_{10}$. All values represent the population marginal mean from six vines each with two shoots.

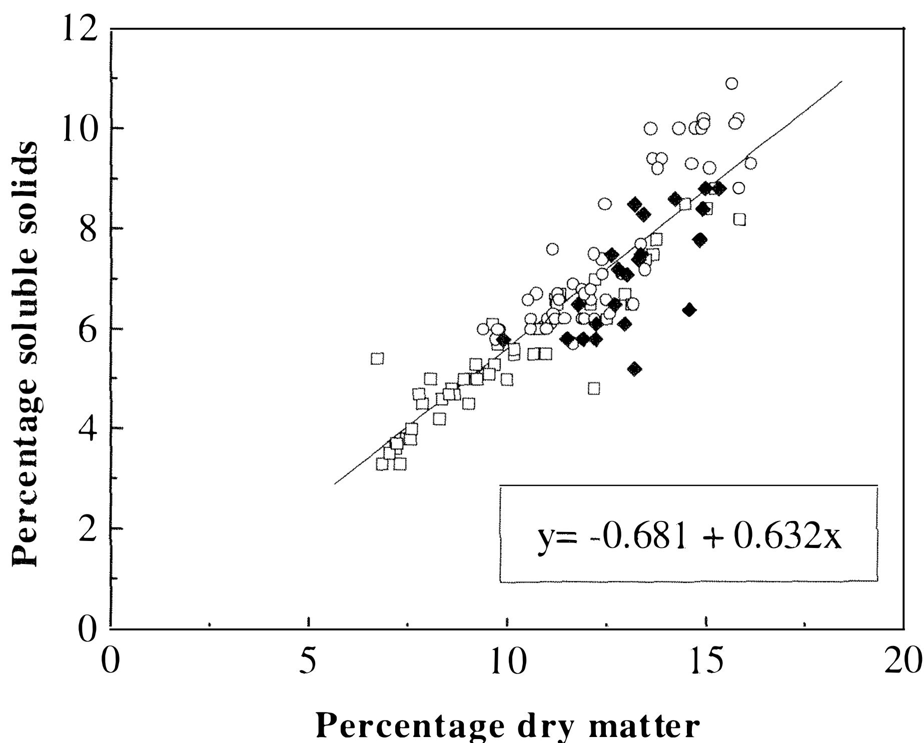


Figure 3.3 The relationship between soluble solids and percentage dry matter at harvest (160 days after full bloom) of kiwifruit on non-girdled shoots (◆) or shoots girdled between 0 and 56 days after full bloom with a leaf:fruit ratio of four (O) or one (□).

Fruit weight was significantly higher at all girdling dates compared with control fruit, but showed a consistent decrease at successively later girdling dates, apart from fruit girdled at full bloom and at 14 DAFB (Figure 3.4). The positive effect of girdling on fruit weight was more pronounced than in the previous year, with fruit on shoots girdled at seven DAFB averaging 54g higher than that of the control fruit (means back-transformed from $\log_{10}$). The percentage dry matter of fruit was significantly higher than the control treatment (contrast, $P<0.05$) at every date girdled after 14 DAFB, and was significantly higher overall for fruit from girdled shoots compared with control fruit (Table 3.2). The fruit percentage dry matter appeared to show a steady increase with increase in time of girdling after full bloom (Figure 3.4) and a significant linear trend was shown by orthogonal contrast ($P=0.0317$) between the earliest and latest times of girdle application.

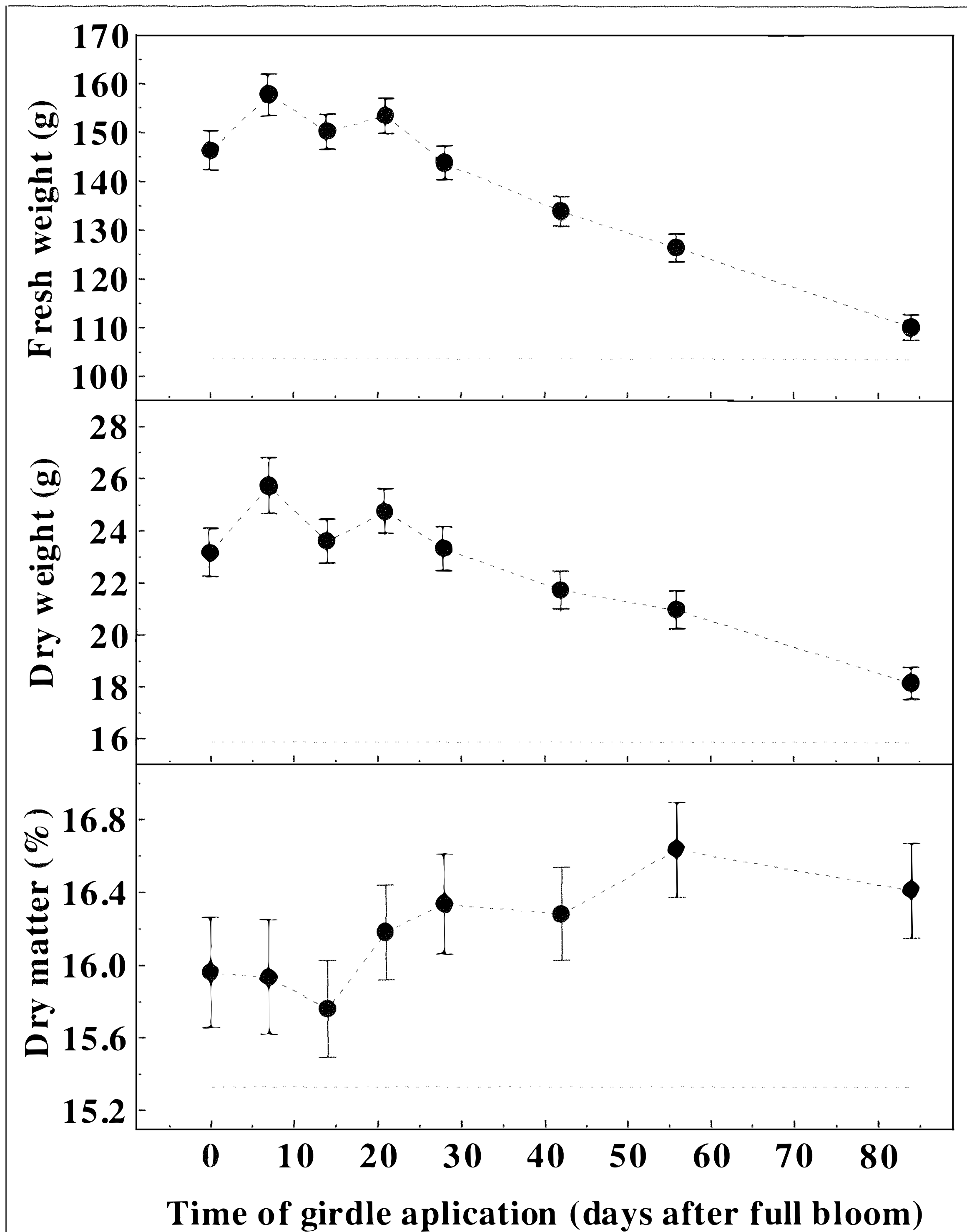


Figure 3.4 Fresh weight, dry weight and percentage dry matter of fruit from shoots which were girdled at eight dates between full bloom and 84 days after full bloom in season two. Shoots were pruned to a high (four) leaf:fruit ratio at full bloom. Fruit from non-girdled control shoots are represented by a horizontal line. Fresh and dry weight were back-transformed from $\log_{10}$. All values represent the population marginal mean from six vines each with four replicate shoots.

Table 3.2 The percentage dry matter of kiwifruit on control shoots or shoots girdled up to 56 days after full bloom in season one or 84 days after full bloom in season two. A low (one) or high (four) leaf:fruit ratio was imposed on the shoots to provide a low or high carbohydrate supply respectively available for fruit. Means and standard errors were estimated from linear combinations of GLM parameters.

| Treatment | Season | Low leaf:fruit ratio on shoot | High leaf:fruit ratio on shoot |
|---|---|---|---|
| Control | one | 13.4 a | 13.2 a |
| Girdled | one | 10.8 b | 13.4[1] a |
| Control | two | - | 15.3 c |
| Girdled | two | - | 16.2[1] d |

Means with common letters are not significantly different at p=0.05 (contrast)
[1] Excluding fruit from shoots girdled at 14 days after full bloom.

There was a trend towards higher seed numbers fruit on shoots girdled later (Figure 3.7), and a significant linear trend was demonstrated by orthogonal contrast (P=0.0048). Fruit from shoots girdled at full bloom had particularly low seed numbers, averaging only 590 seeds per fruit, compared with 960 for control fruit.

### 3.3.2 Growth curves of fruit on girdled shoots

A double sigmoid shaped growth curve was evident for fruit from all treatments,. This was characterised by a period approximating exponential growth between 7 and 42 DAFB (stage I), followed by a brief 'lag' phase during which fruit growth slowed (stage II), and finally a relatively steady rate of growth until harvest (stage III) (Figure 3.5, Figure 3.6). During the 'exponential phase', fruit growth rates on girdled shoots with a low leaf:fruit ratio were dependent on the time of girdling, with a reduced growth rate at earlier girdling times compared with later girdling (Figure 3.5). However after the 'lag' phase, the growth of fruit on shoots girdled at either 28 or 56 DAFB with a low leaf:fruit ratio was strongly inhibited, continuing at only 0 to 0.2 $g.day^{-1}$ (Figure 3.5). Fruit from shoots with a low leaf:fruit ratio girdled at 7 DAFB did not appear to be as strongly inhibited after the lag phase, and fruit weight continued to increase at a rate of around 0.5 $g.day^{-1}$ (Figure 3.5).

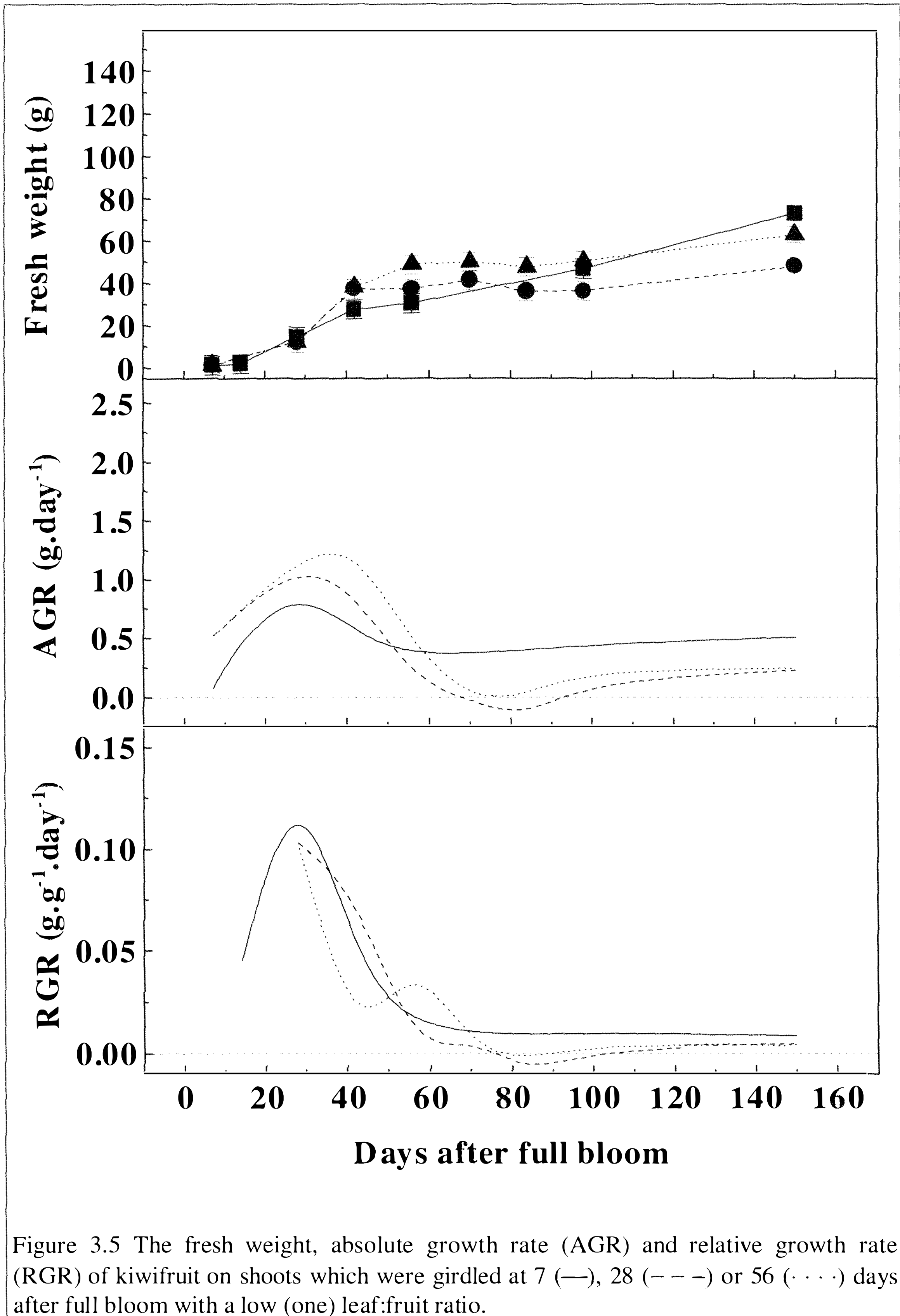


Figure 3.5 The fresh weight, absolute growth rate (AGR) and relative growth rate (RGR) of kiwifruit on shoots which were girdled at 7 (—), 28 (– – –) or 56 (· · · ·) days after full bloom with a low (one) leaf:fruit ratio.

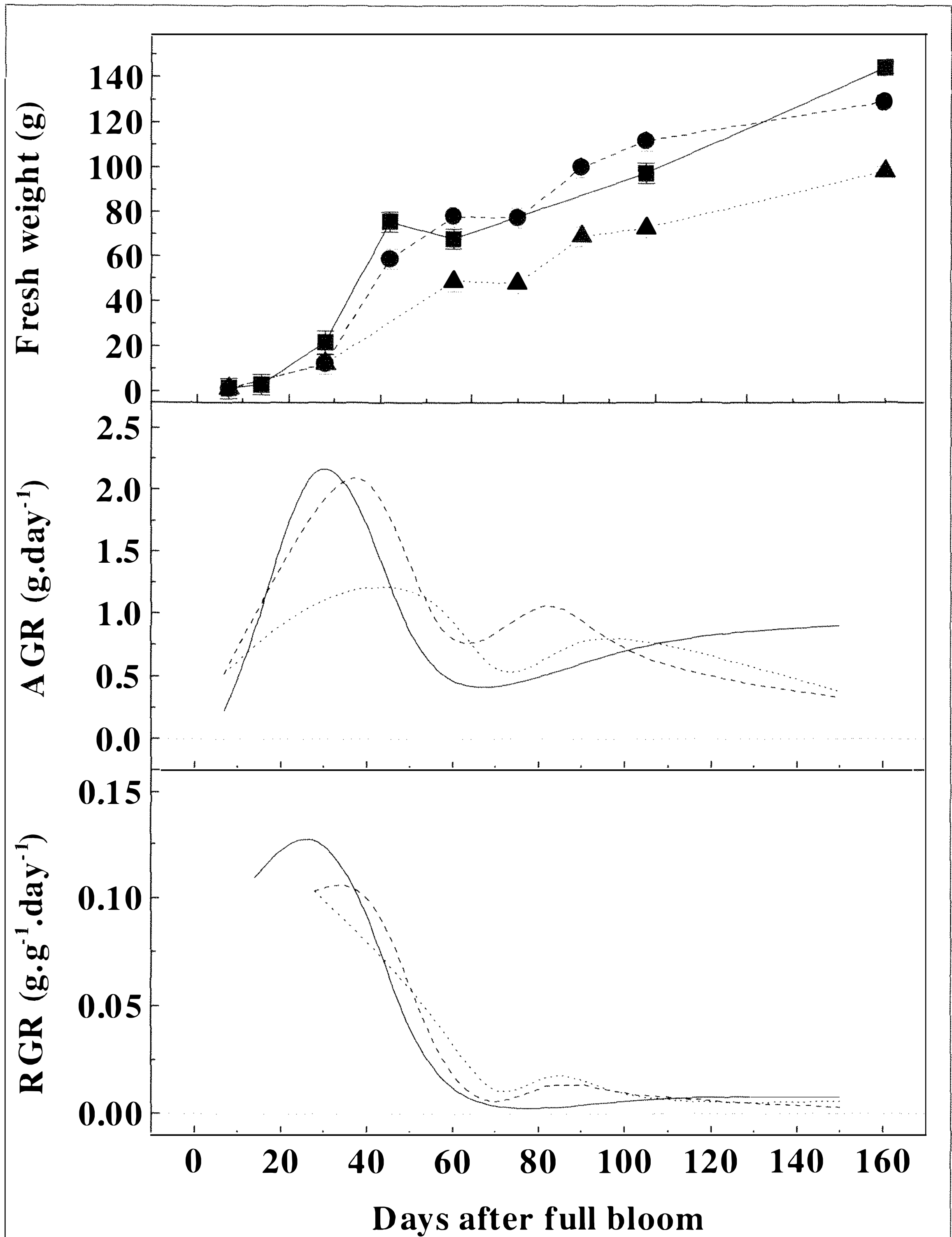


Figure 3.6 The fresh weight, absolute growth rate (AGR) and relative growth rate (RGR) of kiwifruit on shoots which were girdled at 7 (—), 28 (– – –) or 56 (· · · ·) days after full bloom with a high (four) leaf:fruit ratio.

At a high leaf:fruit ratio, fruit size continued to increase throughout the season in all treatments (Figure 3.6). Within the realms of experimental error, there appeared to be no obvious difference in growth of fruit from shoots girdled at either 7 or 28 DAFB which were at a high leaf:fruit ratio. During the 'exponential phase', the growth rates of fruit from shoots with a high leaf:fruit ratio, girdled at either 7 or 28 DAFB were considerably higher than for fruit from shoots girdled at 56 DAFB (Figure 3.6). However after the 'lag phase', fruit from all treatments at a high leaf:fruit ratio appeared to grow at a similar rate of between 0.5 to 1.0 $g.day^{-1}$ (Figure 3.6).

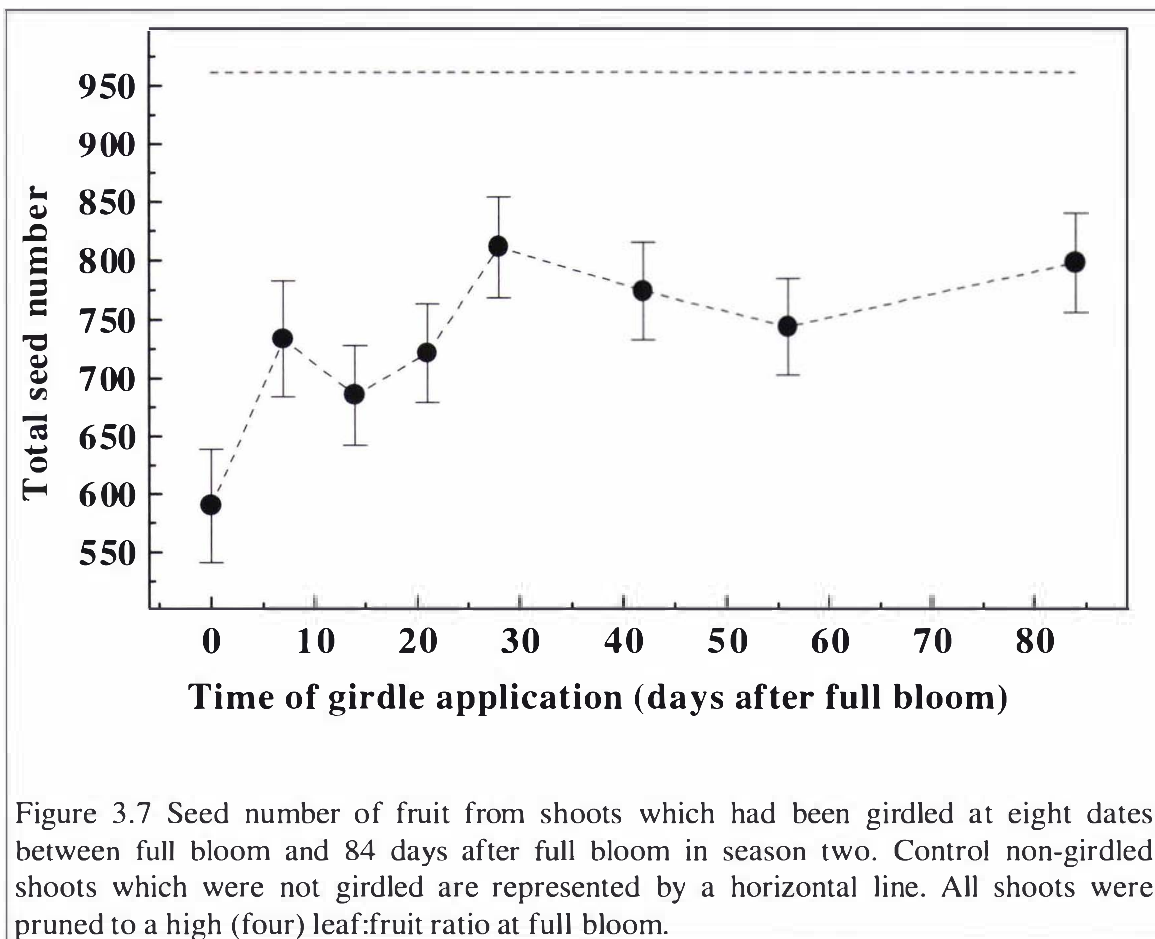


Figure 3.7 Seed number of fruit from shoots which had been girdled at eight dates between full bloom and 84 days after full bloom in season two. Control non-girdled shoots which were not girdled are represented by a horizontal line. All shoots were pruned to a high (four) leaf:fruit ratio at full bloom.

### 3.3.3 The effect of leaf:fruit ratio on cell numbers

For the purpose of increasing replication, all cell number measurements taken after 56, 70 or 98 DAFB were pooled for analysis of treatment differences in outer pericarp, inner

pericarp or core cell numbers respectively, as cell division in each tissue had practically ceased by these dates.

It appeared that post-anthesis cell division in outer pericarp tissues had ceased by 42 to 56 DAFB (Figure 3.8), and there was no difference between any of the treatments (Table 3.3). Over all treatments, the radius of outer pericarp tissue increased from a total 27 cells at seven DAFB to an average of 54 cells after 56 DAFB.

Table 3.3 Total cell numbers counted in a straight line across individual tissues from kiwifruit in transverse sections. Kiwifruit were from shoots which had been girdled at 7, 28 or 56 days after full bloom and had a low (one) or high (four) leaf:fruit ratio. Data was pooled from measurements made on sections of fruit harvested after the cessation of cell division between 56-150 (outer pericarp), 70-150 (inner pericarp) or 98-150 (core and total) days after full bloom. Due to the presence of missing values, means presented are population marginal means.

| Girdling date (days after full bloom) | Leaf:fruit ratio on shoot | Core (half) ($D_{max}$) | Core (half) ($D_{min}$) | Inner pericarp ($D_{min}$) | Outer pericarp ($D_{min}$) | Total (half fruit) ($D_{min}$) |
|---|---|---|---|---|---|---|
| 7 | low | 69.3 b | 54.9 ab | 48.0 c | 53.5 a | 157.3 b |
| 28 | low | 66.0 b | 53.4 b | 54.1 b | 55.5 a | 159.4 b |
| 56 | low | 69.0 b | 50.8 b | 53.5 bc | 53.8 a | 152.3 b |
| 7 | high | 71.9 ab | 59.1 a | 60.2 a | 52.2 a | 173.3 a |
| 28 | high | 79.1 a | 58.4 a | 60.1 a | 56.5 a | 177.6 a |
| 56 | high | 70.3 b | 55.2 b | 53.9 b | 53.4 a | 161.3 b |

$D_{max}$: Measurements of cell diameter were made along a straight line transect in the direction of the maximum equatorial diameter.

$D_{min}$: Measurements were made along a straight line transect in the direction of the minimum equatorial diameter.

Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).

In contrast, cell division in the core and inner pericarp tissue was influenced by the time of girdling and leaf:fruit ratio. Inner pericarp cell division appeared to continue until between 56 and 70 DAFB. The inner pericarp increased from 27 cells wide at seven DAFB to between 54 and 63 cells wide by 70 DAFB, which represents 1 to 1.17

doublings in cell numbers in this tissue. At the high leaf:fruit ratio, inner pericarp cell numbers were significantly higher level in the earlier girdling times than at the latest girdling time (Table 3.3). There was also some evidence that inner pericarp cell numbers were reduced at the low leaf:fruit ratio when a girdle was applied at seven DAFB (Table 3.3).

The peak period of cell division in the core was up until around 56 DAFB, and continued slowly thereafter until some time between 98 DAFB and fruit maturity. Core cell numbers measured across the minimum diameter showed only 0.38 to 0.59 doublings, while across the maximum diameter there were 0.65 to 0.98 doublings. Thus it appears that there was around twice as much cell division in the maximum diameter of the core. Core cell numbers were higher at a high leaf:fruit ratio than at a low leaf:fruit ratio at all times of girdling, although when shoots were girdled at 56 DAFB, the difference was small (Table 3.3).

### 3.3.4 The effect of leaf:fruit ratio on cell shape and size

The average cell diameter in all tissues were increased at the high leaf:fruit ratio, however the increase in cell diameter was accentuated at the earlier girdling dates (Figure 3.8 to Figure 3.10, Table 3.4). The effect on cell diameter at maturity was greatest in the outer pericarp and in the maximum axis of the core (Table 3.4). Core tissue was composed of relatively evenly sized cells, although the shape varied from isodiametric reasonably circular shaped to cells which were more elliptical (see chapter one, Plate 1.5). Cells in the core appeared to enlarge to a greater extent across the maximum axis, as cell diameter approximately doubled in this direction, whereas across the minimum axis, cell diameter increased only by around 1.5 times (Figure 3.8 to Figure 3.10). Although core cell diameter in the minimum axis was higher at the high leaf:fruit ratio at all sampling dates after 63 DAFB and all dates of girdling (Figure 3.8 to Figure 3.10), this was not statistically significant in fruit harvested at maturity (Table 3.4). However core cell diameter in the maximum axis were significantly increased by a high leaf:fruit ratio at the earlier girdling dates (Table 3.4).

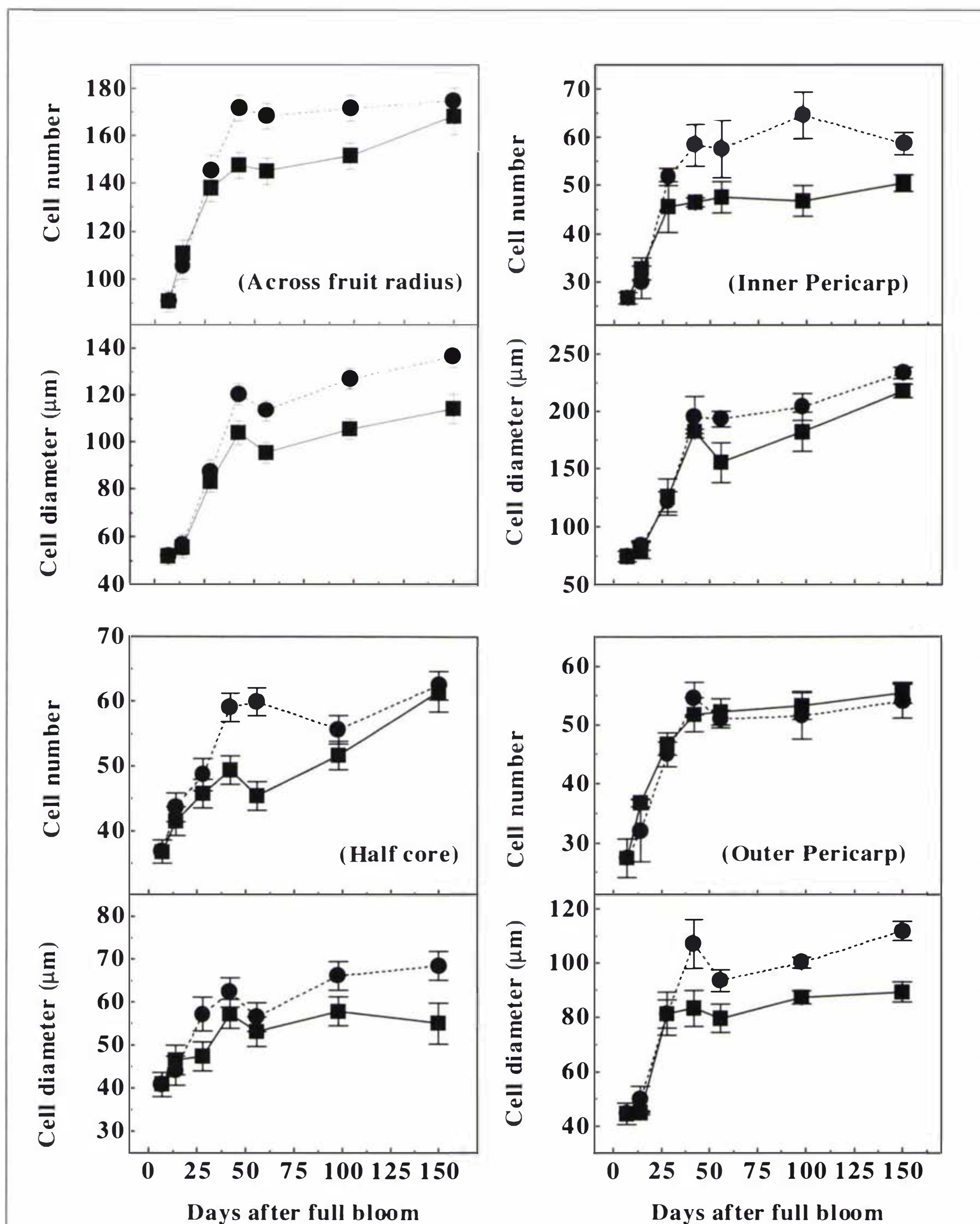


Figure 3.8 The effect of a leaf:fruit ratio of one (—■—) or four (– –●– –) on shoots which were **girdled 7 days after full bloom** on kiwifruit cell number or cell diameter. Measurements are of the inner pericarp and outer pericarp on one side of the fruit, across half the diameter of the core, or total of these three tissues. Cellular measurements represent counts of cell numbers, or average cell diameter along a straight line transect across the minimum equatorial diameter. (n=4).

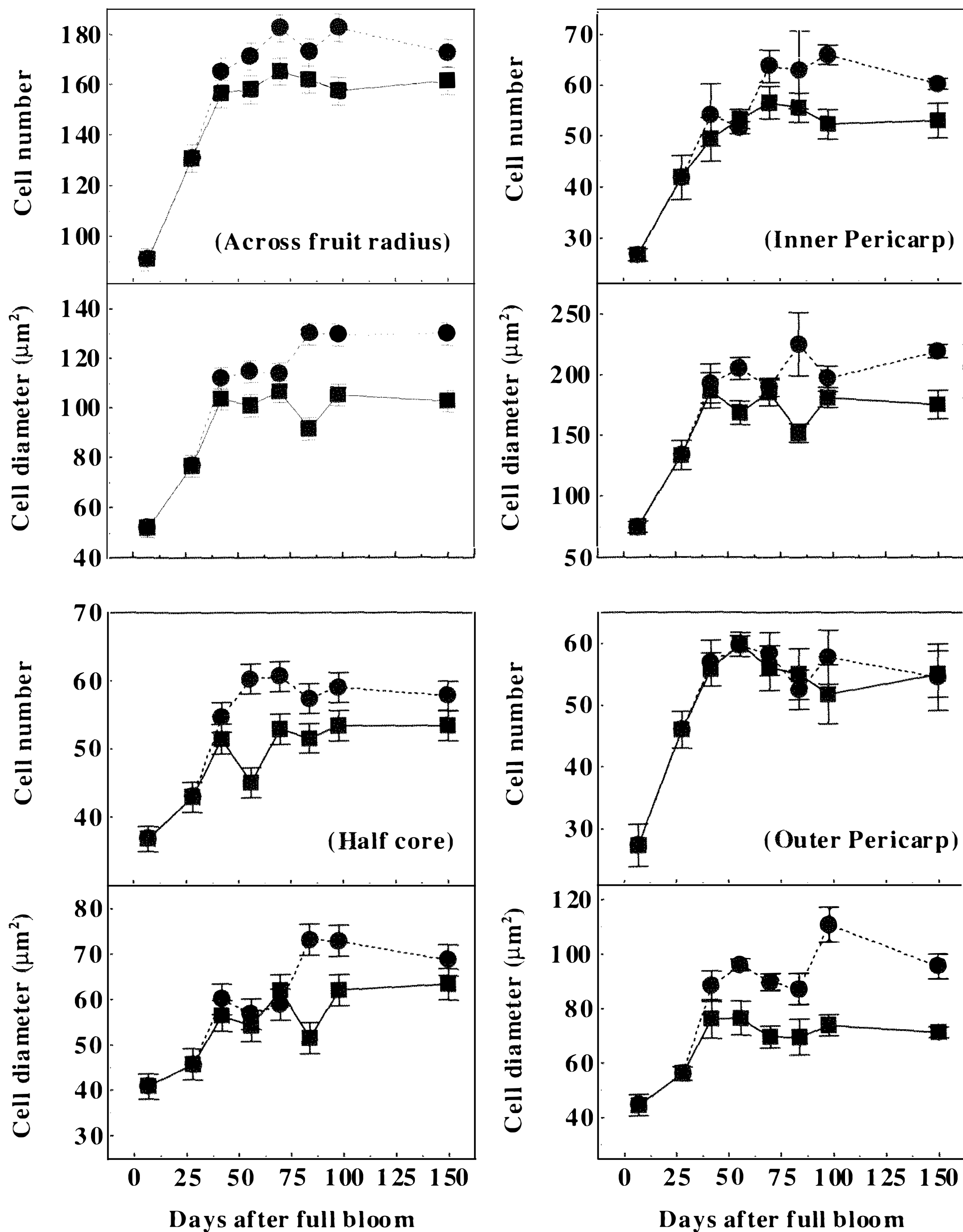


Figure 3.9 The effect of a leaf:fruit ratio of one (—■—) or four (– –●– –) on shoots which were **girdled 28 days after full bloom** on kiwifruit cell number or cell diameter. Measurements are of the inner pericarp and outer pericarp on one side of the fruit, across half the diameter of the core, or total of these three tissues. Cellular measurements represent counts of cell numbers, or average cell diameter along a straight line transect across the minimum equatorial diameter. (n=4).

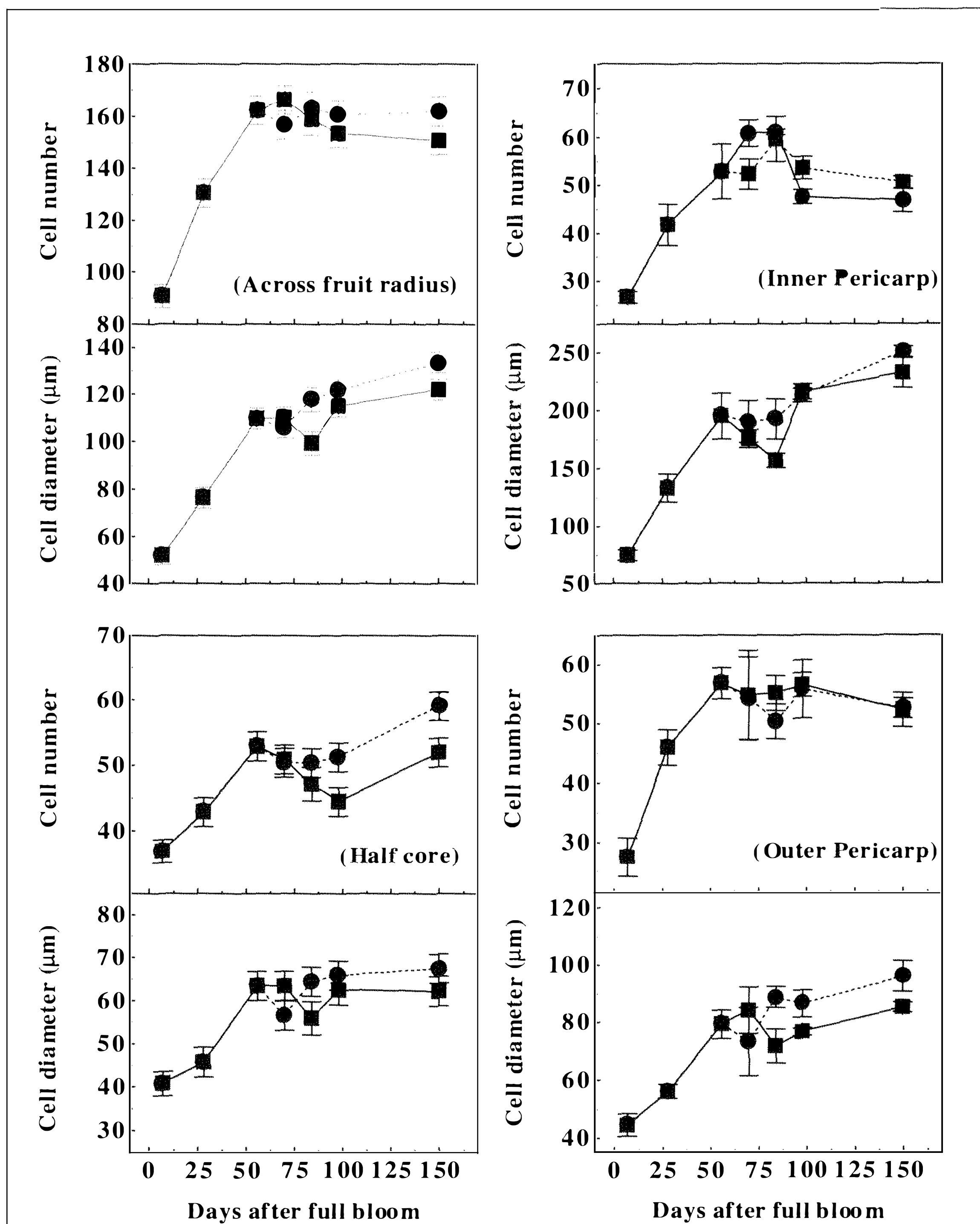


Figure 3.10 The effect of a leaf:fruit ratio of one (—■—) or four (– –●– –) on shoots which were **girdled 56 days after full bloom** on kiwifruit cell number or cell diameter. Measurements are of the inner pericarp and outer pericarp on one side of the fruit, across half the diameter of the core, or total of these three tissues. Cellular measurements represent counts of cell numbers, or average cell diameter along a straight line transect across the minimum equatorial diameter. (n=4).

Table 3.4 Average cell diameter (μm) at maturity (150 days after full bloom) measured across individual tissues of kiwifruit in transverse sections. Kiwifruit were from shoots which had been girdled at 7, 28 or 56 days after full bloom and had a low (one) or high (four) leaf:fruit ratio. Due to the presence of missing values, means presented are population marginal means.

| Girdling date (days after full bloom) | Leaf:fruit ratio on shoot | Core ($D_{max}$) | Core ($D_{min}$) | Inner pericarp ($D_{min}$) | Outer pericarp ($D_{min}$) | Total (half fruit) ($D_{min}$) |
|---|---|---|---|---|---|---|
| 7 | low | 100 cd | 55 a | 217 bc | 89 bc | 115 c |
| 28 | low | 92 d | 63 a | 175 d | 71 d | 103 d |
| 56 | low | 107 bc | 62 a | 232 c | 85 c | 122 bc |
| 7 | high | 121 a | 69 a | 233 bc | 111 a | 137 a |
| 28 | high | 117 ab | 69 a | 219 c | 96 b | 130 ab |
| 56 | high | 109 abc | 68 a | 251 ab | 96 b | 134 a |

$D_{max}$: Measurements of cell diameter were made along a straight line transect in the direction of the maximum equatorial diameter.
$D_{min}$: Measurements were made along a straight line transect in the direction of the minimum equatorial diameter.
Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).

Inner pericarp tissue was the most diverse of all three fruit tissues. At seven DAFB, it was composed of fairly evenly sized elongated cells over its entire radius. However as fruit developed, some of the cells nearer the outer pericarp began differentiating into very large cells (see chapter one, Plate 1.3). Between the locules, cells composing the locule wall elongated disproportionally in the radial direction leading to a population of very long thin cells (see chapter one, Plate 1.4), while near the core, cells remained relatively small and evenly shaped. This differentiation of cell type in the inner pericarp made cell measurements in this tissue very difficult and probably not as informative as measurements made in the other tissues. Average inner pericarp cell diameter increased around two to three fold from 75 μm at 7 DAFB to 175-250 μm at 150 DAFB. The effect of leaf:fruit ratio on the diameter of inner pericarp cells was not clear. Inner pericarp cell diameter was larger at the high leaf:fruit ratio than at the low leaf:fruit ratio at all sampling dates (Figure 3.8 to Figure 3.10) and significant differences in individual comparisons of final cell diameter were found (Table 3.4). However the main and

interaction model effects on final inner pericarp cell diameter were non-significant, thus results for the inner pericarp in Table 3.4 should be treated with caution.

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. Kiwifruit were harvested at maturity (150 days after full bloom) from shoots which had been girdled at 7, 28 or 56 days after full bloom and had a low (one) or high (four) leaf:fruit ratio. Due to the presence of missing values, means presented are population marginal means.

| | | | Large cells | | Small cells | |
|---|---|---|---|---|---|---|
| Girdling date (days after full bloom) | Leaf:fruit ratio on shoot | Fresh weight (g) | diameter (μm) | number | diameter (μm) | number |
| 7 | low | 73 d | 231 b | 8.7 a | 62 c | 43.7 a |
| 28 | low | 48 f | 191 c | 7.3 a | 56 c | 45.8 a |
| 56 | low | 63 e | 197 c | 8.3 a | 68 bc | 45.0 a |
| 7 | high | 144 a | 290 a | 8.3 a | 82 a | 47.5 a |
| 28 | high | 129 b | 255 b | 8.0 a | 78 ab | 41.0 a |
| 56 | high | 98 c | 247 b | 9.3 a | 79 ab | 41.0 a |

Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).

The outer pericarp was also composed of two cell populations. Some cells expanded into very large spherical cells. These were interspersed with smaller cells which became more prevalent (and smaller) towards the epidermis (see chapter one, Plate 1.3). The average cell diameter approximately doubled from 44 μm at 7 DAFB to 90-110 μm at 150 DAFB. In outer pericarp tissue, large cells made up 16% of the total number of cells in the straight line transect, but there were no differences in numbers of large or small cells in the outer pericarp tissue transect due to the girdling and leaf:fruit ratio treatments (Table 3.5). The outer pericarp cell diameter was higher at the high leaf:fruit ratio than at the low leaf:fruit ratio (Table 3.4). The differences in average cell diameter in outer pericarp was reflected in an increase in size of both large and small cells (Table 3.5).

## 3.4 Discussion

### 3.4.1 The interaction between girdling and leaf:fruit ratio

In this study, girdling of shoots has been used as a tool to investigate source-sink relations in kiwifruit. Specifically, high or low leaf:fruit ratios were applied to shoots to monitor kiwifruit development in the presence of carbohydrate deficit or excess. We investigated how the leaf:fruit ratio affects fruit development at different times, and whether effects on fruit size are mediated by cell expansion, cell division or cell density. In addition, fruit growth curves were generated in order to determine key times at which growth was affected.

The results of this study support those obtained by others who have girdled shoots of kiwifruit. Fruit growth was enhanced at a leaf:fruit ratio of four, while it was inhibited at a leaf:fruit ratio of one. Although kiwifruit normally obtain carbohydrate from nearby leaves on their own lateral shoot, carbohydrate can be readily obtained from other shoots some distance away when there is a shortage (Lai et al., 1989b). Snelgar et al. (1986) found little difference in the weight of kiwifruit on individual defoliated canes compared with those on non-defoliated canes, which suggested that fruit are able to obtain carbohydrate from other canes. However on a girdled shoot, phloem linkages are interrupted and the fruit become entirely dependent on the carbohydrate produced by leaves within the shoot. At a low leaf:fruit ratio, fruit growth is probably inhibited by a shortage of carbohydrate, whereas at high leaf:fruit ratio, excess carbohydrate cannot be exported out of the shoot, and probably becomes available to stimulate fruit growth. A leaf:fruit ratio of around two on girdled shoots has been found to produces similar sized fruit to non-girdled shoots, suggesting that this is the minimum number of leaves required to support average fruit growth (Snelgar et al., 1986; Lai et al., 1989a). Although carbohydrate can be transported to non-girdled shoots with a low leaf:fruit ratio (Lai et al., 1989b), fruit growth was reduced in severely defoliated parts of vines (Buwalda and Smith, 1990). Thus it appears that in non-girdled vines, carbohydrate can

be transported readily to fruit which have a poor carbohydrate source in close proximity, unless vine carbohydrate resources are limited overall.

On shoots with a low leaf:fruit ratio which were girdled at either 28 or 56 DAFB, fruit growth rates were inhibited throughout the season compared with growth rates of fruit at a high leaf:fruit ratio. However the inhibition was particularly severe between 56 and 100 DAFB, and fruit appeared to be in an extended 'lag' phase (Figure 3.5). In addition, there were no significant differences in fresh weight between fruit on shoots at the low leaf:fruit ratio girdled at six dates between 0 and 56 DAFB (Figure 3.2), which suggests that fruit growth in all six girdling times was inhibited severely after 56 DAFB. Although the growth rate of fruit on shoots at the low leaf:fruit ratio which were girdled at seven DAFB did not appear to be inhibited (Figure 3.5), this could be due to chance variation in fruit from the last two harvests. This seems likely as fruit weight from the corresponding treatment in the time of girdling experiment was not significantly different to other girdling times (Figure 3.2). The time at which the severe inhibition of growth occurred (56 DAFB) corresponds to the beginning of the lag phase in the double sigmoid growth curve of kiwifruit (Hopping, 1976; Lai, 1987), and also coincides with the complete cessation of cell division in pericarp tissues (Figure 3.8 to Figure 3.10). It is likely that an alteration in partitioning of imported carbon occurs at this time as kiwifruit cells switch from division to expansion and storage. The alteration in metabolism may be associated with the increased conversion of carbohydrates to storage forms which begin to accumulate after 40 DAFB (Okuse and Ryugo, 1981). Derivation of data presented by Okuse and Ryugo (1981) showed that starch and soluble sugars only made up approximately 22% of the 7g dry weight of a normal kiwifruit at 60 DAFB, while at maturity, these composed approximately 40% of the 16g dry weight. Thus over half (4.9g) of the 9g increase in dry weight of a kiwifruit between 60 DAFB and maturity was composed of starch and soluble sugars. As fruit from girdled shoots at a low leaf:fruit ratio did not undergo very much cell expansion after 56 DAFB (Figure 3.8 to Figure 3.10), little imported carbon would have been utilised in structural dry matter. Instead, imported carbon was probably utilised mainly in respiration, and storage. The percentage dry matter was considerably lower in fruit on shoots at leaf:fruit ratio of one than at a leaf:fruit ratio of four, as has also been found by (Seager and Hewett, 1995). This

suggests that carbohydrate was probably also in limited supply for storage as starch or soluble sugar.

It has been suggested that two leaves are required on a girdled shoot to allow a kiwifruit to attain a similar size to fruit on non-girdled shoots (Snelgar et al., 1986; Lai et al., 1989a). As the shoot itself is a weak sink (Lai, 1987), and as there was very little growth of fruit on girdled shoots with a low leaf:fruit ratio after 56 DAFB (Figure 3.5), it seems plausible that a large proportion of the photo-assimilate exported by the leaves must have been mostly used in respiratory maintenance of the fruit and supporting shoot tissue. As the net photosynthetic rate of leaves on girdled shoots at a low leaf:fruit ratio has been found to be unaffected compared with control shoots at any time throughout a growing season (Lai et al., 1989a; Woolley and Lai, 1990), it seems unlikely that the leaf output of carbohydrate was affected on girdled shoots at a low leaf:fruit ratio. Seager (1993) measured the respiration rate of kiwifruit harvested from girdled shoots at a leaf:fruit ratio of one or five, and found no trend over time, although the mean respiration rate was slightly higher in fruit from shoots with the high leaf:fruit ratio on four out of five dates. The mean respiration rate of kiwifruit harvested from girdled shoots at a leaf:fruit ratio of one at 20°C, was 286 nmol $CO_2$ $kg.FW^{-1}$ $s^{-1}$ over the period 21 March to 15 May (Seager, 1993). On a dry weight basis (% dry weight averaged 11.7% over this period) this equates to 2.44 nmol $CO_2$ $g.DW^{-1}$ $s^{-1}$. However average kiwifruit respiration rates of fruit from non-girdled shoots at a comparable time were 0.88 nmol $CO_2$ $g.DW^{-1}$ $s^{-1}$ at 25°C measured while fruit were still attached to vines (Walton and De Jong, 1990). Derivation of data (estimated visually from graphs, 15% dry weight assumed) presented by Pratt and Reid (1974) gave similar kiwifruit respiration rates of between 0.4 and 1.0 nmol $CO_2$ $g.DW^{-1}$ $s^{-1}$ for detached fruit. The three fold difference between the reported respiration rates of kiwifruit from girdled shoots (Seager and Hewett, 1995) and non-girdled shoots (Pratt and Reid, 1974; Walton and De Jong, 1990) suggests that the respiration of fruit on girdled shoots may not be equivalent to that of fruit from non-girdled shoots, even at a low leaf:fruit ratio. This could explain the very low growth rates of kiwifruit at low leaf:fruit ratio that we have found. If this is the case, this also casts doubt on the use of girdling experiments to determine the leaf requirement for kiwifruit

growth (Snelgar et al., 1986; Lai et al., 1989a), and suggests that the leaf requirements of a kiwifruit may be somewhat lower than two leaves per fruit.

Maintenance respiration is dependent on biomass (Amthor, 1989), which may explain why fruit on girdled shoots at the low leaf:fruit ratio appeared to undergo an extended lag phase between 60 and 100 DAFB (Figure 3.5). As fruit biomass increased, respiration would have utilised an increased percentage of carbohydrate, particularly if fruit respiration rates are higher on girdled shoots. However respiration rate is also dependent on temperature (Amthor, 1989). This could explain the improvement in the fruit growth rate which occurred later in the season between 100 and 150 DAFB (Figure 3.5). For example, in 1992 average daily maximum temperature decreased steadily from 21°C in January to 12.8°C in May (N.Z. Met. service, 1992).

The response of fruit growth to girdling and leaf:fruit ratio was different between seasons. Fruit responded typically to high leaf:fruit ratios in season two in this study (Figure 3.4). However in season one, fruit size at the high leaf:fruit ratio was lower than had been expected, although fruit were still significantly heavier than control fruit (Figure 3.2). In season two there were higher sunshine hours and higher mean daily maximum and minimum temperature in every month from December to May compared with season one (N.Z. Met. service, 1992; N.I.W.A., 1993). The photosynthetic rate in kiwifruit, as in most plants, is highly dependent on temperature and incident light (Laing, 1985). Thus it is possible that the response to girdling may have been mediated by conditions for photosynthesis which were different between seasons. In addition, lower average temperatures in season one could have reduced root function, affecting the overall performance of vines.

### 3.4.2 A possible inhibitory effect of girdling on kiwifruit growth

In two seasons, the fruit from shoots with a high leaf:fruit ratio girdled at 14 DAFB, grew to a smaller size and had a lower dry weight and percentage dry matter than might be expected compared with fruit from shoots girdled slightly earlier or later. This may be a crucial period in kiwifruit growth, perhaps for cell division or seed formation, and

girdling during this period could therefore limit the fruit growth potential. There are reports that girdling may lead to a transitory reduction in the photosynthetic rate. For example Mayoral et al. (1985) reported a temporary reduction in the photosynthetic rate, associated with increased stomatal resistance within 3 hours of the placement of a steam girdle on cucumber leaves, although leaves recovered within a further 3 hours. If this is the case in kiwifruit shoots, a temporary carbohydrate shortage could have been induced by girdling. However it is difficult to see that such a short-term reduction in the carbohydrate supply could have such a large effect considering the day to day differences in net photosynthesis which occur as a result of weather conditions. Alternatively, girdling has been reported to lead to changes in plant hormones, which could affect fruit development at a critical time. Reduced levels of gibberellin and cytokinin have been found in xylem sap of girdled peach branches, compared with non-girdled branches on the same tree (Cutting and Lyne, 1993). Cell division is thought to be dependant on cytokinin, and fruit may have a requirement for external supply of cytokinin. Placement of a girdle at a critical time could thereby conceivably interrupt cell division processes at this time by lowering the cytokinin supply. However xylem sap cytokinin levels were actually enhanced in girdled kiwifruit canes (Chapter five), which suggests that girdling of shoots is unlikely to result in a deficiency of cytokinins. The most likely explanation is associated with the effect of girdling on auxin transport. Girdling appears to interrupt auxin transport (Dann et al., 1985), which may be essential for growing organs (Bangerth, 1989). Dominant apple and tomato fruits appear to export a high amount of the auxin IAA, and this reduces IAA export from inhibited fruits (Gruber and Bangerth, 1990). Therefore it is possible that the build up of IAA in girdled shoots could inhibit IAA export from kiwifruit, and that this is crucial for fruit growth at 14 DAFB. Dominance relationships have also been found in kiwifruit (Lai et al., 1990), although it has not been established that these are related to IAA transport. However diffusible IAA from kiwifruit was found to be lower in fruit from girdled shoots (chapter six). Similarly, application of an auxin transport inhibitor to pedicels of kiwifruit ovaries, reduced fruit fresh and dry weight most severely when it was applied at 14 DAFB (Chapter seven). Although it was not able to be confirmed that auxin export was crucial for kiwifruit growth, it is possible that auxin export is particularly important during this period of kiwifruit development.

### 3.4.3 The validity of correlations between radial cell measurements and fruit size

Fruit are three dimensional structures, thus the measurement of a single dimension does not accurately represent what may be occurring in all fruit dimensions. A good correlation to kiwifruit weight has been found to the product of Length x maximum equatorial diameter x minimum equatorial diameter (Snelgar et al., 1992), and to the fruit length cubed (Green et al., 1990). However results presented in chapter two have shown that any single fruit dimension individually correlates well to fruit weight, but residual variation is markedly reduced if all three parameters are used. The good correlation obtained between kiwifruit minimum equatorial diameter and fruit weight (chapter two) suggest that the measurement of cellular parameters in one radial dimension are representative of fruit expansion in the radial axis, and possibly also of fruit growth as long as fruit shape is relatively consistent. The most significant deviations in fruit shape appear to be when fruit are flat or fan shaped in which case fruit are significantly wider than normal fruit in the maximum equatorial diameter (Watson and Gould, 1994), and when fruit are poorly pollinated in which case fruit are significantly shorter than normally pollinated fruit (Chapter six).

However there are two main problems associated with the cell counting technique used. Firstly the diversity of cell types which are present in any tissue, especially in the inner pericarp, make cell number and size measurements strictly an average measure and thus they do not provide information as to which types of cells are affected by treatments. In inner pericarp tissue this may be the most information which can be usefully interpreted, due to the very wide diversity of cells present. However measurements in the outer pericarp may benefit from separation of the two component cell types as was done with fruit which were harvested at maturity. Core tissue is made up of fairly uniform cells, and thus can probably be counted with the most confidence. The second problem is associated with demarcation of boundaries between tissues. Although vascular bundles provide a useful boundary, cell types do not abruptly change but instead merge into one another with a gradual change of shape and size. Radial location of outer bundles can also vary even in adjacent bundles, and this will increase the amount of variation necessitating increased replication in order to detect treatment differences.

### 3.4.4 Post-anthesis cell division in kiwifruit

Final cell numbers counted in this study were comparable to numbers in 'Hayward' kiwifruit reported elsewhere (Woolley et al., 1992; Cruz-Castillo, 1994; Lewis, 1994). Cell numbers of fruit on girdled shoots in this study averaged 54 cells across the outer pericarp, from 48 to 60 cells across the inner pericarp and from 51 to 59 cells across half of the core (minimum equatorial diameter). Cruz-Castillo (1994) reported that the outer pericarp averaged 60 to 69 cells, inner pericarp 52 to 57 cells and core (half, minimum equatorial diameter) 52 to 58 cells. However cell numbers were found to be dependent on the time of anthesis, with lower cell numbers recorded in late flowers compared with early flowers (Cruz-Castillo, 1994). Woolley et al. (1992) found 55, 55 and 40 cells in outer pericarp, inner pericarp and core (half, minimum equatorial diameter) respectively, and Lewis (1994) reported approximately 55, 40 and 50 cells (values estimated visually from graphs) in outer pericarp, inner pericarp and core respectively at 40 days after anthesis, although whether counts were made in the minimum or maximum equatorial diameter was not stated. The differences between these studies are likely to be due to both the difficulty of counting cells in inner pericarp tissue, and differences in demarcation of tissue boundaries between core and inner pericarp. However it is possible that some of the differences in cell counts between these studies were due, at least in part, to the particular selection of 'Hayward' used. Although 'Hayward' kiwifruit is the standard cultivar grown in New Zealand (Ferguson et al., 1990), a number of selections have been made from 'Hayward' which have fruit size differences (G.S.Lawes personal communication, 1996). Differences in fruit growth have been found between clonally propagated Hayward selections from a single orchard (Cruz-Castillo, 1994) as well as from the same clonal selection on different rootstock (Cruz-Castillo et al., 1991), and it is possible that some differences in fruit size are due to rates of pre- or post-anthesis cell division within the selections. Alternatively cultural conditions, crop load, orchard location, or vine location within the orchard could affect fruit via cell division.

Cell numbers in kiwifruit approximately doubled across the fruit radius over the period of fruit growth monitored in this study. If the cell division in the radial direction is representative of fruit cell division in all three dimensions, this would represent a total of

three doublings in cell number for an entire kiwifruit. If the shape of a kiwifruit is assumed to approximate a sphere ($4.\pi.r^2$), and the number of cells in a radius at anthesis (90 cells) is imposed into this equation, a kiwifruit at anthesis would contain approximately 9 million cells. Using logic similar to Coombe (1976), this represents about 23 doublings of cell number in the fruit prior to anthesis. Coombe (1976) estimated that in apple, 21 doublings were required prior to anthesis, but only 4.5 doublings of cell number occurred after anthesis, while only 17 and 1.5 doublings occurred prior to and after anthesis respectively in grapes. Extrapolation of the results of fruit cell numbers at anthesis and maturity presented by Cheng and Breen (1992) reveals that in three strawberry cultivars, between 2.4 and 2.8 doublings in cell numbers occur between anthesis and maturity. Similar extrapolation of the results of Scorza et al. (1991) showed that in four peach cultivars between 4 and 4.8 doublings occurred on an entire transverse fruit section between full bloom and maturity. The total fruit cell division in peach would probably be even higher, assuming that longitudinal cell division also occurs. The high total cell number in peach may be due to a long duration of cell division, as this appears to continue up until fruit maturity (Scorza et al., 1991). Thus it appears that the level of post-anthesis cell division in different fruit species varies quite considerably. This is possibly due in part to the duration of cell division. however pre-anthesis cell division is considerably more prolific than the cell division which occurs post-anthesis, and thus probably provides greater scope for influencing the final fruit cell number.

### 3.4.5 The effect of leaf:fruit ratio on cell division and expansion in kiwifruit

As non-girdled control shoots were not included in this experiment, it is not possible to conclude as to whether girdling *per se* affected cells. However although the assumption that there is no interaction between girdling and leaf:fruit ratio cannot be demonstrated here, a comparison of the effects of low versus high carbohydrate supply on cell division and expansion can still be made. Although carbohydrate is known to be essential for both cell division and expansion in tissue culture of plant organs or callus, it is more difficult to demonstrate the influence it has in an intact plant. But as increased crop load almost always leads to a decrease in fruit size (weight or volume), it is useful to establish

whether carbohydrate supply affects fruit size via cell division as well as expansion. If cell division is affected, canopy management to maximise the availability of assimilate during the period of cell division may be crucial to the potential growth rate of fruit throughout the remainder of a season.

The main effect of the leaf:fruit ratio on fruit diameter can be attributed to effects on cell expansion. Fruit from the high leaf:fruit ratio had a higher cell diameter than fruit from the low leaf:fruit ratio at all girdling times (Figure 3.8 to Figure 3.10), although differences were not always statistically significant. In particular, cell diameter was most affected in the inner and outer pericarp cells. Both large and small outer pericarp cells were similarly affected by leaf:fruit ratio (Table 3.5). However at high leaf:fruit ratios, outer pericarp small cell diameters were higher than core cell diameter (both minimum axis) (Table 3.4 and Table 3.5). As both are starch storing parenchyma type cells (Hopping, 1976; Patterson et al., 1993) this suggests that within a kiwifruit, outer pericarp cells may have a greater ability to expand than core cells. An increase in the size of outer pericarp small cells has also been found in response to CPPU application (Patterson et al., 1993).

Increased leaf:fruit ratio during the period of post-anthesis cell division (seven and 28 DAFB) led to an increase in cell number in core and inner pericarp tissues. In other studies, carbohydrate deficit has also been associated with reduced fruit cell numbers. For example, reduction of the availability of assimilates to tomato fruits by partial defoliation immediately following pollination was shown to cause a large decrease in fruit cell numbers (Bohner and Bangerth, 1988b). Cucumbers exposed to limited assimilate supply over their entire growing period via high crop load had strongly decreased growth due to both decreased cell numbers and cell size (Marcelli, 1993). Reducing the crop load of 'Millers seedling' apple by heavy thinning of flowers prior to full bloom increased fruit cortex cell numbers and cell size compared with un-thinned trees (Denne, 1960). However the effect of leaf:fruit ratio on post-anthesis cell numbers in kiwifruit was found to be relatively minor (Table 3.3) compared with the effects on cell diameter (Table 3.4). The level of post-anthesis cell division in kiwifruit, as in most fruits, is low compared with pre-anthesis cell division. Thus it may be more appropriate to focus on influencing

cell division prior to anthesis. If similar effects of leaf:fruit ratio on pre-anthesis cell division occurs, pre-anthesis girdles, or knife width girdles (scoring) of trunks, leaders or canes could potentially increase the number of cells in kiwifruit.

Girdles applied at 56 DAFB in this study, had no effect on cell numbers in the pericarp tissue. This is consistent with the reported time that post-anthesis cell division ceases in kiwifruit. Outer pericarp and inner pericarp cell division in kiwifruit 'Monty' had ceased by 23 and 33 days respectively (Hopping, 1976), while in 'Hayward' kiwifruit cell division was found to continue for around 38 and 45 days in outer and inner pericarp respectively (Woolley et al., 1992). Similarly, fruit from shoots with a high leaf:fruit ratio girdled at 56 DAFB had higher (although non-significant) core cell numbers compared with girdled shoots with a low leaf:fruit ratio in both the minimum and maximum axis (Table 3.3). This is consistent with the assertion that cell division in the core continues slowly for up to 110 days after anthesis (Hopping, 1976), and core cell numbers could therefore be affected by application of a late girdle.

Although the cell number and size influence fruit weight to the greatest extent, Coombe (1976) points out that cell density is also involved in determining weight. The data presented here shows clearly that the percentage dry matter in fruit is strongly affected by leaf:fruit ratio on a girdled shoot. As the effect of leaf:fruit ratio on fruit weight was not solely due to cell volume, cellular measurements made in this study can not account for all of the effects of girdling on fruit weight. At a low leaf:fruit ratio, the percentage dry matter was reduced by around 2.5% compared with control fruit (Table 3.2), although at a high leaf:fruit ratio it appears that the percentage dry matter was only slightly increased over all girdling dates in season two (around 1%), and was not affected in season one. A significant effect of leaf:fruit ratio on the percentage dry matter of kiwifruit was also reported by Seager and Hewett (1995) who suggested that at low leaf:fruit ratio, imported assimilate may be used for respiration, rather than as stored reserves.

In season two there was a significant trend towards higher percentage dry matter in fruit on shoots at a high leaf:fruit ratio when the shoot was girdled at later times (Figure 3.4). This suggests that the extra carbohydrate was being utilised for storage rather than growth in later girdled fruit, leading to fruit with a relatively high percentage dry matter. As late girdling has no significant effect on cell numbers (Table 3.3), expansion of fruit from shoots girdled at later times with a high leaf:fruit ratio may have been limited by low cell numbers. This supports the hypothesis promoted by (Ho, 1988) that the number of cells in a sink organ determines the potential sink size.

## 3.5 Summary

The growth of kiwifruit was not affected by leaf:fruit ratio on the fruiting shoot unless a girdle was also applied to limit carbohydrate flow into and out of the shoot. On girdled shoots, fruit growth was generally stimulated at a leaf:fruit ratio of four. Although the stimulation of fruit growth was generally greater when girdles were applied early in the season, girdling at 14 DAFB resulted in a lower response to girdling. This may be due to an inhibitory effect of girdling on seed formation or cell division at a crucial time, due to a build up of auxin in girdled shoots which could an cause inhibitory response similar to those found in correlative dominance. At a leaf:fruit ratio of one, fruit growth was severely inhibited on girdled shoots. However the girdling time had very little on fruit weight at a low leaf:fruit ratio, and fruit weight only increased at a very low rate after the 'lag' period of the double sigmoid growth curve at around 60 DAFB. This may be due to cessation of cell division and expansion in the bulk of fruit tissues, and a change in metabolism towards storage. In addition there is evidence that fruit on girdled shoots have a higher respiration rate than fruit on non-girdled shoots, which may further restrict their ability to accumulate dry matter.

Although most of the increase in size of fruit on girdled shoots at the high leaf:fruit ratio compared with shoots at the low leaf:fruit ratio was due to increased cell expansion, there was some increase in cell numbers when shoots were girdled during the period of active cell division. As the percentage dry matter was increased in fruit girdled after the period of cell division on shoots with a high leaf:fruit ratio, this suggested that cell

expansion may have reached an upper limit, and fruit were compelled to increase storage of starch.

At a high leaf:fruit ratio, fruit size on girdled lateral shoots was increased by up to 57g compared with fruit on intact lateral shoots. Increases of this size have the potential to be of commercial benefit to kiwifruit growers.

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# 4. Cane Girdling of kiwifruit I: Potential use to Increase Fruit Size

## 4.1 Introduction

In recent years the New Zealand kiwifruit marketing board (NZKMB) placed considerable emphasis on obtaining large size fruit. This has been a response to an over-supply of kiwifruit in the world market, both from New Zealand kiwifruit growers, and from other producing countries and financial encouragement has been provided by the NZKMB to growers to produce large size fruit. Manipulation of crop load can achieve increase in fruit size (Cooper and Marshall, 1992), although the response does vary between authors and seasons. For example, calculations from linear models fitted to fruit number and weight data suggested that halving fruit numbers from 1000 to 500 fruit per vine (approximately 40 and 20 fruit/$m^2$ respectively) increased fruit weight by 25 g in one season but only 11 g in the following season (Burge et al., 1987). Visual estimation from graphs presented by Richardson and McAneney (1990) reveals that a decrease in crop load from 40 to 20 fruit/$m^2$ resulted in an increase in fruit weight of less than 10 g. Calculations from linear models fitted to crop load data by Cooper and Marshall (1992) suggest that the decrease in crop load from 40 to 20 fruit/$m^2$ increased fruit weight by 10, 6 and 11 g in three consecutive seasons. However girdling of individual kiwifruit laterals at high leaf:fruit ratio has been shown to produce large increases in fruit size (Snelgar et al., 1986; Lai et al., 1989a; Woolley et al., 1992), and interest has been shown by growers in developing girdling for commercial use in New Zealand kiwifruit orchards. The potential for large increases in fruit size compared with normal crop loading management was demonstrated by Snelgar et al. (1986) who showed that an increase in leaf:fruit ratio on whole vines of one leaf per fruit resulted in an increase in mean fruit weight of only 6.9g, whereas on a girdled lateral shoot the same increase in leaf:fruit ratio resulted in an increase of 30g.

The girdling of lateral shoots is unlikely to be satisfactory for grower use. It is time consuming, and shoots have a tendency to break during a growing season. In grapevines,

where girdling is practised commercially, girdling is usually done on trunks, main leaders or individual canes. However where girdles are applied to trunks or main leaders, care has to be taken that girdles are relatively narrow, so that they will heal quickly enough to allow supply of photosynthetically fixed carbon to be transported to the root system, otherwise vine death will occur (Winkler, 1954). A previous report has suggested that girdling of kiwifruit canes can lead to increased fruit weight (Patterson and Mason, 1988).

The girdling of individual canes on kiwifruit vines was chosen as an experimental tool to investigate the effects on source-sink relations and overall vine performance. Cane girdling was chosen for the following reasons:

1. A high level of control can be maintained by varying the numbers of canes girdled per vine.
2. Some leaf area can be left in uninterrupted phloem vascular contact with the root system to supply it with carbohydrate.
3. Replacement canes for future seasons can grow and develop unaffected by a possible vascular interruption between the cane and storage areas in the stem and root system. Girdled canes can be removed completely during the normal winter pruning operation.
4. A girdled cane provides a useful experimental unit within a vine.

The aim of the experimental work described was to investigate the feasibility of a large scale girdling procedure on kiwifruit vines for commercial viability in terms of fruit growth potential, to check for any effects on fruit storage potential and return bloom of vines, and as a method for investigating source-sink relations in kiwifruit vines. Specifically two experiments were set up, the first to optimise the timing and position of girdles on canes, and the second to check the effects of girdling different percentages of canes on any one vine.

## 4.2 Materials and Methods

### 4.2.1 The effect of time and placement of cane girdling on fruit weight

Vines with at least 15 full length canes tied down in the previous winter, were selected at the Massey University fruit crops unit (MUFCU). Girdling was carried out similarly to girdling of lateral shoots as described in chapter three, except that girdles were applied to fruiting canes instead of lateral shoots. Five cane girdling treatments were applied in a randomised complete block design with each treatment replicated twice per vine, on each of three adjacent vines. Vines were considered as blocks and a replicate consisted of a single cane. Treatments were allocated to canes randomly as follows (also see Figure 4.1):

1. Girdle placed on cane immediately adjacent to the main leader at full bloom (FB).
2. Girdle placed on cane with two indeterminate shoots between the girdle and main leader at FB.
3. Girdle placed on cane immediately adjacent to the main leader at three weeks after full bloom (WAFB).
4. Girdle placed on cane with two indeterminate shoots between the girdle and main leader at three WAFB.
5. No girdle applied (Control ).

At three WAFB, obviously poorly pollinated fruits were removed from the vine. Girdles were maintained throughout the season. Fruit were harvested and weighed at maturity.

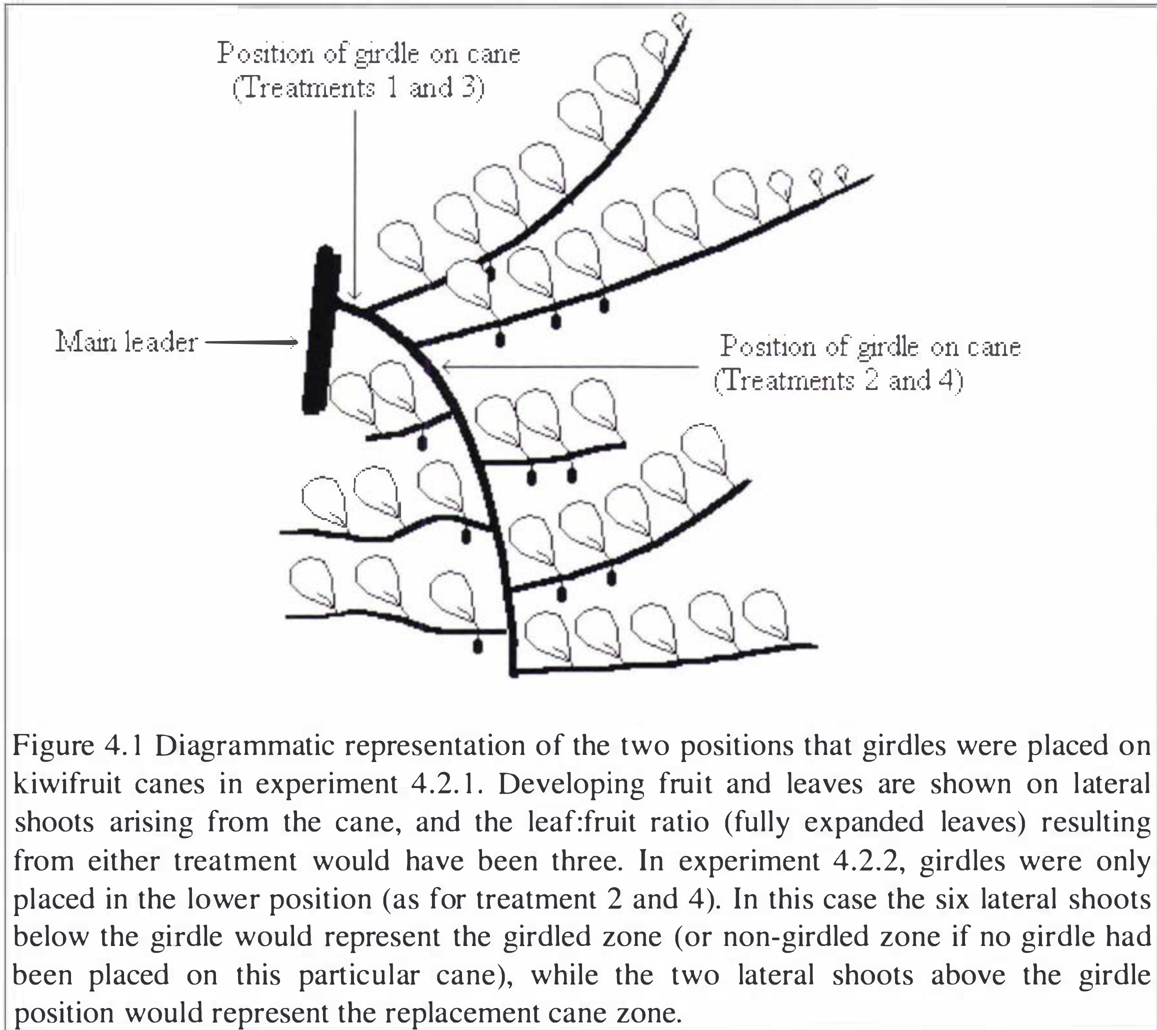


Figure 4.1 Diagrammatic representation of the two positions that girdles were placed on kiwifruit canes in experiment 4.2.1. Developing fruit and leaves are shown on lateral shoots arising from the cane, and the leaf:fruit ratio (fully expanded leaves) resulting from either treatment would have been three. In experiment 4.2.2, girdles were only placed in the lower position (as for treatment 2 and 4). In this case the six lateral shoots below the girdle would represent the girdled zone (or non-girdled zone if no girdle had been placed on this particular cane), while the two lateral shoots above the girdle position would represent the replacement cane zone.

### 4.2.2 The effect of girdling a different percentages of the canes on a vine on fruit size and vine performance

Two adjacent rows of kiwifruit were used at the MUFCU. Three treatments were applied to vines:

1. No girdled canes (0G).
2. Every second cane girdled (50G).
3. Every cane girdled (100G).

The experimental design was a randomised complete block design, with rows as blocks, and vines as replicates. Each treatment was replicated on six vines per row.

Girdles were applied to canes at FB (Plate 4.1, Plate 4.2) as described in section 4.2.1. The girdle was positioned above the second wire on the T bar where the canes are tied down vertically, with two replacement shoots between the girdle and the main leader (Figure 4.1). This position is similar to that used by Buwalda and Smith (1990b) to arbitrarily separate the replacement cane zone (RCZ) where next years replacement canes arise, from the fruiting zone (FZ) which is the main fruit producing part of the vine. The aim of girdle placement was to leave at least two replacement lateral shoots above the girdle to form next seasons replacement canes, while allowing complete removal of the girdled cane below the girdle at the end of the experiment. In addition, the long, indeterminate well exposed laterals present in the replacement cane zone provide a significant source of carbohydrate for the non-girdled parts of the vine.

Fruit were to be thinned once fruit set was completed at two WAFB, with the aim of ensuring leaf:fruit ratios on individual canes of four leaves per fruit. However counts of fruit and leaf numbers indicated that leaf:fruit ratios on canes were mainly close to 4:1 or greater without additional thinning, so this was not carried out. Lateral, misformed and obviously poorly pollinated fruit were removed as is normal management practice. Vines were managed as for commercial cropping, with irrigation schedules based on tensiometer readings and regular summer pruning of vegetative growth. Split application of urea and potassium chloride fertilisers were made twice during the cropping cycle, however no phosphorus and micro-nutrient fertilisers were used due to adequate existing levels (Shane Max, personal communication, 1993).

Fruit were harvested at commercial maturity (6% soluble solids). Fruit were harvested separately from lateral shoots arising above the girdling position on all canes (replacement cane zone, RCZ), from girdled canes below the girdling position (girdled zone, GZ) and from non-girdled canes below the girdling position (non-girdled zone, NGZ) (see Figure 4.1). Fruit fresh weights were measured immediately following harvest, and the total number of fruit in each zone counted.

For two vines per treatment in each row, fruit was harvested, counted and weighed for each individual cane, and leaf numbers on each cane were also counted. Leaf number divided by fruit number on each cane gave a leaf:fruit ratio. Leaf numbers in the RCZ were also counted on these vines. This enabled an average leaf:fruit ratio in the RCZ of these vines to be calculated.

Storage behaviour of fruit was assessed as follows. A random sample of 375 GZ fruit were taken from three 100G vines and 375 NGZ fruit from three 0G vines in one row. Fruit from the two treatments were placed in 15 trays each, with 25 count plix tray inserts and polyethylene liners. Trays were placed in a coolstore maintained at 0-1°C. One tray from each treatment was selected at 0, 8, 15, 28, 43, 72, 103, 128, and 153 days after harvest. A 2mm thick slice was removed from two sides of each fruit, and firmness was measured with a penetrometer. The same penetrometer was used for all testing, and was calibrated on a top pan balance before use on each date.

In the winter following the experimental work, all vines received normal winter pruning. This involved complete removal of all canes tied down in the previous year, and replacement with new canes (replacement canes) which had developed during the previous summer. This process removed all canes which had been girdled. Measurements of return bloom therefore relate to treatments applied in the previous summer but which were no longer physically present on vines. Return bloom was measured in the following spring, approximately one month prior to flowering in the following spring. Total numbers of compound (king plus lateral) and single (king only) flower clusters were counted and the total length of cane tied down was measured on each vine.

## 4.3 Results

### 4.3.1 Time and placement of cane girdles

All of the cane girdling treatments had a significantly higher fruit weight compared with fruit from control canes (Table 4.1). Cane girdling resulted in an increase in fruit weight of between 20 and 30g compared with fruit on control canes, but there were no significant differences between the time of application or between the position of the cane girdle.

Table 4.1 The effect of timing and position of a girdle on a cane, on the fresh weight of kiwifruit at maturity.

| Time of girdle application | Position of girdle on the cane | Mean fresh weight (g) | Standard error |
|---|---|---|---|
| Control | - | 97.4 b | 4.95 |
| Full bloom | Next to cordon | 121.1 a | 5.64 |
| Full bloom | Two replacement canes from cordon | 121.8 a | 7.38 |
| 3 WAFB[1] | Next to cordon | 128.7 a | 8.52 |
| 3 WAFB | Two replacement canes from cordon | 123.0 a | 7.50 |

Means with common letters are not significantly different at P= 0.05 (LSD).
[1] Weeks after full bloom.

Plate 4.1 A fresh girdle on a fruiting kiwifruit cane. Girdles were 10mm wide, and were positioned with two indeterminate shoots between the girdle and the cordon The cane is tied onto a 'second wire' which can be seen at the lower right corner.

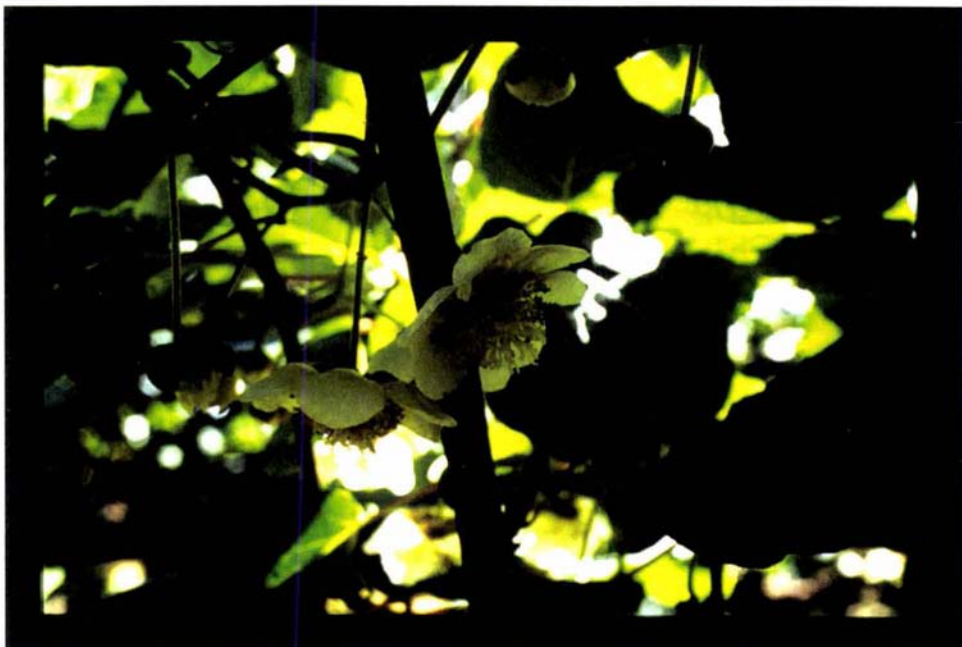

Plate 4.2 Kiwifruit flowers in bloom. Girdling was carried out when 80% of flowers were in bloom (full bloom).

### 4.3.2 Mean fruit weight over whole vines

There were no significant model or treatment effects for the total number of fruit per vine or crop load (Table 4.2), thus differences in mean fruit weight cannot be attributed to crop load. There were significant model effects for average fruit weight over the entire vine. The average fruit weight over the whole vine (i.e. RCZ, GZ and NGZ combined) was significantly increased by girdling either 50% or 100% of the canes on a vine compared with control vines. The increase in average fruit weight was relatively small and represented an approximate 5% increase. However, there was no significant difference in fruit weight between either of the two girdling treatments 50G and 100G (Table 4.2). The effect of cane girdling on the distribution of fruit in export size grades appeared to be a shift of fruit from the 30 to 36 size grades (116-127g) into 25 to 27 (127-160g) and jumbo (over 160g) size grades (Figure 4.2). In the 50G and 100G treatments, 5.6% and 5.8% of all fruit were jumbo size respectively, whereas almost no fruit of this size grade (0.3%) were found in the control (0G) treatment.

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. Values are the mean of 12 replicate vines.

| Percentage of canes girdled | Mean fruit weight (g) | Crop load (fruit.m$^{-2}$ canopy) | Yield (Kg.m$^{-2}$ canopy) | Gross return[1] (\$.m$^{-2}$ canopy) |
|---|---|---|---|---|
| 0 | 111.51 b | 22.5 a | 2.50 a | $ 1.73 a |
| 50 | 116.49 a | 19.0 a | 2.20 a | $ 1.86 ab |
| 100 | 117.91 a | 20.8 a | 2.44 a | $ 2.10 b |

Means in a column with common letters are not significantly different at P=0.05 (LSD).

[1] Gross return is based on NZKMB returns to growers after packing costs in 1993 season, but does not include orchard labour costs associated with application of girdles.

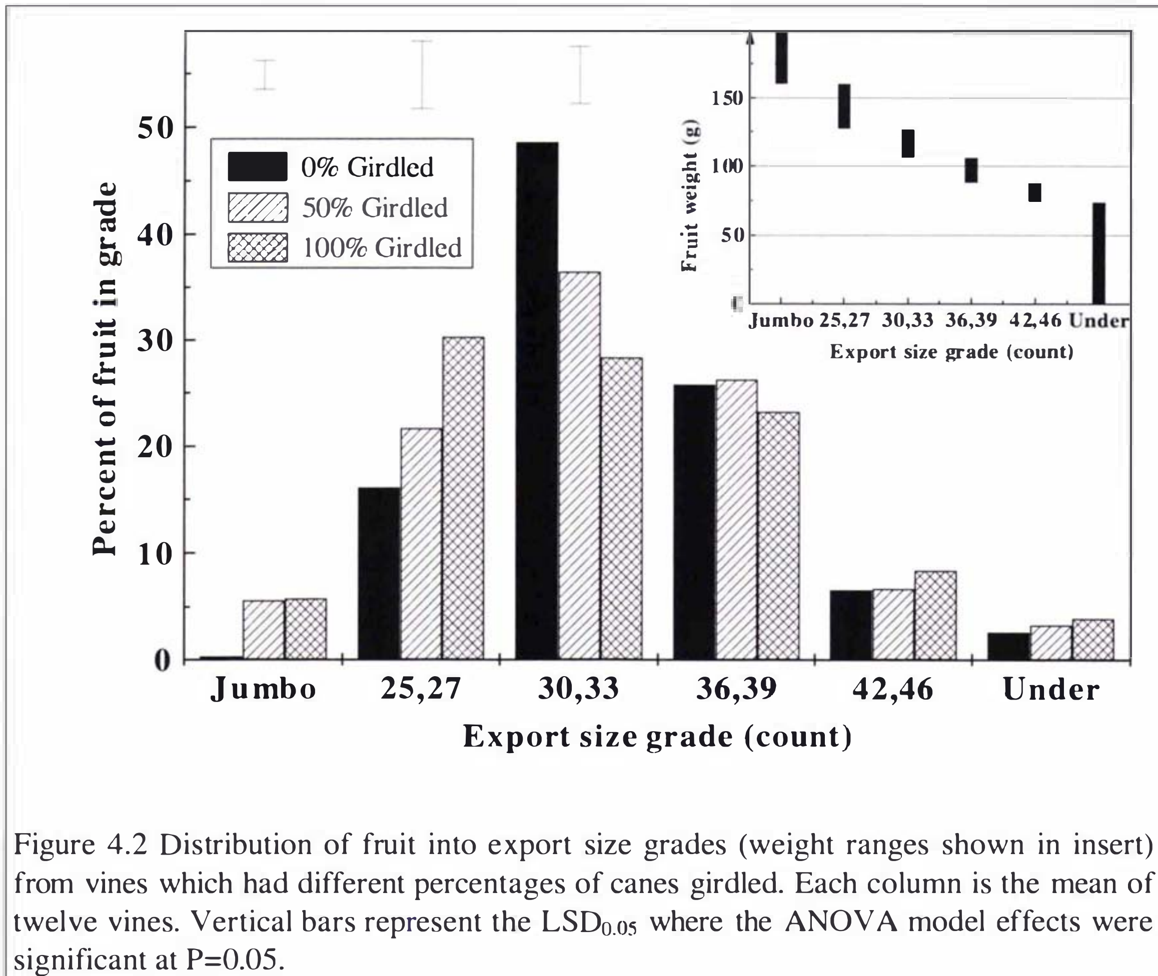


Figure 4.2 Distribution of fruit into export size grades (weight ranges shown in insert) from vines which had different percentages of canes girdled. Each column is the mean of twelve vines. Vertical bars represent the $LSD_{0.05}$ where the ANOVA model effects were significant at P=0.05.

### 4.3.3 Mean fruit weight from different parts of a vine

Although the two girdling treatments applied to vines had a positive effect on the mean fruit weight on the vine (Table 4.2), there was no increase in mean fruit weight when all the fruiting canes were girdled (100G) compared with when half the canes were girdled (50G). For this to be the case, either the weight of fruit on girdled canes (GZ) or fruit in the RCZ must have been reduced in the 100G treatment compared with the 50G treatment. Thus analysis of fruit weight in the different parts of the vines was necessary (see Figure 4.1 for description of the zones).

In order to analyse the treatment effects on fruit weight within the different zone, a simple factorial model of zone x treatment is not appropriate. This is because the three possible zones RCZ, GZ and NGZ were not allocated randomly in combination with the treatment applied to the whole vine, but instead were formed *post-hoc* as a result of

these treatments. However the zones can be considered to be equivalent between the three treatments, and any differences in fruit weight in a particular zone between the treatments can thus be considered due to the treatment.

In the RCZ which was present on all treatments, there were significant treatment effects on the mean fruit weight (Figure 4.3). Mean fruit weight in the RCZ was significantly lower when the percentage of the canes girdled was increased from 50 to 100% (contrast, P=0.0183), or from 0 to 100% (contrast, P=0.0001). Fruit weight in the RCZ was decreased when the percentage of canes girdled was increased from 0 to 50%, although the difference was only significant at the 10% level (contrast, P=0.0593). In addition, the mean fruit weight in the NGZ was decreased significantly as the percentage of canes girdled was increased from 0 to 50% (contrast, P=0.0026). Similarly in the GZ, mean fruit weight was significantly decreased as the percentage of canes girdled was increased from 50 to 100% (contrast, P=0.0023) (Figure 4.3).

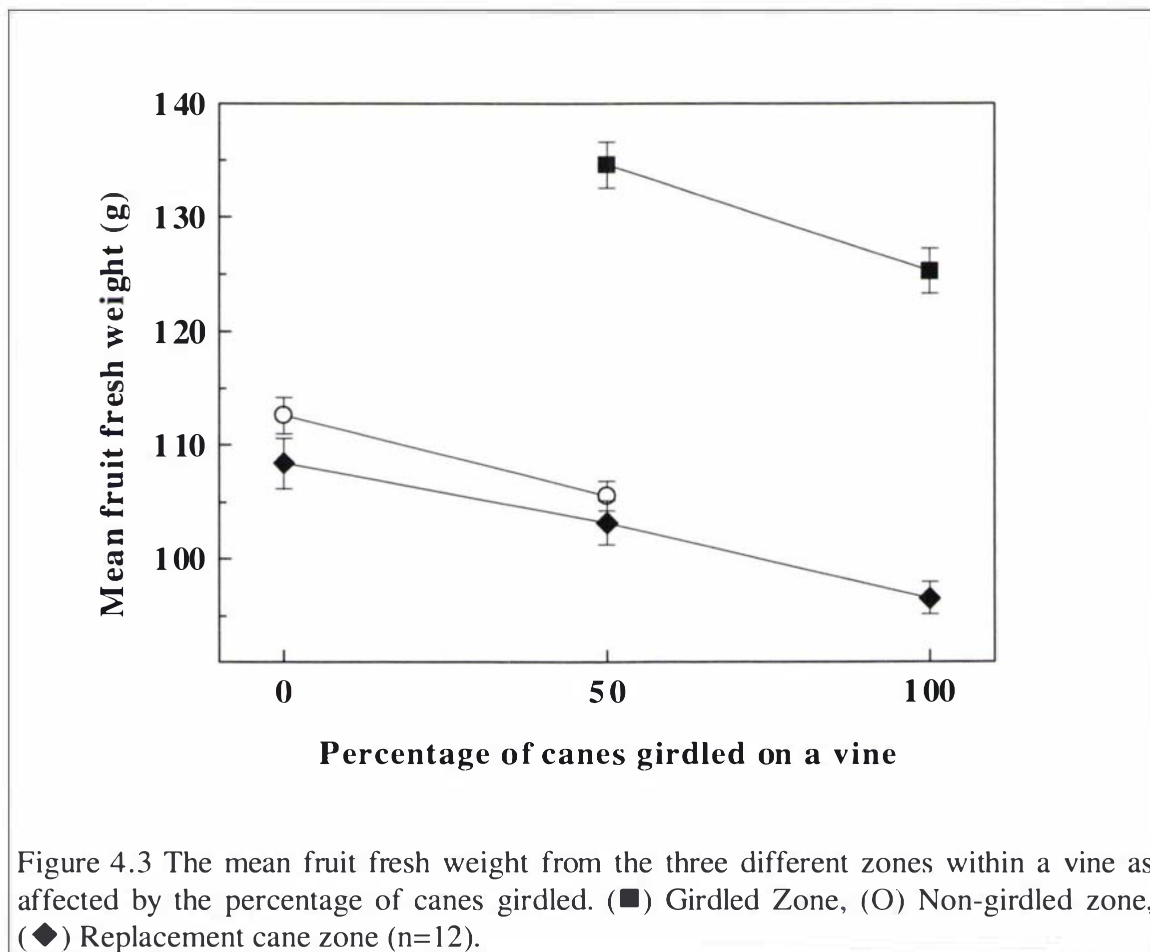


Figure 4.3 The mean fruit fresh weight from the three different zones within a vine as affected by the percentage of canes girdled. (■) Girdled Zone, (O) Non-girdled zone, (◆) Replacement cane zone (n=12).

### 4.3.4 The leaf:fruit ratio within canes and the replacement canes zone

The leaf:fruit ratio on girdled and non-girdled canes exceeded four on 81% of all canes sampled, and was not below two on any of these canes. The mean leaf:fruit ratio was 7.3, however the distribution was skewed and a few canes had a very high leaf:fruit ratio due to low numbers of fruit on those particular canes (Figure 4.4). In order to establish the relationship between leaf:fruit ratio and fruit weight on girdled canes, individual linear regression lines were fitted for each vine from which canes were sampled. The slope of fitted lines was significantly different from zero (P=0.05) on only one vine out of twelve. The average slope of fitted lines from each treatment was not significantly different from zero (Table 4.3), and visual appraisal of scatter plots did not reveal any evidence of either a linear or non-linear trend in the relationship (Figure 4.5).

In the RCZ, the leaf:fruit ratio was high in all vines which were sampled. The average RCZ leaf:fruit ratio in these vines was 11.4 leaves per fruit, and the lowest leaf:fruit ratio measured on any vine was 9.3. There were no significant ANOVA model or treatment effects for RCZ leaf:fruit ratio (Table 4.4).

Table 4.3 Summary of the relationships between leaf:fruit ratio and fruit weight on girdled and non-girdled canes. The slope was calculated as the mean of individual slopes of linear regression lines from four replicate vines. The mean slope was not significantly different to zero (P=0.05) for any of the treatments.

| Percentage of canes girdled | Slope of the relationship between leaf:fruit ratio and fruit weight ($\frac{g}{fruit}.(\frac{leaves}{fruit})^{-1}$) | |
|---|---|---|
| | girdled | non-girdled |
| 0 | - | 0.22 (0.64) |
| 50 | 1.06 (0.95) | 0.89 (0.49) |
| 100 | 0.61 (0.24) | - |

Standard error of mean in parentheses.

Table 4.4 Leaf measurements in the replacement cane zone of vines with different percentages of fruiting canes girdled. Measurements were taken at the time of fruit maturity. Values are means of four replicate vines.

| Percentage of canes girdled | Total leaf number | Estimated total leaf area ($cm^2$) | Mean leaf:fruit ratio |
|---|---|---|---|
| 0 | 759 (194) | 113 888 (29072) | 10.7 (0.9) |
| 50 | 825 (186) | 123 675 (27828) | 12.9 (1.0) |
| 100 | 869 (51) | 130 313 (7619) | 10.7 (0.6) |

Standard error of mean in parentheses.

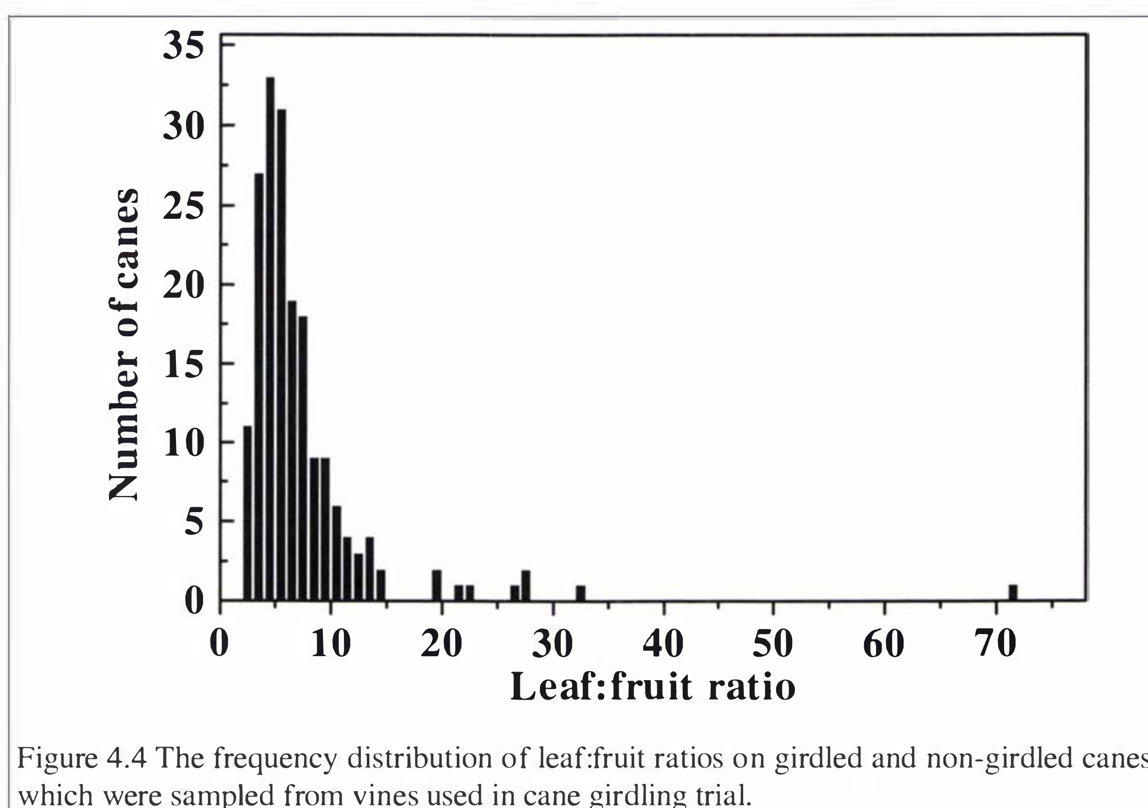


Figure 4.4 The frequency distribution of leaf:fruit ratios on girdled and non-girdled canes which were sampled from vines used in cane girdling trial.

### 4.3.5 The return bloom of vines which were cane girdled in the previous year

Over all the vines measured, there was an average of 18.6 king flowers and 4.9 compound flower clusters per metre of cane. Although there were no significant model effects for either parameter of return bloom, there was some indication (unprotected LSD) of a negative effect of cane girdling on the number of compound flowers. The

number of compound flower clusters per metre of cane was significantly lower on 100G vines than on control (0G) vines (Table 4.5). This result must be viewed with caution due to the lack of significant model effects, and the low proportion of variation which could be accounted by model parameters (ANOVA model $R^2 = 0.096$).

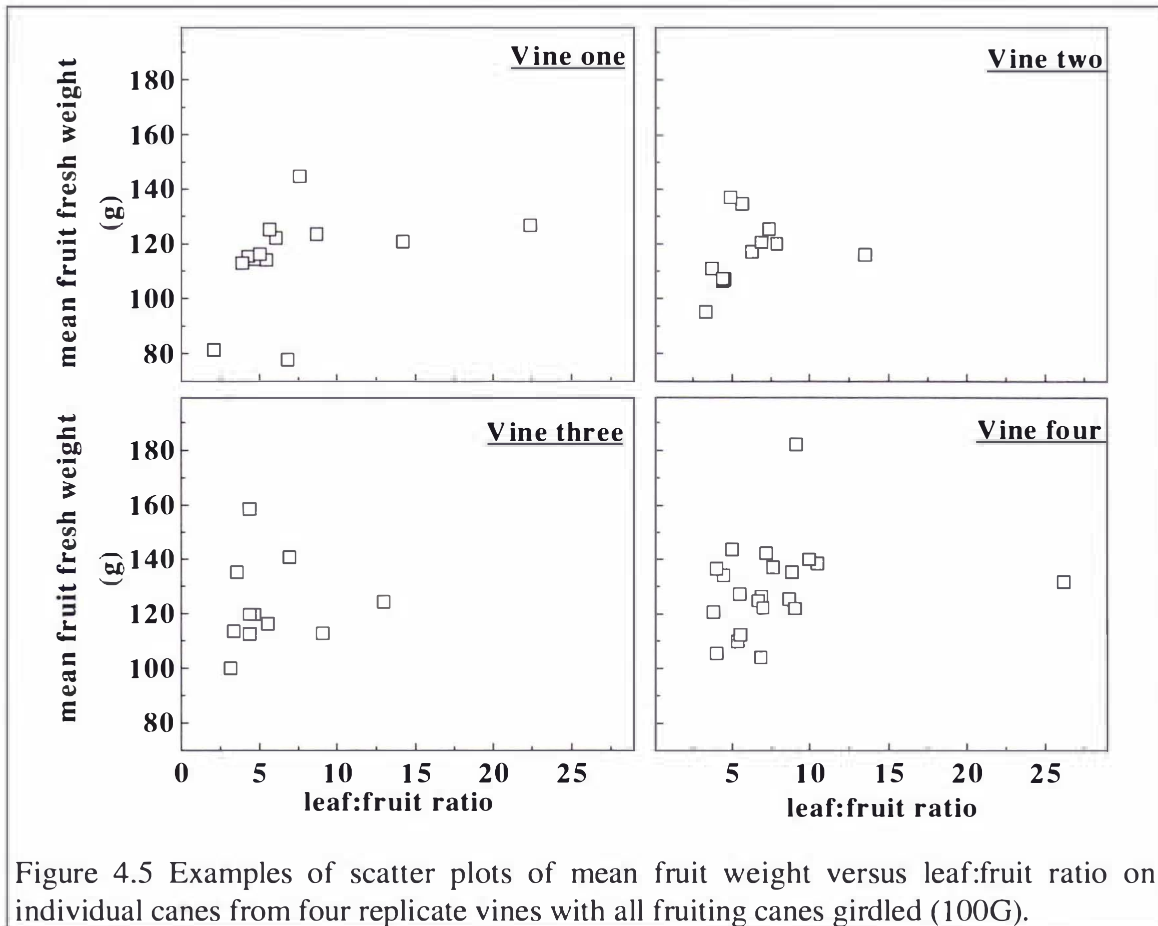


Figure 4.5 Examples of scatter plots of mean fruit weight versus leaf:fruit ratio on individual canes from four replicate vines with all fruiting canes girdled (100G).

Table 4.5 The effect of cane girdling in the previous season on the return bloom of king flowers or compound flower clusters (n=12).

| Percentage of canes girdled | King flowers[1] (flowers.m$^{-1}$ cane) | Compound flower clusters (clusters.m$^{-1}$ cane) |
|---|---|---|
| 0 | 18.6 a | 5.6 a |
| 50 | 19.6 a | 5.1 ab |
| 100 | 17.5 a | 3.9 b |

Means in a column with common letters are not significantly different at P=0.05 (unprotected LSD).
[1] The number of king flowers includes those present in compound flower clusters.

### 4.3.6 Storage of fruit from girdled and non-girdled canes

Fruit from both the GZ and NGZ were found to have high firmness at the time of harvest, although fruit from GZ (8.97 KgF) were significantly ($p<0.01$) firmer than fruit from NGZ (8.02 KgF) at this time. However within one week of storage at 0°C, the difference in firmness between these two treatments had disappeared. The firmness of fruit from both treatments dropped rapidly during the initial period of storage, but levelled off at a firmness close to 1 KgF after 72 days (Figure 4.6). Average fruit firmness dropped below 1 KgF at 103 days in fruit from NGZ, whereas in fruit from GZ this was not observed until the following date (128 days) (Figure 4.6).

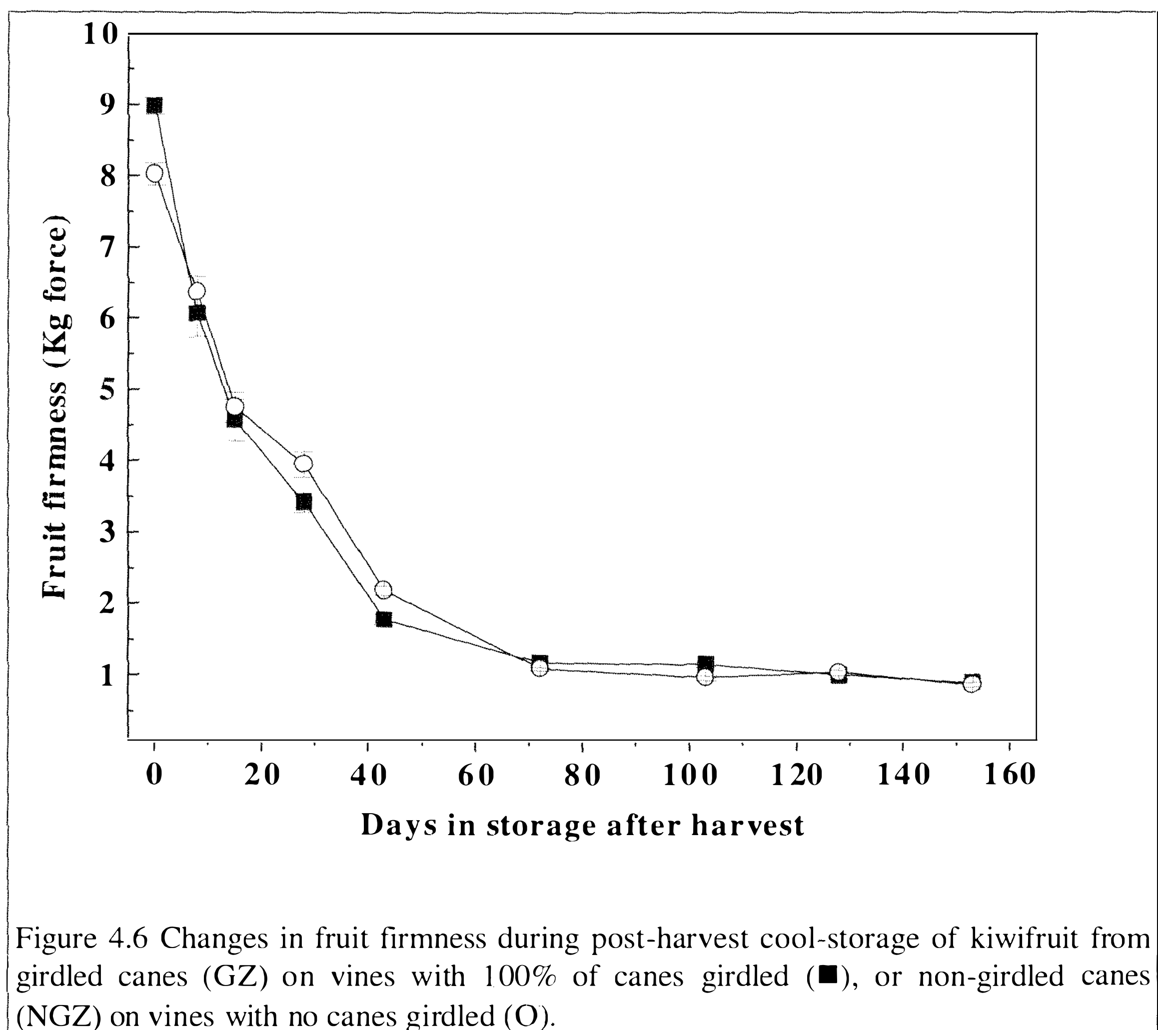


Figure 4.6 Changes in fruit firmness during post-harvest cool-storage of kiwifruit from girdled canes (GZ) on vines with 100% of canes girdled (■), or non-girdled canes (NGZ) on vines with no canes girdled (O).

## 4.4 Discussion

### 4.4.1 The effect of cane girdling on kiwifruit size

Girdling of kiwifruit canes has been reported to result in increased fruit weight of up to 10g per fruit compared to non-girdled canes (Patterson and Mason, 1988). Other previous reports of kiwifruit girdling have dealt with girdling of the trunk (Davison, 1980), or individual lateral shoots (Snelgar et al., 1986; Snelgar and Manson, 1992; Lai et al., 1989a; Seager and Hewett, 1995). Fruit on canes which had been girdled grew to a higher weight than fruit on non-girdled canes or in the replacement cane zone of the same vine. The increase in fruit weight on a cane due to girdling was between 24g and 31g, and was not affected by the position on the cane or timing of application (Table 4.1, Figure 4.3).

Although there was a significant increase in fruit size on girdled canes, the increase was up to 31g which is lower than occurs on girdled lateral shoots at a similar leaf:fruit ratio (4:1). For example, an increase in fruit weight of 43g was obtained from girdling lateral shoots at FB with a 4:1 leaf:fruit ratio in an experiment conducted in the same orchard and in the same year as the cane girdling was carried out (chapter three, season two). Fruit on girdled canes may have been at their maximum growth potential, and unable to take further advantage of the high level of carbohydrate available. This hypothesis is supported by the lack of any relationship between leaf:fruit ratio and fruit weight on girdled canes (Figure 4.5). This would suggest that the photo-assimilate supply was not a limitation to fruit growth at the leaf:fruit ratios in this experiment. The higher mean fruit weights found on girdled lateral shoots at leaf:fruit ratios of four and over have usually resulted from uniformly selected well exposed lateral shoots, with well pollinated fruit (Woolley et al., 1992; Chapter three), whereas girdled canes were not as uniform.

Although girdling eliminates competition from most other sinks on the vine, there are still alternative sinks present on girdled canes. It is possible that the growth of fruit on girdled canes may have been limited by competition with alternative sinks such as vegetative growth and stem storage. Re-growth from buds present on lateral shoots did occur on

both girdled and non-girdled canes during the experiment, and although this was removed during normal summer pruning operations every six to eight weeks, expanding leaves and active shoot re-growth apices could conceivably compete with fruit growth prior to their removal. Although stem storage of carbohydrate is a weak sink compared with fruit (Lai, 1987), when carbohydrate is not in limited supply for fruit growth, kiwifruit stems have been found to accumulate significant amounts of $^{14}C$ fixed by leaves (Lai et al., 1989b). It has been demonstrated that kiwifruit are strong sinks for a threshold level of carbohydrate, but they are relatively weak sinks for the carbohydrate required for additional fruit size increment (chapter six). Thus the presence of sinks with low competitive ability could still have limited additional kiwifruit growth on girdled canes.

An alternative hypothesis is that vegetative growth could provide an inhibitory signal to fruit on a girdled cane, limiting the ability of fruit to obtain additional carbohydrate. Lai et al. (1989c) found that kiwifruit growth was reduced when there were 8 or more fully expanded leaves distal to the fruit on a lateral shoot, although the effect was most prominent in poorly pollinated fruit. It is possible that a similar leaf inhibitory effect was operating in girdled canes at a high leaf:fruit ratio. This could reduce the potential sink size of a fruit over that able to be attained in a girdled lateral shoot, either by inhibition of cell division after anthesis or reducing metabolism of incoming sugars, therefore making it unable to take full advantage of the available carbohydrate provided by a girdle+high leaf:fruit ratio.

### 4.4.2 Return bloom of kiwifruit vines girdled in the previous season

Although the number of king flowers was not significantly reduced by girdling of 100% of fruiting canes on a kiwifruit vine, there were indications that numbers of lateral flowers may have been reduced by cane girdling. In terms of commercial application, this would be considered a positive feature, as lateral flowers produce small fruit and are usually removed in a manual flower thinning operation prior to bloom. However in terms of vine physiology, such an effect could indicate that cane girdling had a deleterious effect on the vines. All kiwifruit inflorescences are initially composed of both king and

lateral flowers, although a high percentage of lateral flowers abort prior to flowering (Brundell, 1975; Polito and Grant, 1984). Return bloom of lateral flowers appears to be more sensitive to competition than king flowers. For example, thinning 50% of kiwifruit flowers in one season resulted in an increase in total flower numbers of 34% in the following season, due to a 200% increase in numbers of nodes with lateral flowers but only an 18% increase in flowering nodes per flowering shoot (Burge et al., 1987). Abortion of lateral flowers in 100G vines may be due to the dominance of king flowers over lateral flowers as has been found to occur in apple (Gruber and Bangerth, 1990). Lateral flower abortion rather than flower bud evocation was affected by girdling treatments, therefore the affect of girdling is most likely be due to intensification of competition by king and lateral flowers for the stored reserves.

### 4.4.3 Storage potential of fruit from girdled canes

Kiwifruit grown at the MUFCU have shown a tendency to store well compared with fruit grown in other regions of New Zealand, and at 73 days after harvest fruit firmness would be expected to level off at between 2-4 KgF (I. Davie, personal communication, 1996). However at this stage, fruit in this experiment were close to 1 KgF, which suggests that fruit were softening more rapidly than would be expected. Although fruit from girdled canes were significantly firmer than from non-girdled canes at harvest, this is of minor relevance considering the advanced speed of softening which occurred, and the lack of difference in firmness later in post-harvest life. There was very little difference between the curves of fruit softening over time for girdled and non-girdled fruit. Although fruit from girdled canes were slower to reach a firmness of 1 KgF, this is of doubtful relevance, as some fruit from both treatments had reached 1 KgF within 10 weeks of harvest. Additionally some fruit from both treatments were below current minimum firmness requirements between June and August. This was probably due to accelerated softening of these fruit.

The most likely explanation for the accelerated softening of fruit in this experiment is that ethylene concentrations were elevated at some point during cool-storage. There is an indication that this may have occurred between 28 and 43 days after harvest as fruit firmness appeared to be levelling off between 15-28 days, whereas between 28-43 days

the rate of softening had increased again (Figure 4.6). However this speculation is based on deviation of a single data point (28 days) so cannot be viewed with certainty. As the cool-store was used by other researchers, in particular some dealing with *Botrytis cinerea* infection of kiwifruit, it is possible that fruit were inadvertently exposed to elevated ethylene levels by the presence of decomposing fruit. Alternatively, problems have been experienced with the ethylene scrubber used in the particular cool-store (R. Johnstone, personal communication 1993) and there may have been periods during cool-storage in which the scrubber was not functioning properly. In conclusion it appears that girdling had no significant effect on fruit storage although results are somewhat inconclusive due to the speed at which fruit ripened.

### 4.4.4 The effect of girdling different percentages of canes on vine performance

On a whole vine basis, girdling of either 50 or 100% of the canes on a vine resulted in an increase in the mean fruit weight, and a change in fruit size distribution which resulted in potentially higher returns. However the increase in mean fruit weight was only between 5 and 6g, which represented an increase in fruit weight of less than 6%. The small difference in mean fruit weight between treatments appears to be due to two factors. Firstly, fruit size was decreased in the non-girdled parts of the vine as a result of cane girdling. This occurred in both the RCZ and NGZ. Secondly, the response of fruit on girdled canes decreased as the percentage of girdled canes on the vine increased. These results were surprising as we had not expected that increased percent girdling would affect fruit size within girdled canes, as they are autonomous for carbohydrate. Although girdling is widely regarded to increase fruit size on girdled branches, reports of the effect of girdling on fruit size often compare fruit on girdled and non-girdled branches from the same tree, without comparison with fruit from completely non-girdled trees. For example girdling of 'Marsh seedless' grapefruit branches resulted in increases in fruit size of up to 33.7% on girdled branches compared with fruit from non-girdled branches, however no mention of non-girdled branches from control trees was made (Cohen, 1984). Fruit from limb girdled 'Sturdeespur Delicious' apple trees were found to be heavier and have higher dry weight at similar crop loads than fruit from non-girdled limbs, however only non-girdled limbs on the same tree were used as a control (Schechter et al., 1994). In the

report of cane girdling of kiwifruit made by Patterson and Mason (1988), there was no mention of whether non-girdled canes were from the same vine or from control vines.

It seems unlikely that decreased fruit growth in non-girdled parts of the vine was due to increased competition from the root system or storage in leaders and trunk for the following reasons. Competition for a limited supply of carbohydrate usually favours proximal competing sinks, particularly if they are strong sinks (Wardlaw, 1990). Fruit in non-girdled parts of vines are strong sinks for carbohydrate compared with roots and stem storage (Buwalda and Smith, 1990a) and were in close proximity to source leaves, whereas the root system and trunk storage sinks are relatively distant. In experiments described by (Buwalda and Smith, 1990b), complete defoliation of fruiting canes from kiwifruit vines did not affect weight of fruit in the RCZ despite the major decrease in the vine source-sink ratio. As the leaf:fruit ratio averaged over seven in non-girdled fruiting canes and 11 in the RCZ, it is unlikely that distant sinks could reduce fruit growth by a limitation to carbohydrate availability.

An alternative hypothesis is that the reduced supply of carbohydrate available to the root system caused the inhibition of root processes such as absorbtion of water and minerals. In particular, water deficits can reduce fruit expansion (Judd et al., 1989) and leaf photosynthesis (Chartzoulakis et al., 1993). This could explain both the reduction in fruit size found in non-girdled as well as girdled parts of the vines when an increased percentage of canes were girdled. Girdling of other crops has often been found to reduce carbohydrate levels in roots. For example girdling of one year old sour orange seedlings was found to reduce starch levels in roots, and within one month leaf nitrogen and phosphorus levels in girdled seedlings were lower than in control seedlings (Wallerstein et al., 1978). The application of a stem girdle to *Betula pendula* or *Tilia cordata* severely inhibited root and shoot growth in both species, although root carbohydrate was only reduced in *B. Pendula* (Abod et al., 1991). The root system of kiwifruit is generally thought to be a poor competitor for carbohydrate (Buwalda and Smith, 1990a), although defoliation of up to 75% of leaves in the RCZ had no effect on root starch levels (Cruz-Castillo, 1994). However, kiwifruit root growth appears to be quite sensitive to vine source-sink ratio. For example kiwifruit vines with an overall leaf:fruit ratio of two had

only half the root growth (white root length density) of vines with a ratio of five or ten leaves per fruit (Buwalda, 1991) and partial defoliation of kiwifruit vines was found to reduce root growth to a greater extent than fruit growth (Buwalda and Smith, 1990b). This indicates that cane girdling could potentially have a negative effect on root growth in kiwifruit, particularly if leaf:fruit ratio in the RCZ was low. However nutrient and water uptake by kiwifruit roots is not normally limited by root length (Buwalda and Smith, 1990a). As vines used in this study were well irrigated and supplied with fertiliser, even if root growth were reduced by a reduction in carbohydrate supply to the root system, this may not have affected fruit growth. In addition it is uncertain whether or not girdling would have resulted in carbohydrate deficit in roots. The RCZ of the girdled vines used in our study averaged over 11 leaves per fruit, and most of these leaves would have been in very well exposed positions, and therefore higher than average net exporters of carbohydrate. As approximately two leaves are required to fulfil the growth requirements of an export sized fruit (Lai et al., 1989a), the remaining photosynthetic output of at least nine leaves per fruit was available to supply the root system with carbohydrate.

It has been suggested that some of the effects of girdling can be attributed to altered levels of growth regulators in the girdled plant (Wallerstein et al., 1973; Dann et al., 1984). Dann et al. (1984) proposed that the interruption by girdling of a signal, probably auxin, transmitted basipetally in phloem associated tissue, leads to a reduction in a co-ordinating signal from the root system, which consequently leads to reduced vegetative growth of the shoot system. Auxins are synthesised in young leaves, fruit and shoot tips, and are transported in the plant basipetally. Transport occurs in the living tissue associated with the phloem (Goldsmith et al., 1974; Morris and Thomas, 1978), and is thus effectively isolated from nearby xylem elements. The application of a girdle should effectively eliminate auxin transport out of girdled canes. In support of this hypothesis, Dann et al. (1985) found a transient increase in IAA concentration in bark immediately above a girdle, along with a permanent decrease in IAA concentration immediately below the girdle, and concluded that the basipetal transport of IAA past the girdle had been eliminated. Girdling of a large proportion of the canes on a kiwifruit vine could substantially reduce the amount of auxin received by the roots. Cutting and Lyne (1993) have reported convincing evidence in support of a co-ordinating signal from the root

system in response to a girdle, finding a reduction in levels of cytokinins and gibberellins in xylem sap of girdled peach branches. Although it is not certain if xylem sap is a source of plant hormones for fruit growth (see chapter eight), it is possible that reduced levels in xylem sap could reduce the total amount of hormones in fruit, and therefore limit sink strength. Alternatively reducing auxin translocation to roots by girdling can reduce root initiation (Stoltz and Hess, 1966), therefore reduced auxin transport from girdled canes could lead to a direct effect on the root growth, and may have caused a reduction in root growth or function and thus affected water and mineral ion transport to shoots.

## 4.5 Summary

Girdling of individual fruiting canes resulted in an increased average weight of the fruit from girdled canes, which was expected from results obtained from previous work on the girdling of lateral shoots. Girdling 50 or 100% of canes on a vine would have resulted in increased revenue compared with non-girdled vines due to a shift of fruit size into size grades with higher returns. However when the percentage of canes girdled on a vine was increased from 0 to 50 and to 100% there were specific effects on fruit weight, which reduced the benefit of girdling on average fruit weight:

1. The response of fruit weight to girdling was diminished
2. The weight of fruit from non-girdled parts of the vine was reduced.

The effects of cane girdling on return bloom were minimal or non-existent, and carbohydrate shortage did not provide a suitable explanation for reduced fruit growth. Two alternative hypotheses are proposed here to explain the negative responses to girdling:

1. *A reduced amount of carbohydrate was availabile to the root system in cane girdled vines which reduced root function, growth, and/or absorption and transport of water*

*and mineral nutrients to the shoots, which reduced fruit growth either directly, or indirectly by an effect on leaf photosynthesis.*

2. *Interruption of the auxin signal out of canes by girdling reduced auxin signals from shoots to roots, which either reduced root function as described above or reduced growth hormone signals from roots to shoots.*

## 4.6 References


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# 5 Cane Girdling of Kiwifruit II: Characterisation of Negative Effects on Vine Performance

## 5.1 Introduction

The potential for cane girdling to increase kiwifruit size has been demonstrated in chapter four. Girdling of either half or all of the tied down fruiting canes on a kiwifruit vine was found to increase the average fruit size by approximately 5%. This would have resulted in a significant increase in returns of up to 32%, largely due to increased numbers of 27, 25 and jumbo size grade fruit, which attract a premium price.

However a deleterious effect of cane girdling on the weight of fruit from non-girdled parts of the vine was detected. In addition, when a higher proportion of canes were girdled on a vine, the stimulatory effect within the girdled canes themselves was diminished. These two factors resulted in a lower than expected overall response to cane girdling. A possible negative effect on the return bloom of lateral flowers was also found to result from cane girdling.

The aim of the experimental work described in this chapter was to confirm the response of kiwifruit vines to cane girdling and to investigate further the negative effects on fruit weight which occurred in both the non-girdled and girdled parts of the vine. The source-sink balance on cane girdled vines was altered further using thinning and defoliation treatments and recording the responses of various aspects of vine physiology. In particular the response of the root system was investigated, as a hypothesis was raised in chapter four suggesting that girdling may have reduced carbohydrates or growth regulators transported to the roots, resulting in impaired or altered root function.

As the primary functions of roots are to absorb water and mineral nutrients and transport these to shoots, impairment of root function could affect shoot water and nutrient status. These can influence kiwifruit growth indirectly through effects on the photosynthetic rate (Chartzoulakis et al., 1993) or directly by limiting fruit expansion (Judd et al., 1989). The response of water relations and photosynthetic rate to cane girdling were therefore

described in this chapter. Root length density was also measured to check for effects of girdling on root growth.

In addition to absorbing nutrients and water, roots are a biosynthetic source of cytokinins (Letham, 1994). The concentration of cytokinins, as well as gibberellins in xylem exudate were found to be lower in girdled peach branches (Cutting and Lyne, 1993). A girdle-induced change in growth regulator transport from roots could influence fruit growth directly, or indirectly by effects on vegetative growth. A glasshouse experiment on potted kiwifruit plants was carried out with the aim of altering shoot auxin transport by decapitation, girdling, synthetic auxin application and auxin transport inhibitors and correlating this to concentrations of cytokinins in xylem sap. Cytokinin concentrations in xylem sap from cane girdled vines were also monitored and correlated to growth rates of fruit and vegetative growth.

## 5.2 Materials and Methods

### 5.2.1 The interaction between cane girdling, partial defoliation and thinning

The aim of this experiment was to vary the source-sink balance in the replacement cane zone (RCZ) of vines which had been cane girdled in order to accentuate or to negate any resulting carbohydrate limitation. As girdling effectively eliminates carbohydrate export from girdled canes to anywhere else in the vine, defoliation and fruit thinning in the RCZ directly affects source-sink relationships between RCZ leaves and the root system.

Experiment one

To determine interactions between cane girdling, defoliation and thinning a three way factorial treatment structure was used with four levels of cane girdling, two levels of defoliation and two levels of fruit thinning. Cane girdling treatments were 0% (OG), 20% (20G), 50% (50G) and 100% (100G) of canes girdled. Defoliation treatments were no manipulation (control) or removal of every second leaf on lateral shoots within the RCZ (50% defoliation). Fruit thinning treatments were no thinning (control) or removal of all fruit in the RCZ (thinned). Each treatment combination was replicated on four vines.

Girdle treatments were applied at full bloom (FB). Defoliation and fruit thinning treatments were applied one week after full bloom (WAFB). Lateral and misformed fruits were removed and vines were managed normally with re-growth removed every six to eight weeks. Fruit were harvested at commercial maturity, total fruit numbers counted and fresh weights of every third fruit recorded. Return bloom was measured in the following spring as described in chapter four. Numbers of flowering or vegetative lateral shoots were also recorded.

Experiment two

Defoliation treatments were repeated in 1994, with the aim of increasing the level of defoliation of the RCZ and increasing replication. In this experiment defoliation was

focused only on vines with 100% of canes girdled as this girdling treatment effectively isolates the RCZ as the sole region of the vine supplying the root system, storage and return bloom requirements with carbohydrate, thus it was considered that potential effects would be accentuated.

A randomised complete block design was used with five girdling/defoliation treatments replicated on nine vines. Treatments were as follows:

1. 0G — 0% canes girdled, no defoliation.
2. 50G — 50% canes girdled, no defoliation.
3. 100G — 100% canes girdled with no defoliation (Plate 5.1).
4. 100G+50D — 100% canes girdled plus every second leaf removed from all laterals in the RCZ (Plate 5.2).
5. 100G+75D — 100% canes girdled plus three out of four leaves removed from all laterals in the RCZ (Plate 5.3).

Girdles were applied at full bloom, defoliation was carried out at one week after full bloom and vines were managed as in experiment one. Fruit weights and return bloom was measured as in experiment one.

### 5.2.2 The effect of cane girdling on gas exchange and water relations

Leaf gas exchange parameters and leaf xylem water potential ($\Psi_{xylem}$) were measured on leaves arising on lateral shoots from girdled and non-girdled canes from vines used in experiment one (5.2.1). Measurements were made between 11am and 2pm on five dates from January to March. Two fully expanded leaves exposed to sunlight and not subtending a fruit were selected from each type of cane on each vine. Measurements of gas exchange were made using a Li-Cor 6200 portable photosynthesis system (Lincoln, NE, USA) downloaded directly to a computer. The Li-Cor 6200 system is a closed gas exchange system fundamentally composed of a leaf chamber in which a known area of

leaf is sealed and an infra-red gas analyser (IRGA) determines the $CO_2$ concentration in the system. A pump drives air from the chamber to the IRGA for measurement of $CO_2$ and then diverts a proportion into a desiccant before the air passes back into the chamber (Figure 5.1). Photosynthesis of the leaf area enclosed within the chamber causes a decrease in $CO_2$ concentration in the system over time and along with values for system volume, relative humidity and temperature, the rate of photosynthesis per unit leaf area is calculated. Estimates of stomatal resistance and leaf internal $CO_2$ concentration are also calculated automatically and the level of photosynthetically active radiation at the time of photosynthesis measurement are recorded by a quantum sensor attached to the chamber, parallel to the leaf. Boundary layer resistance was calculated from measurement of the evaporation rate from saturated filter paper. Prior to use, the IRGA was always calibrated using a zero $CO_2$ standard (air passed through soda-lime) and an external gas bottle with known $CO_2$ concentration (N.Z. Industrial Gases, Palmerston North). During measurement, relative humidity was maintained at a near constant level by diverting an appropriate proportion of the flow through the desiccant.

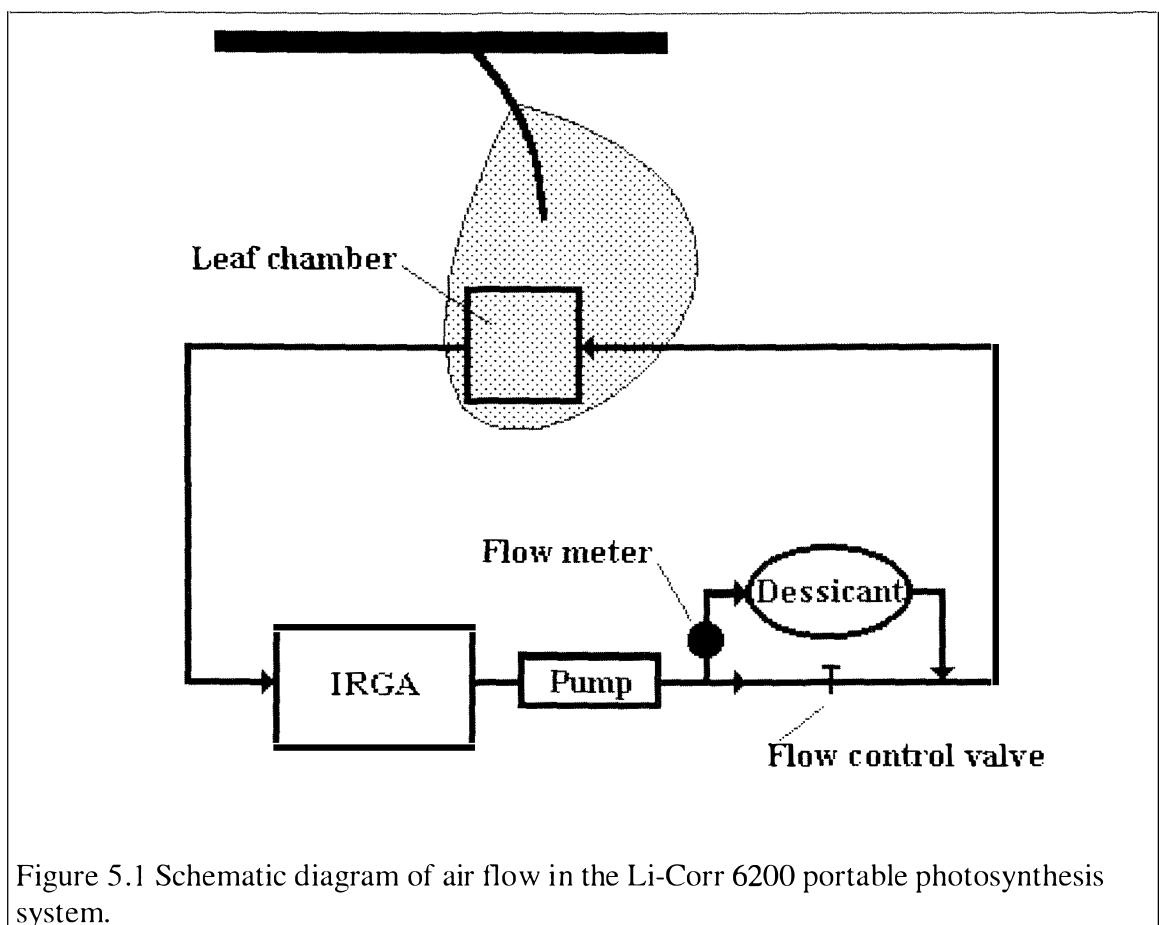


Figure 5.1 Schematic diagram of air flow in the Li-Corr 6200 portable photosynthesis system.

Plate 5.1 An intact kiwifruit canopy (non-defoliated) from below. Fruiting canes can be seen branching from the main leader (centre).

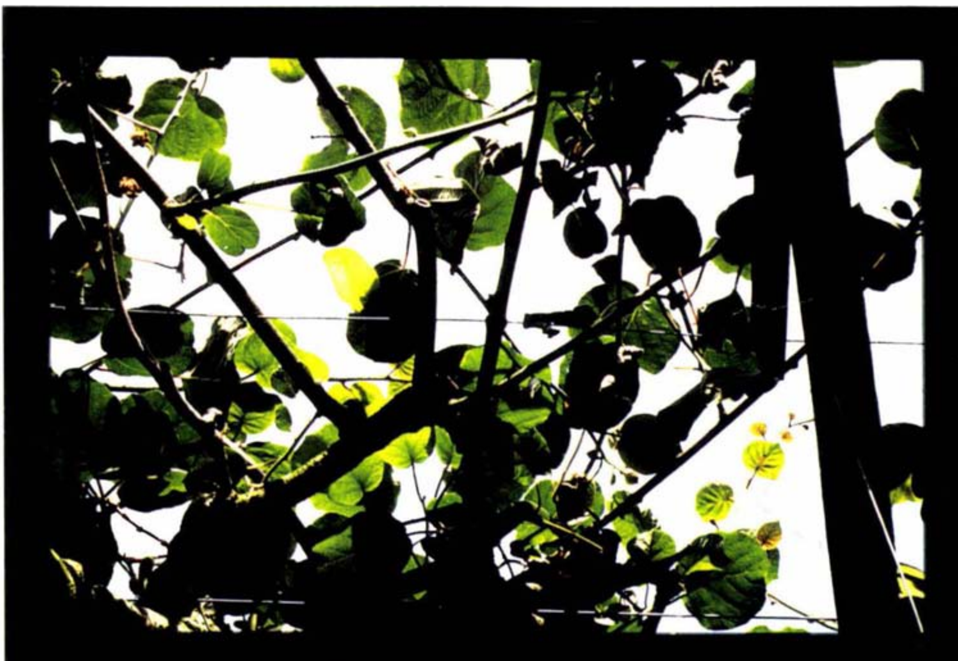

Plate 5.2 A kiwifruit canopy which has had every second leaf removed (50% defoliation) in experiment two (5.2.1).

Plate 5.3 A kiwifruit canopy which has had three out of every four leaves removed (75% defoliation) in experiment two (5.2.1).

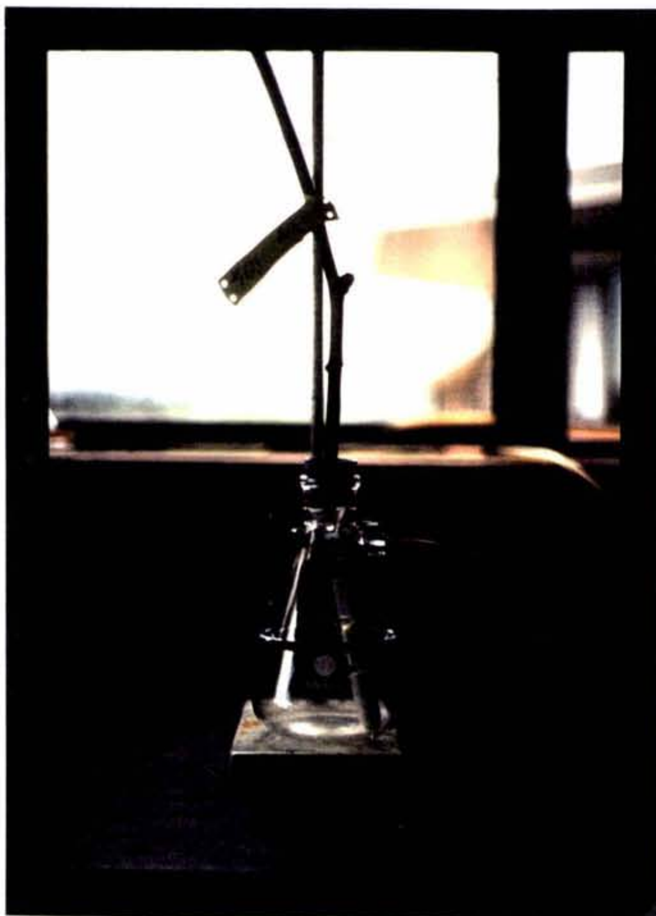

Plate 5.4 Extraction of xylem exudate from a kiwifruit cane for the measurement of abscisic acid and cytokinins. A drop of sap can be seen exuding from the base of the cane to be collected into the plastic tube below.

Leaf xylem water potential was measured using the pressure bomb method (Turner, 1981). Leaves were excised with a sharp scalpel blade and the pedicel immediately sealed into a pressure chamber (Soil Moisture Equipment Corporation, Santa Barbara, CA, USA), with the cut end of the pedicel protruding through a teflon O-ring out of the chamber. A moist cloth was maintained in the bottom of the chamber to reduce transpirational loss of leaf water during measurement. Pressure in the chamber was gradually increased by introducing pressurised $N_2$ gas until a drop of liquid was exuded onto the cut end. The gas pressure in the chamber at this point is assumed to be equal and opposite to leaf xylem water potential when it was cut from the plant.

### 5.2.3 The effect of cane girdling on root growth

The amount of root growth was estimated on selected treatment combinations from vines used in experiment one (5.2.1) using methods similar to those described by Buwalda and Hutton (1988). Although the peak period of root growth in kiwifruit has been reported to occur in March (Buwalda and Hutton, 1988), soil underneath vines was found to be too hard for penetration of a manual corer in March and coring was delayed until sufficient irrigation was applied to soften the soil in early April. Cores were taken by hand using a 25mm internal diameter soil core sampler, to a depth of 60cm. On each vine, six root cores were taken at a one metre radius from the trunk of the vine. Soil was washed off roots in a 1mm screen sieve with a jet of water. Following the assumptions made by Buwalda and Hutton (1988), white roots were assumed to be new root growth formed within the previous month. White roots were separated from the total root mass and total length of the white roots was estimated using the modified line intersect method of Tennant (1975). The total length of fibrous brown roots was estimated using a Cormair automatic root-length scanner (Commonwealth Aircraft Corporation Ltd., Melbourne, Australia). For analysis, all root lengths were converted to $Km.m^{-2}$ soil surface and white root lengths were $log_{10}$ transformed to stabilise variance.

### 5.2.4 The effect of cane girdling on fruit mineral content

Mineral deficiencies in kiwifruit vines have been demonstrated to lead to reduction in yield. In general, deficiencies of nutrients have the effect of reducing fruit numbers by affecting flowering, although reductions in average fruit size have been found in some cases (Smith et al., 1987; Buwalda and Smith, 1990a; Tagliavini et al., 1995). Deficiencies in minerals such as potassium and magnesium have a severe effect on fruit number (Smith et al., 1987; Clark and Smith, 1987) and could be expected to increase the size of the fruit due to the decrease in crop load. However this is not the case and suggests that mineral deficiency also affects leaf function and/or fruit sink strength. The aim this experiment was to establish whether any fruit mineral deficiencies indicative of impairment of root function were induced by cane girdling treatments.

Two fruit were randomly selected from the RCZ, girdled canes and non-girdled canes of three vines per treatment (0G, 50G and 100G) from experiment two (5.2.1). A 10mm slice was taken from the centre of the fruit, dried and ground to pass through a 1mm screen. Samples were analysed for N, P, K, Ca and Mg as described in chapter two.

### 5.2.5 The growth rate of fruit and vegetative growth

On commercial kiwifruit orchards, pruning of vegetative growth takes place from late spring until late summer. However the peak period of growth occurs during the mid-summer period of December and January (Sale, 1981). This is a critical time for vine management, as uncontrolled growth can lead to significant problems with light penetration, access and canopy replacement pruning later in the year. Any effect of cane girdling on vegetative growth could be important horticulturally as well as providing physiological information. Likewise, fruit growth rate could provide information on key times when cane girdling is influencing fruit growth. Therefore growth rates of fruit and shoots arising from lateral buds was investigated on 0G, 50G and 100G treatments on vines used in experiment two.

For measurement of shoot growth rate, 10 lateral shoots from girdled canes and 10 from non-girdled canes with at least six fully expanded leaves and two fruit were selected on three vines from experiment two (5.2.1) per treatment at full bloom. Shoots were pruned above the sixth leaf and fruit were thinned to two fruit per lateral shoot. The length of new shoots arising from buds in leaf axils on the pruned laterals was measured at weekly intervals for six weeks.

For non-destructive measurement of fruit weight, four fruit per vine were randomly selected at full bloom and fruit weight was estimated at regular intervals over the entire season using the relationship between fruit dimensions and fruit weight described in chapter two.

### 5.2.6 The levels of endogenous cytokinins in root xylem exudate, following decapitation of rooted kiwifruit cuttings

Hardwood cuttings were taken during winter. Uniform dormant canes from a single Hayward kiwifruit scion selection were cut to 200 mm lengths. The basal ends of the cuttings were dipped in 2500 ppm indole-butyric acid solution for five seconds and cuttings were allowed to root in well watered sand maintained at 22°C with an air temperature of 7°C for 12 weeks. At this time, 70% of cuttings had produced roots and the rooted cuttings were potted on into 80% peat, 20% pumice media containing fertiliser. Cuttings were hardened off under shade-cloth and then grown for four months over summer to allow a substantial root and shoot system to develop.

The most uniform cuttings were selected for use in a shoot decapitation experiment. Experimental design was a completely randomised design with five cuttings randomly allocated per treatment as follows:

1. Control
2. Lanolin: lanolin applied to cut surface.
3. NAA: 1-Napthaleneacetic acid, 1.5 mM in lanolin applied to cut surface.
4. Girdled: base of cutting girdled 48 hours prior to decapitation

5. NPA: naphthylpthalamic acid, 3.4 mM in lanolin applied to base of the cutting 48 hours prior to decapitation.

Lanolin was fully hydrated before use by warming lanolin, mixing water to saturation and draining off excess water when the lanolin had cooled to room temperature. NAA and NPA were suspended in a small volume of ethanol and mixed into warmed hydrated lanolin. Lanolin used in treatment two had the same volume of ethanol added.

Cuttings from all treatments were decapitated below all shoots at 8 a.m. on the same day and the cut ends allowed to exude xylem sap for two hours into plastic tubing. Lanolin and NAA treatments were applied at two hours after decapitation following the initial collection. Root xylem exudate from control cuttings was also collected at 2-4, 4-6, 6-8 and 14-16 hours after decapitation. Exudate was also collected for a two hour period from all treatments at 24, 48 and 72 hours after decapitation. Root xylem exudate was stored frozen at -70°C until analysed for cytokinins as described in chapter two.

### 5.2.7 The effect of cane girdling on levels of endogenous cytokinins and abscisic acid (ABA) in extracted xylem exudate

Vines from experiment two (5.2.1) were sampled for xylem exudate between full bloom and eight WAFB. One cane per vine (0G and 100G), or two from the 50G treatment (one girdled and one non-girdled) were removed from the vine half an hour before sunrise and taken to the laboratory for extraction of xylem sap.

Xylem exudate was extracted from canes using vacuum (Plate 5.4). The base of canes were re-cut and 3 cm of bark stripped from the base of the cane. The cane was pushed through a hole in a rubber bung and this was inserted tightly into a vacuum flask. Vacuum was applied and 3cm lengths pruned off the top of the cane as required to maintain sap flow (approx. 10 second intervals). Droplets of sap were collected in a funnel draining into a polypropylene test-tube inside the vacuum flask. Sap was immediately frozen at -70°C until required for analysis of cytokinins and ABA as described in chapter two. Data was found to require transformation with $\log_{10}$ prior to analysis in order to stabilise variance.

## 5.3 Results

### 5.3.1 Mean fruit weight over whole vines

In both cane girdling experiments one and two, there were no significant main effects of girdling and defoliation or significant interactions between girdling, defoliation and thinning (note thinning was only conducted in experiment one) on the overall mean fresh weight, yield, crop load or gross returns. In experiment one, mean fruit weight over all treatments was 105.3g while crop load averaged 34.3 fruit.$m^{-2}$ canopy. In experiment two, fruit weight averaged 116.3g with an average crop load of 28.1 fruit.$m^{-2}$ canopy. In experiment two, fruit from 100G vines where 75% of the RCZ leaves were defoliated were 4.2g smaller than fruit from non-defoliated 100G vines, but this was not statistically significant (contrast, P=0.3216).

There were significant differences between thinned and non-thinned vines in experiment one. Thinning of the RCZ resulted in significantly higher mean fruit weight over the entire vine, however crop load was reduced by approximately 50% and this resulted in significantly lower overall yield and lower gross returns (Table 5.1).

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 (NZ$) of kiwifruit vines in experiment one (n=24 vines).

| Treatment | Weight (g) | Crop load (fruit.$m^{-2}$. canopy) | Yield (Kg.$m^{-2}$. canopy) | Gross return[1] ($.$m^{-2}$. canopy) |
|---|---|---|---|---|
| Control | 101.4 b | 31.2 a | 3.15 a | $2.42 a |
| Thinned | 109.2 a | 15.7 b | 1.71 b | $1.49 b |

Means in a column with common letters are not significantly different at P=0.01 (LSD).
[1] Gross returns are based on NZKMB returns to growers after packing costs in 1994 season, but does not include orchard labour costs associated with thinning.

### 5.3.2 Fruit weights from different parts of vines

Analysis of the weight of fruits from the different parts of the vine in experiments one and two revealed similar trends to those described in chapter four. Girdling increased fruit size in the GZ, but as the percentage of girdled canes on a vine was increased, the response to girdling was diminished. Similarly in non-girdled parts of vines, increased cane girdling decreased the average fruit size. Even at the extremely low level of 20G, fruit weights were low compared with 0G and high compared with 50G and 100G treatments (Figure 5.2). Orthogonal contrasts of fruit weights from experiment one showed evidence of significant linear trends. Increased cane girdling decreased fruit size in the RCZ (linear contrast, P=0.009) and GZ (linear contrast, P=0.007), although this was not significant in the non-girdled canes (NGZ) (linear contrast, P=0.18). Trends in fruit weights from experiment two were less clear than in the previous two experiments and negative linear trends with increased cane girdling were only significant in the NGZ (linear contrast, P=0.0207) and possibly the RCZ (linear contrast, P=0.0998).

Thinning of fruit from the RCZ in experiment one was not found to significantly affect the mean weight of fruit within the NGZ or the GZ. As fruit were removed from the RCZ, no comparisons can be made for this zone.

In experiment one, there was no significant effect of 50% defoliation of the RCZ on fruit weight in any of the three zones. However in experiment two, defoliation of 75% of leaves in the RCZ of 100G vines significantly reduced mean fruit weight in the RCZ compared with non-defoliated vines (Table 5.2) and there was a significant negative linear trend of increasing percentage defoliation on fruit weight in the RCZ (linear contrast, P=0.025). In contrast, RCZ defoliation treatments had no significant effect on the weight of fruit in the GZ in experiment two (Table 5.2).

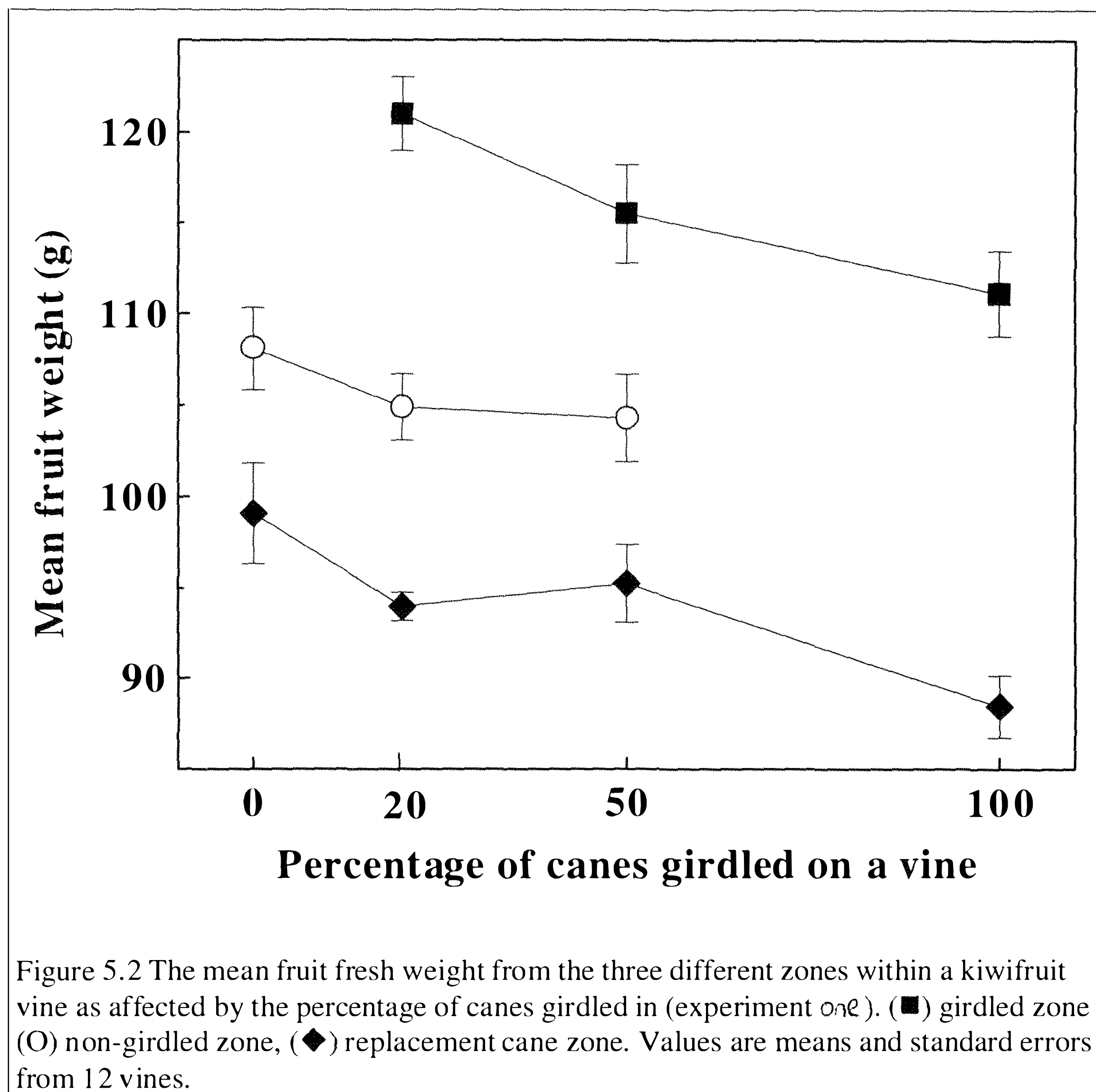


Figure 5.2 The mean fruit fresh weight from the three different zones within a kiwifruit vine as affected by the percentage of canes girdled in (experiment one). (■) girdled zone (O) non-girdled zone, (◆) replacement cane zone. Values are means and standard errors from 12 vines.

Table 5.2 The effect of defoliation of leaves from the replacement cane zone (RCZ) at full bloom on final weight of kiwifruit from girdled canes (GZ) and from the RCZ experiment two). Vines in this experiment had 100% of fruiting canes girdled (n=9 vines).

| Percentage of leaves defoliated | GZ | RCZ |
|---|---|---|
| 0 | 128.3 a | 106.4 a |
| 50 | 125. 9 a | 99.8 ab |
| 75 | 128.0 a | 95.0 b |

Means in a column with common letters are not significantly different at P=0.05 (LSD).

### 5.3.3 Return bloom

In experiment two, the return bloom of king flowers, compound flower clusters and bud-burst were not significantly affected by cane girdling. Return bloom averaged 19.2 king flowers and 3.1 compound flower clusters per metre of cane. On average there were 6.2 shoots per metre of cane of which 5.4 were floral.

In contrast to this and the results presented in chapter four, cane girdling in experiment one significantly affected return bloom of king flowers. There was approximately a 25% decrease in return bloom of king flowers from 100G vines compared with 0G vines, however the number of compound flower clusters was not significantly affected by cane girdling (Table 5.3). The decrease in return bloom of king flowers from vines with girdled canes appeared to be associated with a decreased number of floral shoots (Table 5.3). Return bloom in experiment one was particularly low, probably as a result of gale force winds which occurred in the orchard soon after bud-burst, which caused damage and loss of new lateral shoots. On average return bloom of 10.4 king flowers and 1.3 compound flowers per metre of cane was recorded in experiment one. An average of 4.5 shoots were recorded per metre of cane of which 3.1 were floral shoots.

Table 5.3 The effect of cane girdling on return bloom of king flowers and compound flower clusters in experiment one (n=12 vines).

| Percentage of canes girdled | King flowers[1] (flrs.m$^{-1}$.cane) | Compound flower clusters (flr. clusters m$^{-1}$.cane) | Floral shoots[2] (shoots.m$^{-1}$cane) | King flowers per floral shoot |
|---|---|---|---|---|
| 0 | 12.1 a | 1.61 a | 3.5 a | 3.5 a |
| 20 | 10.4 ab | 1.47 a | 2.9 b | 3.5 a |
| 50 | 9.7 b | 1.18 a | 3.0 b | 3.2 a |
| 100 | 9.4 b | 1.03 a | 2.9 b | 3.3 a |

Means in a column with common letters are not significantly different at P=0.05 (LSD).
[1] The number of king flowers includes those present in compound flower clusters.
[2] Floral shoots are shoots with flowers.

Table 5.4 The effect of partial defoliation of the replacement cane zone (RCZ) of vines which had all canes girdled (100G) on the return bloom of king flowers and compound flower clusters in experiment two (n=9 vines).

| Percentage of RCZ leaves defoliated | King flowers[1] ($flrs.m^{-1}.cane$) | Compound flower clusters ($flr.\ clusters\ m^{-1}.cane$) | Floral shoots[2] ($shoots.m^{-1}cane$) | King flowers per floral shoot |
|---|---|---|---|---|
| 0 | 20.6 a | 3.6 ab[3] | 5.3 a | 3.8 a[3] |
| 50 | 17.2 b | 2.5 bc | 5.0 a | 3.5 ab |
| 75 | 16.2 b | 1.7 c | 4.8 a | 3.4 b |

Means in a column with common letters are not significantly different at P=0.05 (LSD)
[1] The number of king flowers includes those present in compound flower clusters.
[2] Floral shoots are shoots with flowers.
[3] Unprotected LSD.

In experiment one, defoliation of 50% of leaves in the RCZ had no significant effect on any parameters of return bloom measured, however in experiment two 50 or 75% defoliation resulted in a significant reduction in king flower return bloom by 21% and compound flower clusters by 53% (Table 5.4). The reduction in return bloom in this case was associated with a reduction in the number of flowers per floral shoot (Table 5.4).

Thinning of fruit from the RCZ in experiment one had the effect of increasing the return bloom of compound flower clusters and was associated with an increase in the number of compound flower clusters per floral shoot (Table 5.5).

Table 5.5 The effect of thinning all fruit from the RCZ on return bloom of king flowers and compound flower clusters in experiment one (n=24 vines).

| Fruit thinning treatment | King flowers[1] ($flrs.m^{-1}.cane$) | Compound flower clusters ($flr.\ clusters\ m^{-1}.cane$) | Floral shoots[2] ($shoots.m^{-1}cane$) | King flowers per floral shoot |
|---|---|---|---|---|
| Control | 9.8 a | 0.75 b[1] | 3.0 a | 0.25 b |
| Thinned | 10.9 a | 1.90 a | 3.1 a | 0.60 a |

Means in a column with common letters are not significantly different at P=0.05 (LSD)
[1] The number of king flowers includes those present in compound flower clusters.
[2] Floral shoots are shoots with flowers.

### 5.3.4 Photosynthesis and water relations

There were no significant main or interaction effects of the percentage of canes girdled on a vine (0, 20, 50 or 100%), defoliation of the RCZ or thinning of fruit in the RCZ on photosynthetic rate, stomatal resistance, internal $CO_2$ concentration or $\psi_{xylem}$ overall. In addition there were no significant effects of the percentage of canes girdled on any of these parameters for leaves from either girdled or from non-girdled canes (Table 5.6).

When an ANOVA was performed on data separated into leaves from girdled and from non-girdled canes, the photosynthetic rate of leaves from girdled canes was significantly lower than from non-girdled canes on three out of the five dates and was significantly lower over all the dates combined (Table 5.7). The decrease in photosynthetic rate of leaves from girdled canes was associated with significantly increased stomatal resistance (Table 5.7), however internal $CO_2$ concentrations were only significantly altered at one of the dates and overall were not significantly different between leaves from girdled and non-girdled canes (Table 5.7).

Photosynthetic light response curves were plotted for data pooled from all measurements, separated into leaves from girdled and non-girdled canes. The asymptotic exponential model for photosynthetic light response suggested by Peat (1970) has been used to adequately describe a photosynthesis light response curve in kiwifruit (Buwalda et al., 1991). Asymptotic exponential models provide an estimate of the quantum efficiency (quantum yield) and maximum photosynthetic rate ($P_{max}$) and have significant advantages over the Michaelis-Menton type rectangular hyperbole model used by Laing (1985), namely improved fit (Jassby and Platt, 1976) and more accurate estimation of $P_{max}$ (Causton and Dale, 1990). The equation of the model is given by $Pn = P\max - b.c^{I}$; where Pn=net Photosynthetic rate, Pmax=light saturated net photosynthetic rate and I=Light intensity. Quantum yield is derived from the fitted parameters as $b$.ln($c$) (Buwalda et al., 1991). Parameter estimates for the fitted equations suggested that the quantum yield was not different for leaves from girdled and from non-girdled canes (0.038 and 0.039 respectively), however leaves from girdled canes had a lower $P_{max}$ compared with leaves from non-girdled canes (11.3±0.4 and 14.7±0.4 μMol

$CO_2.m^{-2}s.^{-1}$ respectively) (Figure 5.3). The light intensity at which photosynthesis is saturated was suggested to occur at 95% of $P_{max}$ and can be derived from the fitted equation as $\ln(0.05P\max/b)/\ln(c)$ (Buwalda et al., 1991). Calculation of saturating light intensities from the fitted models suggested that girdled leaves were saturated at a light intensity of 812 μMol $PAR.m^{-2}.s.^{-1}$ while non-girdled leaves were saturated at a light intensity of 1046 μMol $PAR.m^{-2}.s.^{-1}$

$\psi_{xylem}$ ranged between -0.105 and -1.0 Mpa on individual leaves and was not significantly different between leaves on girdled and non-girdled canes. However $\psi_{xylem}$ varied significantly between dates (ANOVA $P<0.0001$) and appeared to be closely related to temperature and PPFD. The highest mean $\psi_{xylem}$ was recorded on 13 January (Table 5.7), corresponding to a cloudless day with mean air temperature of 33°C and PPFD of 1547 μMol $PAR.m^{-2}.s.^{-1}$ during measurement. The lowest mean $\psi_{xylem}$ was recorded on 3 February corresponding to an overcast day with mean air temperature of 17.8°C and PPFD of 160 μMol $PAR.m^{-2}.s.^{-1}$.

Table 5.6 The net photosynthetic rate (Pn), stomatal resistance (rs) and xylem water potential ($\psi_{xylem}$ ) on 13 January, of leaves from girdled and non-girdled canes on vines with a different percentage of canes girdled (n=8 vines).

| Percentage of canes girdled | Pn ($\mu$Mol $CO_2.m^{-2}s.^{-1}$) | | rs ($s.cm^{-1}$) | | $\psi_{xylem}$ (MPa) | |
|---|---|---|---|---|---|---|
| | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled |
| 0 | - | 12.0 a | - | 1.1 a | - | -0.64 a |
| 20 | 7.6 b | 11.0 a | 1.5 b | 1.0 a | -0.59 a | -0.66 a |
| 50 | 6.0 b | 12.4 a | 1.9 b | 1.2 a | -0.61 a | -0.65 a |
| 100 | 6.3 b | - | 1.7 b | - | -0.59 a | - |

Means in a column with common letters are not significantly different at P=0.05 (LSD).
Means of a parameter in a row with common letters are not significantly different at P=0.05 (LSD).

Table 5.7 The mean photosynthetic photon flux density (PPFD) and net photosynthetic rate (Pn), internal $CO_2$ concentration (Ci), stomatal resistance (rs) and xylem water potential ($\psi_{xylem}$) of leaves from girdled and non-girdled canes measured at mid-day on five different dates in experiment one. Due to the presence of missing values, means presented are population marginal means of the average of two leaves per vine from up to 24 vines.

| | PPFD ($\mu$Mol PAR.m$^{-2}$.s.$^{-1}$) | Pn ($\mu$Mol $CO_2$.m$^{-2}$s.$^{-1}$) | | Ci (PPM) | | rs (s.cm$^{-1}$) | | $\psi_{xylem}$ (MPa) | |
|---|---|---|---|---|---|---|---|---|---|
| Date | | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled |
| 13 January | 1547 | 6.7 | 11.8 ** | 296 | 280 ** | 1.71 | 1.09 ** | -0.60 | -0.65 * |
| 27 January | 509 | 8.3 | 9.8 * | 303 | 302 ns | 1.50 | 1.29 * | -0.41 | -0.41 ns |
| 3 February | 160 | 5.2 | 5.7 ns | 324 | 326 ns | 1.61 | 1.38 * | -0.22 | -0.23 ns |
| 16 February | 1403 | 10.9 | 13.6 ** | 291 | 288 ns | 1.10 | 0.84 ** | -0.42 | -0.44 ns |
| 3 March | 1581 | 15.6 | 16.6 ns | 285 | 283 ns | 0.75 | 0.66 ns | - | - |
| All | 1054 | 11.4 | 9.2 ** | 300 | 296 ns | 1.34 | 1.05 ** | -0.41 | -0.43 ns |

Means of girdled and non-girdled canes are significantly different at P=0.05 (*) or P=0.01 (**) (contrast).
Means of girdled and non-girdled canes are not significantly different at P=0.05 (contrast).

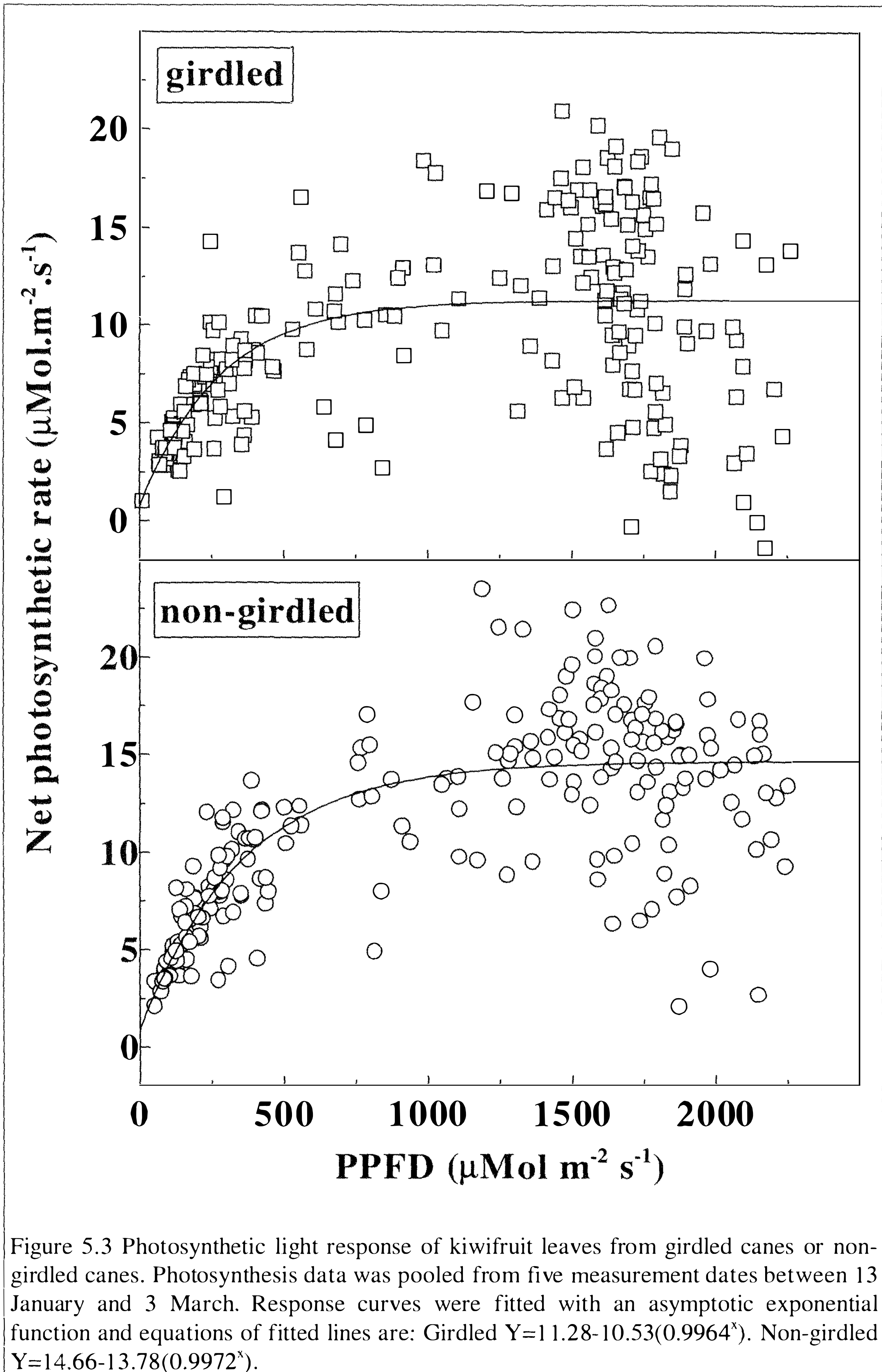


Figure 5.3 Photosynthetic light response of kiwifruit leaves from girdled canes or non-girdled canes. Photosynthesis data was pooled from five measurement dates between 13 January and 3 March. Response curves were fitted with an asymptotic exponential function and equations of fitted lines are: Girdled $Y=11.28-10.53(0.9964^x)$. Non-girdled $Y=14.66-13.78(0.9972^x)$.

### 5.3.5 Root growth

There were no significant main or interaction effects of girdling, defoliation or thinning on total length of white roots or brown roots (Table 5.8, Table 5.9). There were significant row effects, with the total white root length higher in rows more distant from a shelter belt. Average white root length density was 0.146 $km.m^{-2}$soil surface, while the average total brown root length density was 8.54 $km.m^{-2}$soil surface. White root length density also differed with increasing soil depth and on average 94% of all white roots were found to occur in the upper 30cm. However brown roots were more evenly distributed with an average of 57% occurring in the upper 30 cm.

Table 5.8 The effect of cane girdling on root length densities in the top 60 cm of soil samples at a one metre radius around kiwifruit vines in April 1994 (n=12 vines).

| Percentage of canes girdled | Total white root length ($km.m^{-2}$) | Total brown root length ($km.m^{-2}$) |
|---|---|---|
| 0 | $0.13_{-0.88\,(0.18)}$ [1] | 8.6 (0.6) |
| 100 | $0.16_{-0.79\,(0.15)}$ | 8.4 (0.8) |

White root lengths are back-transformed from $\log_{10}$.
Standard error of the mean in parentheses.
[1] Mean and standard error of $\log_{10}$ transformed data are presented as a subscript.

Table 5.9 The effect of thinning and defoliation of the replacement cane zone on root length densities in the top 60 cm of soil samples at a one metre radius around kiwifruit vines in April 1994. Means are the average total root lengths from nine vines.

| Replacement cane zone treatment | Total white root length ($km.m^{-2}$) | Total brown root length ($km.m^{-2}$) |
|---|---|---|
| Control | $0.15_{-0.82\,(0.20)}$ [1] | 8.3 (0.9) |
| 50% defoliated | $0.10_{-0.99\,(0.23)}$ | 8.2 (0.7) |
| 100% thinned | $0.20_{-0.69\,(0.17)}$ | 9.2 (1.1) |

White root lengths are back-transformed from $\log_{10}$.
Standard error of the mean in parentheses.
[1] Mean and standard error of $\log_{10}$ transformed data are resented as a subscript.

### 5.3.6 Fruit mineral content

There were no significant effects of cane girdling treatments on the concentration of the mineral nutrients K, Ca, Mg, N or P in fruit from girdled canes, non-girdled canes or from the RCZ. For simplicity in comparing of the effects of canes girdling on mineral concentration, only the values obtained for fruit from the RCZ have been presented (Table 5.10). In terms of total mineral content per fruit, fruit from girdled canes on 50G vines had consistently higher mineral contents than non-girdled canes, however this was only statistically significant for Mg (Table 5.11).

Table 5.10 The concentration of mineral nutrients ($mg.g^{-1}dwt$) in mature kiwifruit from the replacement cane zone on vines where 0, 50 or 100% of the fruiting canes were girdled (n=3 vines).

| Percentage of canes girdled | K | Ca | Mg | N | P |
|---|---|---|---|---|---|
| 0 | 28.6 (3.0) | 2.2 (0.05) | 0.91 (0.09) | 7.7 (1.65) | 1.5 (0.35) |
| 50 | 24.9 (2.4) | 2.5 (0.29) | 0.85 (0.08) | 9.2 (1.22) | 1.8 (0.22) |
| 100 | 25.5 (1.5) | 2.6 (0.30) | 0.86 (0.06) | 8.7 (0.68) | 1.6 (0.19) |

Values in parentheses are standard errors of the mean.

Table 5.11 The total quantity of mineral nutrients ($mg.fruit^{-1}$) in mature kiwifruit from girdled or from non-girdled canes on vines where 50% of the fruiting canes were girdled (n=3 vines).

| Cane type | Dry weight (g) | K | Ca | Mg | N | P |
|---|---|---|---|---|---|---|
| girdled | 23.4 | 591 | 47.7 | 22.3 | 209 | 44.9 |
| non-girdled | 17.5 * | 505 | 39.1 | 14.5 * | 191 | 37.6 |

* Means of girdled and non-girdled fruit are significantly different at P=0.05 (contrast).

### 5.3.7 Fruit growth

Fruit growth curves generated in experiment two showed very similar patterns for all treatments. An exponential period of growth occurred in all treatments until around seven WAFB. This was followed by a lag period of slow fruit growth, after which a second period of growth occurred at a relatively linear rate (Figure 5.4). Final fresh weight, dry weight and percentage dry matter of fruit from girdled canes was not significantly different between 50G or 100G treatments (Table 5.12). Inspection of growth curves suggested that there was also no difference in growth rates of the girdled fruit during the growing season (Figure 5.4a). In contrast, fresh weight and dry weight of fruit from girdled canes was significantly higher than from non-girdled canes on the 50G treatment (Table 5.12), due to consistently higher growth rate over the entire season (Figure 5.4c). Percentage dry matter was higher in fruit from girdled canes than in fruit from non-girdled canes, although this was not statistically significant (contrast, P=0.17).

Table 5.12 Final weights and percentage dry matter of fruit from girdled canes and non-girdled canes on vines with different percentages of canes girdled. Fruit had been measured non-destructively over a growing season (1995 experiment). Means are from three vines.

| Percentage of canes girdled | Fresh weight (g) | | Dry weight (g) | | Percentage dry matter | |
|---|---|---|---|---|---|---|
| | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled |
| 0 | - | 139 (4) | - | 20.7 (0.9) | - | 14.9 (0.2) |
| 50 | 155 (10) | 130 (3) | 24.1 (1.1) | 18.5 (0.5) | 15.7 (1.1) | 14.3 (0.1) |
| 100 | 151 (2) | - | 23.6 (0.5) | - | 16.1 (0.6) | - |

Standard error of mean in parentheses.

The final fresh weight of fruit from non-girdled canes from 50G vines was almost 10g lower than non-girdled canes from 0G vines (Table 5.12) and although this was not statistically significant (contrast, P=0.13), it was similar to the differences found in the main experiment which were significantly different. Dry weight and percentage dry

matter of fruit from non-girdled canes were also lower in the 50G treatment than in the 0G treatment (Table 5.12) and were statistically significant at the 10% level (contrast, P=0.10, 0.08 respectively). Inspection of growth curves suggested that the growth of fruit from these two treatments was identical up until the lag phase, after which the growth rate of non-girdled fruit from 50G was comparatively slower than non-girdled fruit from 0G (Figure 5.4b).

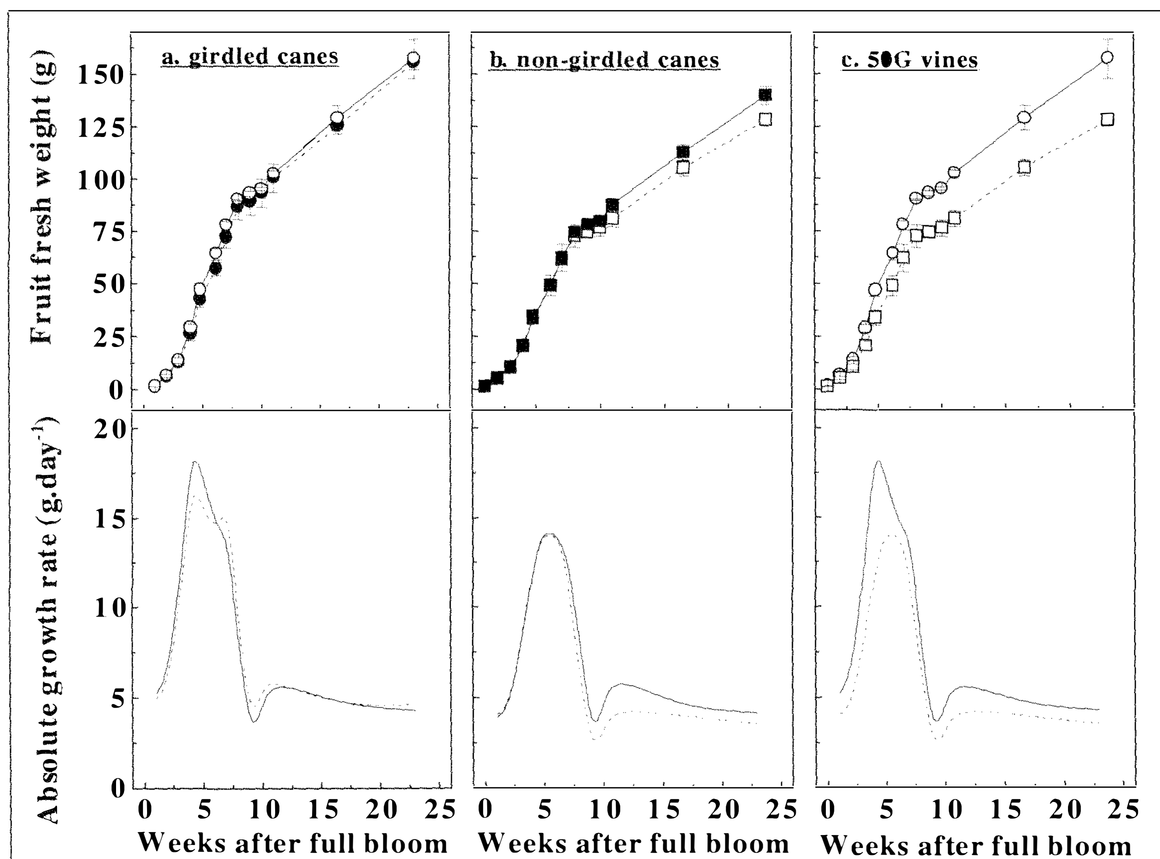


Figure 5.4 Comparisons of fresh weight growth curves for kiwifruit from girdled and non-girdled canes from vines with 0% (0G), 50% (50G) or 100% (100G) of canes girdled. Mean fruit weights were estimated from sequential non-destructive measurements on the same fruits made between anthesis and maturity. Absolute growth rate curves were fitted using β-spline fitting.

a. Girdled canes: 100G (– – ● – –), 50G (—O—).
b. Non-girdled canes: 0G (—■— ), 50G (– – □– –).
c. 50G vines: girdled canes (—O—), non-girdled canes (– – □– –).

### 5.3.8 Shoot growth

Almost all re-growth from pruned lateral shoots occurred in the bud from the leaf axil immediately below the pruning cut. On a few laterals, re-growth occurred from more than one bud, however the more distal regrowth was removed and only the apical most re-growth was measured. Over all lateral shoots which were included in this experiment, 93% of any re-growth which occurred within the 42 day period of measurement had commenced within 14 days after the pruning cut was made on the lateral shoot.

On 50G vines, non-girdled canes had a lower percentage bud-burst than girdled canes, although differences were not statistically significant (contrast, P=0.2456), however this resulted in a significantly lower average length of re-growth per lateral shoot from the non-girdled canes (contrast, P=0.0345) (Table 5.13). Percentage bud-burst of re-growth from girdled canes was 30% higher on 100G vines compared with 50G vines and this was statistically significant at the 10% level (contrast, P=0.078) (Table 5.13). This resulted in an almost two-fold increase in the average length of regrowth from girdled canes on 100G vines compared with 50G vines (contrast, P=0.053) (Table 5.13). There were no significant differences in bud-burst and regrowth of non-girdled canes between 0G and 50G vines (Table 5.13).

The natural log of re-growth length vs. time was found to have a linear relationship for individual shoots in this experiment (Figure 5.5). The relative growth rate (RGR) of an organ can be estimated as the slope of a curve fitted to natural log of the size of an organ size against time. In the case of a straight line relationship, growth is exponential and the slope is constant over time, therefore RGR remains constant over time. RGR's were calculated for each shoot measured as the slope of a linear regression fitted to $\log_e$(length) vs. time. Analysis of variances of shoot RGR's showed no significant model or treatment effects (Table 5.13).

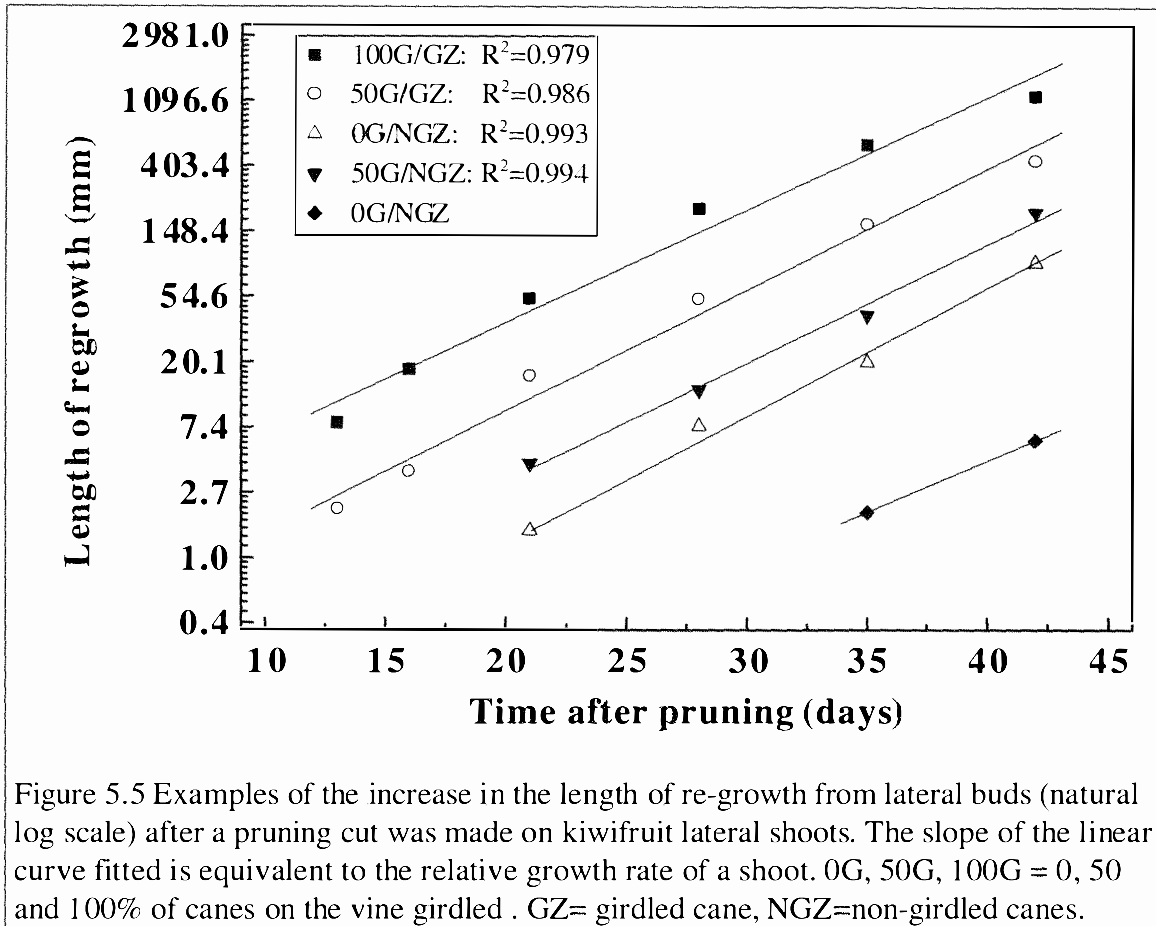


Figure 5.5 Examples of the increase in the length of re-growth from lateral buds (natural log scale) after a pruning cut was made on kiwifruit lateral shoots. The slope of the linear curve fitted is equivalent to the relative growth rate of a shoot. 0G, 50G, 100G = 0, 50 and 100% of canes on the vine girdled . GZ= girdled cane, NGZ=non-girdled canes.

### 5.3.9 The cytokinins in root xylem exudate from decapitated kiwifruit cuttings

Cytokinins were putatively identified using HPLC separation and RIA of purified sap samples (chapter two). Retention times were consistently the same as standard cytokinins and the specificity of the RIA strongly suggests that the putative cytokinins were indeed zeatin (Z), isopentenyl-adenine (2iP) and their respective ribosides.

Following decapitation of rooted kiwifruit cuttings, sap immediately began to exude from cut surfaces at an average flow rate of 2.8 $ml.hr^{-1}$. Sap continued to exude over the 72 hours during which collection of sap was made, although the average flow rate had decreased slightly to 2.3 $ml.hr^{-1}$ by 72 hours after decapitation. At the time of decapitation, the concentration of Z, 2iP and their respective ribosides in the root xylem exudate of control cuttings was low and was not significantly different (contrast P=0.05) to the concentration of these cytokinins from cuttings treated with lanolin or NAA. Concentrations of Z and 2iP were low compared with their ribosides and either did not respond (2iP) or responded only slowly (Z) to decapitation (Figure 5.6).

Table 5.13 Percentage of laterals which produced re-growth, final length of re-growth from all laterals at 8 weeks after full bloom and relative growth rate of re-growth. Re-growth arising from pruned fruiting laterals on girdled and un-girdled canes from vines with nil, 50% or 100% of tied down canes girdled in experiment two. Means are from three vines.

| Percentage of canes girdled | Percentage bud-burst of the apical bud on pruned lateral shoots | | Final length of regrowth[1] ($mm.shoot^{-1}$) | | Relative growth rate ($mm.mm^{-1}.day^{-1}$) | |
|---|---|---|---|---|---|---|
| | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled |
| 0 | - | 45 (12) | - | 146 (91) | - | 0.186 (0.015) |
| 50 | 61 (11) | 34.5 (16) | 253 (43) | 82 (33) | 0.158 (0.012) | 0.159 (0.012) |
| 100 | 93 (7) | - | 475 (69) | | 0.156 (0.007) | - |

Standard error of mean in parentheses.
[1] Includes shoots where no re-growth occurred.

At the time of decapitation, the concentration of zeatin riboside (ZR) in root xylem exudate from control cuttings was 0.73 ng.ml$^{-1}$. Within 14 hours of decapitation, the concentration of ZR in the root xylem exudate from control cuttings was significantly higher (contrast, P=0.0212), than at the time of decapitation and concentrations increased over ten-fold to a maximum of 9.9 ng.ml$^{-1}$ by 24 hours, after which the concentration declined to 4.5 ng.ml$^{-1}$ by 72 hours (Figure 5.6). The concentration of Isopentenyl-adenosine (IPA) in root xylem exudate from control cuttings also increased over the first 24 hours, after which a decline similar to that of ZR was found (Figure 5.6).

Root xylem exudate from cuttings which had a girdle applied to the base of the cutting 48 hours prior to decapitation, contained elevated concentrations of both ZR and IPA compared with controls at the time of decapitation (Table 5.14, Table 5.15). ZR levels in root xylem exudate from girdled cuttings followed a similar pattern to control cuttings, but with a 48 hour shift forwards (Table 5.14). For example, the concentration of ZR from girdled cuttings at the time of decapitation was not significantly different (contrast, P=0.1611) to that in control cuttings 48 hours following decapitation. Likewise, the concentration of ZR from girdled cuttings 24 hours following decapitation was not significantly different (contrast, P=0.1390) to that in control cuttings 72 hours following decapitation. In contrast, IPA levels were significantly higher (contrast, P=0.0060) in girdled cuttings at the time of decapitation than in control cuttings at 48 hours following decapitation (8.09 vs. 2.04 ng.ml$^{-1}$ respectively), although at 24 hours following decapitation, girdled cuttings had similar levels (contrast, P=0.7957) to control cuttings at 72 hours (3.44 vs. 4.01 ng.ml$^{-1}$ respectively).

Inhibition of basipetal auxin transport by NPA applied to the stem of the cuttings partly elevated cytokinins in root xylem exudate. The concentration of ZR in root xylem exudate from NPA treated cuttings was elevated at the time of decapitation compared with control cuttings (Table 5.14), however the concentration of IPA was not significantly different from control cuttings (Table 5.15). ZR concentrations from NPA treated cuttings decreased over the 72 hours following decapitation and at all times were lower than from girdled cuttings, although this was not statistically significant at any time (Table 5.14).

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. Treatments applied to cuttings were control (no treatment), Lanolin applied to cut surface, 1.5 mM naphthalene acetic acid (NAA) in lanolin applied to cut surface, girdling of the base of the cutting 48 hours prior to decapitation and 3.4 mM naphthyl-phthalamic acid (NPA) in lanolin applied to the base of the cutting 48 hours prior to decapitation. Due to the presence of missing values, means presented are population marginal means[1] from up to five cuttings per treatment.

| Time after decapitation | Treatment applied to cutting | | | | |
|---|---|---|---|---|---|
| | Control | Lanolin | NAA | Girdled | NPA |
| 0 hours | 0.73 c | 0.84 c | 1.49 bc | 5.37 a | 3.44 ab |
| 24 hours | 9.89 a | 8.67 a | 3.30 b | 6.70 ab | 3.16 b |
| 48 hours | 7.39 a | 4.99 ab | 3.76 bc | 2.27 c | 1.79 c |
| 72 hours | 4.47 a | 2.90 ab | 1.85 bc | 0.35 c | 0.103 c |

[1] For description of population marginal means, see chapter two, section 2.8.3.
Means in a row with common letters are not significantly different at P=0.05 (lsmeans, SAS).

At no time was the concentration of ZR or IPA in root xylem exudate from cuttings treated with lanolin found to be significantly different from control cuttings. Replacement of the apical supply of auxin by NAA applied to the cut surface partly depressed the elevation of cytokinins in root xylem exudate which occurred in control and lanolin treated cuttings following decapitation. At 24 hours after decapitation, the concentration of ZR in root xylem exudate from NAA treated cuttings was significantly lower than control cuttings (contrast, P=0.0054) and lanolin treated cuttings (contrast, P=0.0251) (Table 5.14). In addition, the concentration of IPA was significantly lower than from control (contrast, P=0.0275) although not lower than the lanolin treated cuttings at 24 hours after decapitation (Table 5.15).

### 5.3.10 The cytokinins and ABA in extracted xylem exudate from cane girdled kiwifruit vines

As with section 5.3.9, free bases and riboside cytokinins were putatively identified following HPLC and RIA. ABA was putatively identified following ELISA using extremely high specificity monoclonal antibodies (chapter two).

Table 5.15 The concentration of putative isopentenyl-adenosine in root xylem exudate from rooted kiwifruit cuttings which had been decapitated. Treatments are as described in Table 5.14. Due to the presence of missing values, means presented are population marginal means from up to five cuttings per treatment

| Time after decapitation | Treatment applied to cutting | | | | |
|---|---|---|---|---|---|
| | Control | Lanolin | NAA | Girdled | NPA |
| 0 hours | 0.57 b | 0.20 b | 1.44 b | 8.09 a | 1.51 b |
| 24 hours | 5.55 a | 3.90 ab | 2.27 b | 3.44 ab | 3.19 ab |

Means in a row with common letters are not significantly different at P=0.05 (lsmeans, SAS).

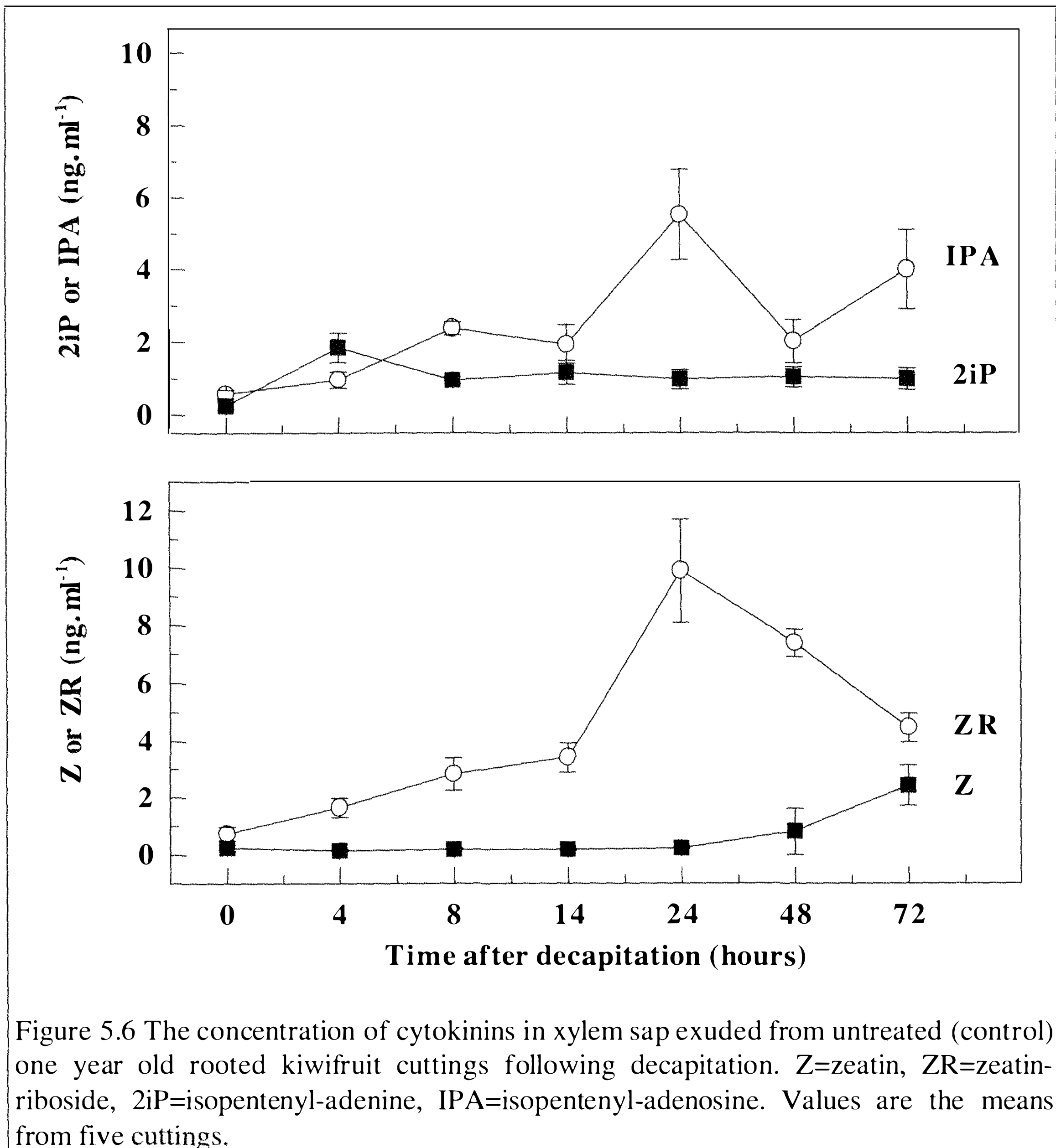


Figure 5.6 The concentration of cytokinins in xylem sap exuded from untreated (control) one year old rooted kiwifruit cuttings following decapitation. Z=zeatin, ZR=zeatin-riboside, 2iP=isopentenyl-adenine, IPA=isopentenyl-adenosine. Values are the means from five cuttings.

The most abundant cytokinin detected in extracted xylem exudate from the excised canes was ZR. In non-girdled canes, the level of ZR varied from between 5 and 13 times the level of IPA which was the next most abundant cytokinin measured. Trace quantities of Z and 2iP were also detected but data is not presented. The concentration of ZR in exudate from non-girdled canes ranged from 1.52 to 3.53 $ng.ml^{-1}$ (Table 5.16), while in girdled canes, levels of ZR reached 7.5 $ng.ml^{-1}$ at one WAFB. The concentration of IPA in exudate from non-girdled canes ranged from 0.19 to 0.60 $ng.ml^{-1}$ while in girdled canes levels reached 2.82 $ng.ml^{-1}$ at two WAFB (Table 5.17). Concentrations of both ZR and IPA in exudate from non-girdled canes appeared to fluctuate with no clear pattern over the first eight WAFB (Table 5.16, Table 5.17).

There were no significant treatment differences in extracted xylem exudate cytokinin concentrations at the time of girdling (0 WAFB), or after four WAFB. However at one and two WAFB there were large differences between treatments, although these were not all statistically significant at the 5% level. The concentration of ZR from 100G vines was three times higher than from 0G vines at one WAFB (contrast, P=0.151) and four times higher at two WAFB (contrast, P=0.080) (Table 5.16). Similarly, the concentration of IPA from 100G vines was over four times higher than 0G vines at one WAFB (contrast, P=0.080) and 13 times higher at two WAFB (contrast, P=0.002) (Table 5.17). There were no significant differences between cytokinin concentrations in non-girdled canes from 0G and 50G vines at any time.

The concentration of cytokinins in extracted xylem exudate in girdled canes from 50G vines was also elevated compared with non-girdled canes at two WAFB, however the increase was smaller than in 100G vines and was only statistically significant for IPA (contrast, P=0.020). The concentration of both ZR and IPA in girdled canes of 100G vines was almost double that in girdled canes from 50G vines, however this difference was not statistically significant.

Table 5.16 The concentration of zeatin-riboside ($ng.ml^{-1}$) in extracted xylem exudate from girdled or non-girdled canes excised from kiwifruit vines prior to sunrise. Canes were from vines with 0, 50 or 100% of canes girdled (n=3 vines).

| Percentage of | 0 WAFB[1] | | 1 WAFB | | 2 WAFB | | 4 WAFB | | 8 WAFB | |
|---|---|---|---|---|---|---|---|---|---|---|
| canes girdled | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled |
| 0 | - | 1.52<br>0.18 (0.22) | - | 2.63<br>0.42 (0.09) | - | 1.56<br>0.19 (0.12) | - | 3.53<br>0.19 (0.12) | - | 2.45<br>0.39 (0.04) |
| 50 | - | 2.40<br>0.38 (0.20) | - | - | 3.69<br>0.57 (0.25) | 1.85<br>0.27 (0.28) | 3.63<br>0.56 (0.05) | 2.07<br>0.32 (0.09) | 1.61<br>0.21 (0.23) | 2.60<br>0.41 (0.23) |
| 100 | 1.72<br>0.24 (0.05) | - | 7.50<br>0.88 (0.19) | - | 6.56<br>0.82 (0.20) | - | 2.95<br>0.47 (0.15) | - | 3.85<br>0.59 (0.06) | - |

Means are back-transformed from $log_{10}$.
Values in subscript are $log_{10}$ transformed means with the standard error of the transformed mean in parentheses.
[1] WAFB = weeks after full bloom.

Table 5.17 The concentration of isopentenyl-adenosine ($ng.ml^{-1}$) in extracted xylem exudate from girdled or non-girdled canes excised from kiwifruit vines prior to sunrise. Canes were from vines with 0, 50 or 100% of canes girdled (n=3 vines).

| Percentage of | 0 WAFB[1] | | 1 WAFB | | 2 WAFB | | 4 WAFB | | 8 WAFB | |
|---|---|---|---|---|---|---|---|---|---|---|
| canes girdled | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled | girdled | non-girdled |
| 0 | - | 0.19<br>-0.73 (0.13) | - | 0.28<br>-0.56 (0.16) | - | 0.22<br>-0.65 (0.17) | - | 0.60<br>-0.22 (0.17) | - | 0.19<br>-0.72 (0.20) |
| 50 | - | 0.30<br>-0.52 (0.40) | - | - | 1.50<br>0.18 (0.18) | 0.29<br>-0.54 (0.07) | 0.81<br>-0.09 (0.15) | 0.43<br>-0.37 (0.15) | 0.37<br>-0.43 (0.25) | 0.43<br>0.37 (0.16) |
| 100 | 0.71<br>-0.15 (0.03) | - | 1.08<br>0.03 (0.20) | - | 2.82<br>0.45 (0.24) | - | 0.59<br>-0.23 (0.13) | - | 0.39<br>-0.41 (0.05) | - |

Means are back-transformed from $log_{10}$.
Values in subscript are $log_{10}$ transformed means with the standard error of the transformed mean in parentheses.
[1] WAFB = weeks after full bloom.

The concentration of ABA in extracted xylem exudate was not significantly affected by the girdling treatments between zero and eight WAFB. ABA concentration increased to a peak of 19 $ng.ml^{-1}$ at one WAFB at which time it was significantly higher (contrast, $P<0.05$) than at any other date measured. ABA levels then declined to a minimum of 7.5 $ng.ml^{-1}$ at eight WAFB (Figure 5.7).

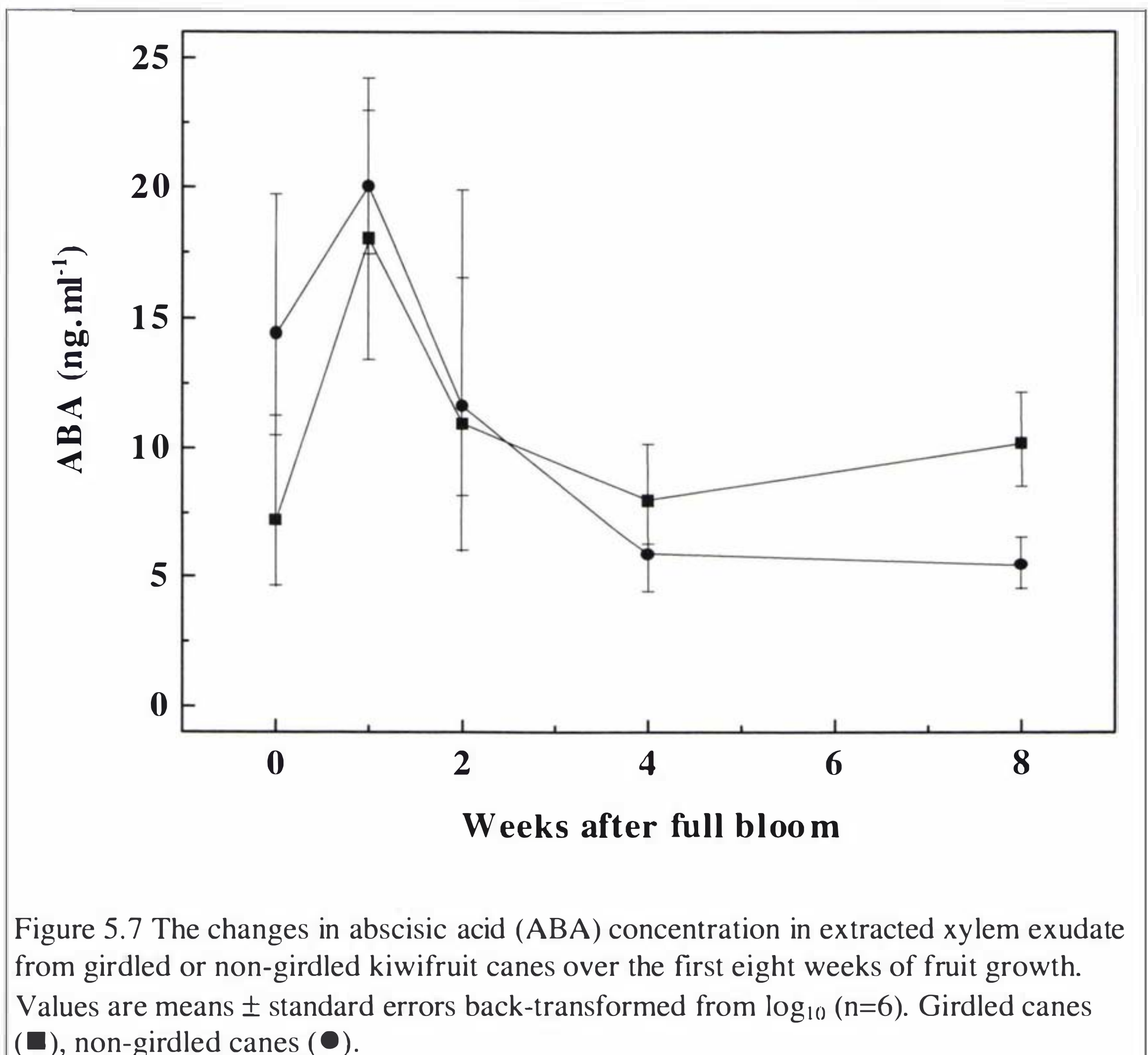


Figure 5.7 The changes in abscisic acid (ABA) concentration in extracted xylem exudate from girdled or non-girdled kiwifruit canes over the first eight weeks of fruit growth. Values are means ± standard errors back-transformed from $log_{10}$ (n=6). Girdled canes (■), non-girdled canes (●).

## 5.4 Discussion

### 5.4.1 Fruit growth

The effect of cane girdling on fruit growth of kiwifruit described in chapter four were confirmed in these experiments. By increasing the percentage of fruiting canes girdled on a vine, the weight of fruit in the non-girdled parts of the vine was reduced and in the girdled canes themselves the response to girdled was reduced. At the comparatively low level of 20G, the weight of fruit from girdled canes was significantly higher than in 50G vines, while fruit in the RCZ and non-girdled canes showed evidence of being smaller than fruit in comparable zones from 0G vines (Figure 5.2). However unlike the experiment described in chapter four, cane girdling did not lead to increased potential revenue, due to the decreased size of fruit from non-girdled parts of the vine and a smaller response to girdling.

The depression of fruit growth from non-girdled canes appears to occur after the lag phase which occurs at around seven weeks after fruit set. Growth curves showed that the growth rate of fruit from 50G vines was consistently lower between the beginning of the lag phase and harvest (Figure 5.4b). At this time, post-anthesis cell division has practically ceased and fruit growth is almost solely due to cell expansion and storage of starch and sugars (Chapter three). Thus the negative effect of girdling on non-girdled fruit does not appear to occur during the cell division stage. Unfortunately it was not clear from our results at which stage of fruit growth that the depression of fruit growth from girdled canes occurs.

In order to increase or decrease the source-sink balance of whole kiwifruit vines, defoliation and fruit thinning treatments were carried out in two experiments in combination with cane girdling. Defoliation of 50% or 75% of leaves from the RCZ of 100G vines decreased the weight of fruit from the non-girdled parts of vines. However the weight of fruit from girdled canes was not affected by defoliation. In another study, defoliating 50 or 75% of leaves in the RCZ of non-girdled kiwifruit vines reduced fruit weight in both the RCZ and fruiting canes (Cruz-Castillo, 1994), which suggests that

defoliation results in an increase in competition between fruit sinks. As leaves in the RCZ of cane girdled vines were solely able to supply the root system with carbohydrate, defoliation would almost certainly reduce the carbohydrate available to be translocated to the root system. The lack of a response in fruit growth from girdled canes on defoliated vines that we have found, suggests that a major decrease in carbohydrate available to the roots does not necessarily result in any effect on fruit growth. It also seems unlikely from the evidence presented in this chapter that cane girdling itself causes a shortage of carbohydrate in the RCZ or the root system. If fruit growth is inhibited by cane girdling in the RCZ due to a lack of carbohydrate, return bloom and root growth would have been expected to be severely inhibited as these are poor competitors for carbohydrate (Buwalda and Smith, 1990a) and this was not the case. Although return bloom was possibly affected, the effect was small and was not able to be confirmed.

### 5.4.2 Decapitation of cuttings and hormones in xylem exudate

Removal of the shoot system of kiwifruit cuttings by decapitation resulted in an almost immediate increase in the concentration of ZR and IPA in root xylem exudate exuded from the cut surfaces. Similar experiments have demonstrated substantial increases in the cytokinin concentration of root xylem exudate after decapitation of bean seedlings (Bangerth, 1994) and in stem tissue immediately below the cut surface after decapitation of pea seedlings (Li et al., 1995). In both of these experiments, the rise in cytokinin concentration did not occur if stems were treated with the synthetic auxin NAA suggesting that root produced and exported cytokinins are under the control of auxin translocated from shoots (Bangerth, 1994; Li et al., 1995). Although NAA applied to decapitated kiwifruit stems reduced the concentration of cytokinins in the root xylem exudate compared with control plants, they were not maintained at levels found at the time of decapitation (Table 5.14, Table 5.15). This supports the hypothesis that auxins and cytokinins are root-shoot co-ordinating signals, however experimental conditions may not have been optimal, as additional plants were not available to test higher concentrations of NAA. The inability of NAA application to maintain the concentration of cytokinins in root xylem exudate to levels found at the time of decapitation could have been due to poor uptake of NAA. Concentrations of NAA used by Bangerth (1994)

were 100-fold higher than we used, as high concentrations are required to allow uptake of physiological concentrations (Bangerth, personal communication 1995).

The application of a girdle to a kiwifruit cutting mimicked the response of decapitation resulting in elevated cytokinin levels in root xylem exudate. This provides strong evidence that girdling of kiwifruit eliminates the auxin signal from shoots past the position of a girdle, as has been found in peach (Dann et al., 1985). This would suggest that girdling of tree trunks could significantly alter an auxin signal received by roots and it is likely that root-shoot signals may be altered in a manner similar to that found in kiwifruit cuttings. Application of an auxin transport inhibitor (NPA) to stems of kiwifruit cuttings resulted in a change in cytokinin concentration in a similar manner to girdling. However cytokinin concentrations were generally low, which suggests that some NPA may have been transported to the roots either in the bark or possibly in condensation which dripped into the media. As NPA is known to inhibit root growth and cause root death (Brunn et al., 1992), this could have reduced their cytokinin biosynthetic capability.

Analysis of extracted xylem exudate obtained from mature vines confirms that ZR is the major sap cytokinin in kiwifruit as was reported by Lewis (1994). ZR and Z appear to be the dominant sap cytokinins in a variety of perennial trees including apple (Tromp and Ovaa, 1994), peach (Cutting and Lyne, 1993) and birch (Rinne and Saarelainen, 1994). Other cytokinins are often present in sap including glucosides, nucleotides, isopentenyl- and dihydrozeatin- cytokinins although usually at much lower concentrations. The concentration of ZR in xylem exudate measured by Lewis (1994) ranged between 0.2 and 4.1 $ng.ml^{-1}$ (values estimated visually from graph) over the first 40 days after flowering, which is in agreement with the range 1.5-3.5 $ng.ml^{-1}$ measured in control (0G) vines in this study (Table 5.16). However we have found no evidence to support the report of a general decrease in xylem cytokinin levels over this period (Lewis, 1994). The reported general decrease appeared to have been made largely on the basis of a high concentration at flowering, and the estimate was associated with high standard errors.

Significant quantities of ABA were found in extracted xylem exudate from mature kiwifruit vines in this study. ABA at 0.9 $ng.ml^{-1}$ has been reported to occur in bleeding xylem sap of *Actinidia chinensis* using gas-liquid chromatography (Davison, 1973). This

is considerably lower than the levels that we have measured using ELISA based on monoclonal antibodies (Figure 5.7). However the ABA levels reported by Davison (1973) were from bleeding sap collected in early spring, at which time vines would be unlikely to experience any water stress as temperatures are low and canopy development is minimal. ABA has also been found in kiwifruit stem tissue (Tafazoli and Beyl, 1993) and fruits (Smith et al., 1995). The concentration of ABA in kiwifruit was characterised by high initial concentrations which declined up until seven weeks after anthesis, although the earliest measurements were made at three weeks after anthesis (Smith et al., 1995). ABA levels in extracted xylem exudate peaked at one WAFB (Figure 5.7) and steadily decreased thereafter. Although the peak in ABA measured at one WAFB may have been due to a temporary water deficit as a result of soil drying (Zhang et al., 1990), this is relatively unlikely as irrigation was used to avoid soil moisture deficits. Declining ABA concentrations in xylem sap after anthesis could explain declining concentrations which were found to occur in fruit (Smith et al., 1995), although more extended sampling is required to confirm the trend that we have reported.

### 5.4.3 Root-shoot signals and growth of fruit and shoots

The concentration of cytokinins in extracted xylem exudate was increased in girdled canes compared with non-girdled canes, rather than depressed as we had expected based on the results of Cutting and Lyne (1993). Part of the second hypothesis proposed in chapter four was that "*Interruption of the auxin signal out of canes by girdling reduced growth regulator signals from roots to shoots, which reduced the sink strength of the fruit*". As xylem cytokinins were increased rather than depressed following girdling, this hypothesis is rejected.

Although it is not certain whether kiwifruit obtain cytokinins from xylem, it is possible that some of the response to girdling of kiwifruit could be due to increased accumulation of cytokinins by fruit from girdled canes. Kiwifruit growth responds to application of the cytokinin compound CPPU (Woolley and Lai, 1990; Chapter eight). In addition, application of endogenous cytokinins is effective at stimulating fruit growth when applied in combination with other growth regulators (Hopping, 1976; Costa and Ryugo, 1978

cited in Hopping, 1990). This suggests that increasing cytokinin levels in fruit could be stimulatory for kiwifruit growth, although correlations between the total amount of endogenous cytokinins and kiwifruit growth have not been found (Lewis et al., 1996b; Lewis et al., 1996a; Chapter eight). The levels of cytokinins in xylem exudate taken up by kiwifruit (based on fruit transpiration rate) are sufficient to account for the increase in total cytokinin in fruit over the first 40 days after anthesis (Lewis, 1994). Additionally, during the early period of fruit growth the total amount of cytokinin in kiwifruit is independent of seeds (Lewis et al., 1996a), which are thought to be the most likely biosynthetic source of cytokinins in fruit (Letham, 1994). The removal of leaves or increasing the number of fruit per cluster reduced the cytokinin content of tomato fruit, which suggested that fruits were competing with each other and with leaves for xylem cytokinins (Varga and Bruinsma, 1974). Substantial amounts of $^{3}$H-ZR and $^{3}$H-dihydrozeatin-riboside supplied to shoot xylem of lupin plants were recovered in the fruit, almost all of which was in the pod wall (Zhang and Letham, 1990), which suggests that fruits may obtain at least some of their cytokinins from imported xylem sap. Although the continued growth of fruit on rootless plants has been reported (Peterson and Fletcher, 1973), substantial amounts of cytokinins are stored in stems (Jameson et al., 1987) and could transfer cytokinins into xylem after roots are removed. Further experimental work is required in order to clarify the source of cytokinins in kiwifruit and to establish correlations between fruit cytokinin levels and growth. As girdling effectively alters xylem sap cytokinin concentrations, this would be a useful experimental technique in this area of research.

In general, re-growth of new vegetative growth was well correlated to the concentration of cytokinins in extracted xylem exudate. Girdled canes on 100G vines had the highest concentration of ZR and IPA in extracted xylem exudate at one and two WAFB (Table 5.16, Table 5.17) and also a significantly higher percentage bud-burst and mean final length of re-growth than any other treatment (Table 5.13). On 50G vines, girdled canes had higher concentrations of ZR and IPA than non-girdled canes at two WAFB (Table 5.16, Table 5.17) and a significantly higher percentage bud-burst and mean final length of re-growth. As there were no differences in the relative growth rate of re-growth from any of the treatments (Table 5.13), differences in re-growth can be entirely attributed to

the percentage and the timing of bud-burst. Cytokinins have been implicated in the release of apical dominance of buds in a number of studies (Cline, 1991) and bud-break has been associated with high concentrations of cytokinins in xylem exudate (Cutting et al., 1991). Although cytokinins in lateral buds were not measured here, cytokinins supplied to rootless bean plants are transported to lateral buds (Woolley and Wareing, 1972), which suggests that increased cytokinins in xylem sap of girdled canes could also be reflected in increased cytokinin levels in lateral buds. The results presented in this chapter support a role for xylem cytokinins transported to buds, in the promotion of bud development after they have been released from apical dominance. Possible roles of xylem cytokinins could be stimulation of cell division in buds (Mauseth, 1976) or induction of increased synthesis and transport of IAA out of buds (Li and Bangerth, 1992). It may also be useful to measure xylem cytokinins in kiwifruit canes between the time of bud-break and flowering to see if correlation's also exist at this time, which could confirm the results obtained from girdling.

The response of xylem cytokinins and re-growth to cane girdling treatments provides an explanation for reduced responses of fruit growth to girdling when increased percentages of canes are girdled on a vine. Although fruit cytokinin levels were not measured in this study, it is difficult to imagine a negative effect of increased cytokinins on fruit growth on girdled canes when a higher proportion of canes were girdled, unless increased fruit cytokinin concentration is inhibitory for fruit growth. It seems more likely that the reduced response of fruit on girdled canes from vines with a higher percentage of canes girdled could be due to interactions with vegetative growth. The correlation obtained here between increased vegetative growth and reduced response to girdling is similar to the results of Dann et al. (1984) and Cutting and Lyne (1993) although in the opposite direction and supports the notion that vegetative growth is inhibitory to fruit growth (Garcia-Martinez and Beltran, 1992). In another instance, kiwifruit growth was found to be reduced by large numbers of fully expanded leaves (Lai et al., 1989). Although the inhibitory effect of leaves on fruit growth reported by Lai et al. (1989) were found after anthesis when leaves were fully expanded, it is possible that the inhibitory effects had already occurred during pre-anthesis ovary development, when shoots were undergoing active growth. Competition for carbohydrate with vegetative growth is unlikely to explain the effect of vegetative growth on fruit growth on girdled canes, as there was an abundant carbohydrate supply due to a high leaf:fruit ratio. It seems more likely that the

rapidly growing shoot apex or expanding leaves on the re-growth could provide an inhibitory signal which reduces fruit growth. Dominance relationships have been found between kiwifruit (Lai et al., 1990; Chapter six) and both apical dominance of lateral buds and correlative dominance of fruit growth are both thought to operate by an inhibitory auxin signal (Bangerth, 1989). This suggests that vegetative growth on girdled canes may inhibit kiwifruit growth by emission of an inhibitory auxin signal.

In contrast to our results which showed increased vegetative growth after girdling of kiwifruit, a number of species including avocado (Ibrahim and Bahlool, 1979), peach (Dann et al., 1984), grape (Orth et al., 1989) and apple (Schechter et al., 1994), demonstrate reduced vegetative growth as a result of girdling. Dann et al. (1984) attributed the effects of girdling on increased fruit size of peaches to the reduction in vegetative growth which occurred, as there was no build up of carbohydrate associated with girdling. Cytokinin levels in apple were decreased in xylem exudate from apple trees following trunk girdling and this was associated with decreased shoot length (Skogerbo, 1992). Similarly levels of both cytokinins and gibberellins in xylem exudate were reduced in girdled peach branches compared with non-girdled peach branches on the same tree and this was associated with a decreased growth rate of shoots on girdled branches (Cutting and Lyne, 1993). This is consistent with the association between gibberellins and internode extension (Kaufman and Jones, 1974). Although gibberellins were not measured in cane girdled kiwifruit, the vegetative growth rate was not influenced by cane girdling treatments (Table 5.13), which suggests that xylem gibberellins may not have been affected by cane girdling of kiwifruit. In addition, the effect of cane girdling of kiwifruit on increased levels of xylem cytokinins was transitory with levels returning to normal by four weeks after girdling, while in the work of Cutting and Lyne (1993) the effect extended over at least seven weeks after girdling.

Reduction in the shoot auxin supply by decapitation of shoots has been demonstrated to result in an increase in root xylem exudate levels of cytokinins in kiwifruit cuttings in this study, in bean plants (Bangerth, 1994) and also in pea plant stem tissue (Li et al., 1995). Similarly, removal of buds from tomato and tobacco plants resulted in increased concentration of xylem exudate cytokinins (Colbert and Beever, 1981). Thus girdling would be expected to decrease the auxin signal reaching roots and therefore increase

rather than decrease xylem cytokinins as was suggested by Dann et al. (1984) and confirmed by Cutting and Lyne (1993). The differences between these studies and our own may be due to a different source-sink balance between leaves and roots in girdled peach and kiwifruit. While cane girdling as described here appears to allow adequate carbohydrate for continued root growth, other crops may not have the same luxury, which could result in carbohydrate deficit in roots and therefore reduced root growth and function, resulting in reduced export of growth regulators.

Although changes in xylem growth regulators in response to girdling reported by Cutting and Lyne (1993) were in the opposite direction to those that we have found, both studies demonstrated differences between branches within the same tree. An alteration in root synthesis or export of growth regulators in response to a reduced auxin signal (Bangerth, 1994) would not explain differences on the same tree unless partitioning of growth regulators in xylem between girdled and non-girdled parts of the plant occurs. It has been suggested that individual roots may preferentially supply individual branches, although lateral movement in xylem traces can occur (Kramer, 1995). A 2/5 leaf phylotaxy has been found in kiwifruit (Ferguson, 1984; Lai et al., 1988) as in other species, which demonstrates the possibility that specific vascular connections can occur. Preferential connection between individual branches and roots could in theory lead to a reduction in auxin signals to individual roots and thus return growth regulator signals could be directed to the same branches. Alternatively, cytokinins are known to be stored in bark tissues (Jameson et al., 1987) and may be released into xylem (Tromp and Ovaa, 1990 and references therein). Girdling may have resulted in increased release of these into the xylem, possibly as a result of reduced localised auxin concentrations below the girdle.

### 5.4.4 Return bloom

Increased percentages of cane girdling resulted in a significant reduction in return bloom of king flowers and a significant negative linear trend in compound flower clusters in experiment one (Table 5.3), but had no effect in experiment two. Effects on return bloom in experiment one were associated with a reduction in the number of floral shoots per

metre of cane. This is in contrast to results presented in chapter four, where only compound flower clusters were affected by girdling which suggested that cane girdling treatments increased abortion of lateral flowers. Return bloom in experiment one was particularly low due to a wind storm which damaged tender floral shoots soon after bud-burst. Thus it is possible that the physical strength of floral shoots was affected by cane girdling treatments, leading to increased breakage of floral shoots. Alternatively non-girdled vines may have been in less exposed positions in the orchard by chance. Due to apparent conflicting results, the effect of cane girdling treatments on return bloom cannot be considered to be conclusive. However it can be concluded that any effect of cane girdling on return bloom is probably small.

When all fruiting canes were girdled, the most severe treatment of 75% defoliation in this study, resulted in a reduction in return bloom of compound flower clusters of about 50% compared with non-defoliated vines (Table 5.4). Cruz-Castillo (1994) obtained similar results from defoliation of 50 or 75% of leaves in the RCZ of non-girdled kiwifruit vines, although return bloom of lateral flowers was reduced by over 80% when 75% of leaves were defoliated. This supports the suggestion that abortion of lateral flowers is sensitive to carbohydrate availability (Snelgar and Manson, 1992). However removal of 75% of leaves from the RCZ in vines where all fruiting canes were girdled is a massive reduction in the source of carbohydrate for RCZ fruit and root sinks, as well as return bloom requirements. Thus it is perhaps surprising that the effects on return bloom were relatively small. This suggests that return bloom may not be as sensitive to a change in source-sink balance as was previously thought (Buwalda and Smith, 1990b; Snelgar and Manson, 1992). It has been suggested that the 2/5 phyllotaxy in kiwifruit may effect the response of return bloom to defoliation, as canes with every third leaf removed showed a lower return bloom than canes with every second leaf removed (Snelgar and Manson, 1992). This could explain the more negative effects on return bloom found when canes are completely defoliated (Buwalda and Smith, 1990b; Tombesi et al., 1993), compared with when only a proportion of leaves on each cane are defoliated. For example, in the 75% defoliation treatment described in this chapter, most buds would have a 2/5 vascular connection to at least one or two leaves on most replacement shoots.

### 5.4.5 Leaf gas exchange water relations and root growth

A hypothesis was proposed in chapter four to explain the reduction in fruit growth in both girdled and non-girdled parts of vines where an increased percentage of canes were girdled:

*The negative responses of fruit growth to increased cane girdling are due to reduced carbohydrate availability to the root system which reduced root function, growth and/or absorption and transport of water and mineral nutrients to the shoots, which affected fruit growth directly, or indirectly by an effect on leaf photosynthesis.*

Based on the evidence presented in this chapter, this hypothesis is rejected for the following reasons:

1. Increased defoliation of the RCZ of vines where all canes were girdled could be expected to reduce carbohydrate supply to roots. Defoliation of up to 75% of leaves in the RCZ had no significant effect on fruit size from girdled canes. This suggested that fruit growth was not indirectly inhibited by results of limited carbohydrate supply to roots.
2. There were no significant effects of girdling on white root incidence, which is considered to represent recent root growth (Atkinson, 1989; Buwalda and Hutton, 1988).
3. The rate of photosynthesis (Pn), stomatal resistance (rs) and mid-day leaf xylem water potential ($\psi_{xylem}$) of kiwifruit leaves have been demonstrated to be sensitive to water stress (Chartzoulakis et al., 1993; Van Oostrom, 1985; Smith et al., 1990). These indicators of water stress were not significantly affected by the percentage of canes girdled on a vine, although on individual canes Pn and rs were affected by girdling itself.
4. Evidence is accumulating that xylem ABA plays a key role in signalling water stress from roots to shoots, even when no detectable shoot water deficits have occurred

(Zhang et al., 1990), however there were no significant effects of cane girdling on xylem ABA levels.

The minimum mid-day $\psi_{xylem}$ of leaves on non water stressed kiwifruit vines was reported at -0.65 Mpa, while on water stressed vines, it dropped as low as -1.2 Mpa (Van Oostrom, 1985). Wilting of kiwifruit leaves was apparent at a $\psi_{xylem}$ of -0.75 Mpa (Van Oostrom, 1985). This suggests that leaves with a mid-day $\psi_{xylem}$ of below -0.65 Mpa could be indicative of water stress, although other factors such as location and ontogeny of vines will cause such a value to vary. Mean mid-day $\psi_{xylem}$ in this study reached -0.65 Mpa on 13 January and on this date almost one third of all leaves measured were below -0.65 Mpa and around 10% were below -0.75 Mpa, suggesting that vines were probably under water stress on this date. However there were no significant differences in $\psi_{xylem}$ between vines with different percentages of canes girdled on this or any other date. Stress on this date is probably attributable to the very high light intensity and air temperature which occurred.

Although photosynthesis and stomatal conductance appeared to be consistently lower on leaves arising from girdled canes (Table 5.7), this cannot be attributed to the effect of girdling on root processes, but must be due to an effect of girdling on processes within the cane itself. This is probably as a result of limited sink demand as has been found previously on leaves from girdled plants (Marquard, 1987; Schaper and Chacko, 1993). The contribution of stomatal closure to a decrease in Pn depends on the extent that internal $CO_2$ concentration (Ci) is also decreased (Farquhar and Sharkey, 1982). If increased stomatal resistance is the cause of decreased Pn, then it would be expected that the Ci would be depleted to a lower level. Ci was not significantly depleted on leaves from girdled canes on 3 and 16 February while Pn was significantly lower on leaves from girdled canes on these two dates. However on 13 January when leaves from girdled and non-girdled canes had the greatest difference in Pn, Ci was significantly decreased on leaves from girdled canes. Thus it appears that the decrease in photosynthetic rate of leaves on girdled canes may be mostly due to inhibition of photosynthesis at the mesophyll, although this may be accentuated by increased stomatal resistance under condition of water stress.

In the kiwifruit vines in this study, total brown root length density averaged 8.54 $Km.m^{-2}$ in a 60cm deep core. This is comparable to the total root length density in 100cm deep cores of kiwifruit vines in April of 9.58 $Km.m^{-2}$ reported by (Buwalda and Hutton, 1988) and 5.46 $km.m^{-2}$ (Buwalda and Smith, 1990b). The root length density of white roots averaged 0.15 $km.m^{-2}$, which is somewhat lower than reported kiwifruit white root length densities. For example calculations from data presented by Buwalda and Hutton (1988) and by Buwalda and Smith (1990b) showed that kiwifruit white root length densities measured at a similar time of year were approximately 0.545 and 0.328 $km.m^{-2}$ respectively in 1m deep cores. However root growth is sensitive to external influences such as soil moisture (Gowing and Davies, 1989), aeration (Box et al., 1989) and temperature (Logsdon et al., 1987), thus variation between locations and seasons are not unexpected.

Root measurements were made on treatment combinations which were considered likely to directly affect the availability of carbohydrate from the RCZ for root system. Complete defoliation of the RCZ at full bloom by Buwalda and Smith (1990b) resulted in approximately a two third decrease in white root incidence. Thus 50% defoliation treatments imposed on the RCZ in this study might have been expected to affect root growth. On the other hand, removal of the fruit sink from the RCZ might have been expected to have stimulated root growth. Although not statistically significant, manipulation of the source:sink ratio in the RCZ by thinning and defoliation treatments appeared to have a possible effect on white root incidence, with thinned vines having double the white root incidence of defoliated vines, while control vines had median white root incidence (Table 5.9). This is in accordance with the results of Buwalda and Smith (1990b) and may confirm the sensitivity of kiwifruit root growth to the availability of carbohydrate, although more research of this kind is required to confirm statistical significance as the variability of root growth between vines and between samples is high.

Detection of mineral deficiencies in kiwifruit as well as many other crops is normally confirmed by leaf analysis (Smith et al., 1987). Thus critical values of minerals for fruit growth are not available and it is not clear what effects fruit mineral deficiencies may have, independent of effects of leaf deficiency on the availability of photo-assimilates. A

correlation between kiwifruit leaf and fruit phosphorus levels has been found (Velemis and Manolakis, 1996). Similarly, positive correlations between leaf and fruit levels of several different minerals have been found in other species such as strawberry (May and Pritt, 1993) and olive (Jordao et al., 1990), although such relationships are by no means ubiquitous and frequently no correlation can be found (Marcelle, 1990). However there were no significant differences in fruit mineral contents between any cane girdling treatments in our study. In addition, the mineral content of fruit were found to be similar to those reported elsewhere for kiwifruit not experiencing mineral deficiency. Values were slightly higher than those reported for mature kiwifruit flesh from well fertilised, high yielding vines by Clark and Smith (1988) and were within the range of values for kiwifruit summarised by Beever and Hopkirk (1990). Thus it seems unlikely that cane girdling treatments resulted in any mineral deficiency in vines in this study.

## 5.5 Summary

The aim of experimental work described in this chapter was to investigate the negative responses of fruit growth in girdled and non-girdled parts of vines, when an increased percentage of canes are girdled. The hypothesis that reduced carbohydrate availability to the root system reduced root function was rejected after consideration of evidence presented in this chapter. Firstly, severe defoliation of source leaves available to supply roots with carbohydrate had no effect on fruit growth within girdled canes. Secondly, there was no effect of cane girdling treatments on vine water status, leaf photosynthesis, stomatal resistance, root growth and fruit mineral content. The second hypothesis that interruption of the auxin signal out of canes by girdling reduced growth regulator signals from roots to shoots, therefore reducing the sink strength of fruit, was also rejected as cane girdling actually resulted in increased cytokinin levels in extracted xylem exudate. A new hypothesis is proposed to explain the reduced response to girdling on vines with a higher percentage of canes girdled:

*A severe reduction in the auxin signal from the shoot system to the root system leads to an increase in cytokinin supply to the shoot system via root xylem exudate. Associated*

*with this, or due to remobilisation of cytokinins from stored reserves in the stems, bud-break was higher on girdled canes from vines with a higher percentage of canes girdled and the consequent vegetative growth results in decreased fruit size due either to competition for assimilates or an inhibitory signal.*

The main evidence in support of this hypothesis is as follows:

1. Girdling of kiwifruit cuttings mimicked the effects that removal of shoot auxin supply by decapitation had, by increasing cytokinin levels in root xylem exudate.
2. Cane girdling on whole vines also resulted in increased cytokinins in extracted xylem exudate.
3. Bud-break and subsequent vegetative growth from lateral buds was correlated to cytokinins in extracted xylem exudate.
4. The girdling of a higher percentage of canes on a vine increased cytokinin levels in extracted xylem exudate from girdled canes and also increased bud-break and subsequent vegetative growth on these canes.
5. An inhibitory effect of high numbers of leaves on fruit growth has been reported previously (Lai et al., 1989).

Although this provides an explanation for responses on girdled canes, no explanation was found for the reduction in the weight of fruit from non-girdled parts of vines when increased number of canes are girdled. At this stage we can conclude that a number of obvious *a priori* factors have been eliminated, but we have found no satisfactory explanation for this effect.

In addition to these effects, it was postulated that part of the positive response to cane girdling could have been due to increased levels of xylem cytokinins reaching fruit. Further investigation is needed to clarify the role of endogenous cytokinins for fruit growth and to establish if kiwifruit obtain cytokinins from xylem.

The photosynthetic rate of leaves from girdled canes was reduced compared with non-girdled canes, although this was not affected by the percentage of canes girdled on a vine. It was concluded that kiwifruit leaves are subject to feedback limitation to photosynthetic rate at high leaf:fruit ratio.

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# 6. The Contribution of Seeds to Kiwifruit Growth and Interactions with Genotype

## 6.1 Introduction

Kiwifruit growth is highly dependent on the formation of a high number of viable seeds. In commercial orchards, pollination is one of the most crucial events in the production cycle, which is highlighted by the considerable effort placed into developing systems for both insect pollination (Jay and Jay, 1984; Matheson, 1991; Goodwin et al., 1991) and artificial pollination techniques (Hopping, 1982; Hopping and Jerram, 1980; Stevenson, 1990). There is a well established positive relationship between seed number in *A. deliciosa* kiwifruit, especially for *A. deliciosa* 'Hayward' (Hopping, 1976a; Pyke and Alspach, 1986; Lai et al., 1990; Snelgar et al., 1992). A 100g 'Hayward' kiwifruit contains approximately 1000 to 1200 seeds on average (Pyke and Alspach, 1986), which requires deposition of 1000 to 3000 pollen grains on the stigma of the flower (Matheson, 1991). However there is significant variation in the relationship and fruit with low seed numbers can grow to a larger size than fruit with high seed numbers (Lai, 1987). Hopping (1976a) reported a similar relationship for the *A. deliciosa* cultivars 'Monty' and 'Standard' to that found in 'Hayward'. Wu et al. (1994) reported a new variety of kiwifruit 'Xyangzhou 83802' (species not stated) which averaged 56g and had only 27-32 seeds per fruit. However there have been no reports published on the relationship between seeds and fruit growth in species of kiwifruit other than for *A. deliciosa*. With the recent effort placed into the selection of new varieties of kiwifruit, information on relationships of seed number and fruit weight for the new cultivars will be important for optimising production.

The relationship between seeds and fruit weight can be further improved if fruit weight is plotted against total weight of the seeds in the fruit (Pyke and Alspach, 1986; Lai, 1987). Seeds are thought to be a source of growth regulators which have a role in promoting fruit growth (Crane, 1969) and it is possible that larger seeds may produce a higher level of growth regulators, thus enabling increased fruit growth. However in this case it is difficult to establish whether there is a causal relationship, or whether large fruit also

have large seeds due to improved supply of assimilates. Grant and Ryugo (1984) found that kiwifruit from exposed lateral shoots, had a higher weight per seed and had larger seeds than fruit from shaded lateral shoots. Although this might suggest that seeds were larger because the fruit were from a well exposed canopy position, it is also possible that seed growth was stimulated independently and fruit growth increased as a result.

Kiwifruit appear to be subject to dominance, specifically growth of kiwifruit with low seed numbers is inhibited by the presence of nearby kiwifruit with high seed numbers (Lai et al., 1990). Similarly, dominance has been found to be related to the presence or number of seeds in pea (Tamas et al., 1986), zucchini (Stephenson et al., 1988) and persimmon (Kitajima et al., 1992). Dominance relationships in other species are often related to the time of fruit set, with fruits which set first often dominant over later setting fruits (Bangerth and Ho, 1984; Bohner and Bangerth, 1988b). Although kiwifruit ovaries which arise early grow to a larger size than ovaries which arise late (Lai et al., 1990; Cruz-Castillo, 1994), this is not attributed to dominance but to the larger size of early ovaries at anthesis. The mechanism by which dominance is exerted is thought to be inhibitory signals emitted from dominant organs. Bangerth (1989) has proposed that polar indole-3-acetic acid (IAA) export from dominant organs causes autoinhibition of polar IAA transport from inhibited organs and that polar IAA export is essential for a growing organ. This is supported by evidence that both dominant apple and tomato fruits show higher total amounts of IAA diffused through pedicels than inhibited fruit (Gruber and Bangerth, 1990) and is consistent with the correlation between seeds and IAA export (Bangerth, 1989 and references therein).

It has been suggested that fruit cell number is an important factor which limits the sink strength of fruit and therefore the size which they attain (Ho, 1988; Chapter three). Variation between fruit size of different kiwifruit genotypes could be due to different numbers of cells present in the ovary. For example, fruit from large fruited peach cultivars were found to have up to 3.7 times as many cells in cross section as fruit from small fruited peach cultivars (Scorza et al., 1991). Similarly, differences in fruit size of four different strawberry cultivars could be attributed almost entirely to the number of receptacle cells (Cheng and Breen, 1992). A comparison between *A. deliciosa* 'Monty' (Hopping, 1976b) and 'Hayward' (Woolley et al., 1992; Cruz-Castillo, 1994) suggests

that differences in fruit size between these cultivars could be due to cell number differences (chapter one, section 1.2.5). Although measurements of cell size have recently been made in one selection of *A. chinensis* (Clayton, 1996), no attempt has been made to determine cell numbers in any *Actinidia* species other than *A. deliciosa.*

The aim of experimental work described in this chapter was to obtain further information on relationships between seed number or seed weight and kiwifruit growth in 'Hayward' and extend this to other kiwifruit selections from three *Actinidia* species. The dominance relationship reported by Lai et al. (1990) was further investigated and the relationship of seed number and inter-fruit competition to IAA export was ascertained. Correlations were also established between fruit cell number and fruit size of several kiwifruit selections. The relationship between fruit cell number and seed weight in different kiwifruit selections suggested that cell division may be limited at low seed weight and this hypothesis was tested by controlled reduction of seed numbers in 'Hayward' kiwifruit and subsequent measurements of cell numbers from both transverse and longitudinal fruit sections.

## 6.2 Materials and Methods

### 6.2.1 The effect of inter-fruit competition and leaf:fruit ratio on fruit size of kiwifruit

Pairs of flowers on indeterminate well exposed lateral shoots were selected prior to opening and fruit seed number was manipulated by style excision as described in chapter two, although hand pollination of flowers was not performed. Low seed numbers (L-fruit) were induced in fruit by reducing the number of styles to five. High seed numbers (H-fruit) were induced by leaving all styles intact.

Experiment one: High seeded fruit

Experimental design was a randomised complete block design with a factorial treatment structure. Two levels of inter-fruit competition treatment were imposed by inducing either high seed numbers (H) or low seed numbers (L) in adjacent pairs of fruit on a lateral shoot resulting in either HL or HH pairs. Inter-fruit competition treatments were applied randomly in combination with two levels of leaf:fruit ratio (1:1, 5:1) on six lateral shoots on each of four vines. Half of the lateral shoots were girdled at 21 days after full bloom (DAFB) as described in chapter three, while the other half were left intact. At commercial maturity fresh weight, dry weight and seed numbers were estimated as described in chapter two for the H-fruit only.

Experiment two: Low seeded fruit

Experimental design was as in experiment one described above, except that inter-fruit competition treatments consisted of LL or LH pairs of fruit on lateral shoots. Measurements were made as in experiment one but for the L-fruit only.

### 6.2.2 The effect of inter-fruit competition, girdling and seed number on IAA exuded by kiwifruit

Flowers were selected and styles were manipulated as described in section 6.2.1 to give pairs of fruit on lateral shoots with either two adjacent high seeded fruit (HH), a high and a low seeded fruit (HL) or two low seeded fruit (LL). At 21 DAFB, leaf:fruit ratio was adjusted to two and girdles were randomly applied to half of the lateral shoots.

At mid-day on 22, 29 and 49 DAFB, six pairs of fruit per competition type with the pedicels attached were harvested by cutting the pedicel near the base with a sharp razor blade. Fruit were immediately placed with the pedicels in vials containing 5ml Phosphate buffer (0.01M pH 6.2). IAA was allowed to diffuse basipetally from the fruit via the pedicel into the buffer solution for 10 hours at 20°C. Vials and fruit were held at 100% humidity to avoid transpirational xylem uptake of buffer.

Buffer solution was lyophilised and stored under $N_2$ gas. RIA of IAA was performed by D. Woolley in the laboratory of Dr. F. Bangerth, Hohenheim, Germany.

### 6.2.3 Generation of growth curves for low and high seeded kiwifruit

Flowers were selected and styles manipulated prior to opening as described in section 6.2.1 to give either low or high seeded fruit, but leaf:fruit ratio was not manipulated and girdles were not applied. From 14 DAFB up until harvest, regular measurements were made on each fruit of length, maximum equatorial diameter ($D_{max}$) and minimum equatorial diameter ($D_{min}$). At 157 DAFB, other fruit on the vines reached commercial maturity and fruit being progressively measured were harvested, fresh weight and dry weight obtained and surface seed numbers counted. The product of $LxD_{max}xD_{min}$ correlates extremely well to fruit weight over the entire season (chapter two) and was used to predict fruit weight for growth analysis.

### 6.2.4 Seeds, cell number and cell size in *A. deliciosa*, *A. chinensis* and *A. arguta* selections

This experiment was designed to ascertain the contribution of seeds and cell number to fruit size of different kiwifruit selections. Kiwifruit from three different species were used:

1. Three selections of *A. deliciosa* differing in fruit size were obtained from the Massey University fruit crops unit, Palmerston North (lat. $40^{0}23'$). The selections were 'Hayward' (normal sized fruit), '87-9-106' (small fruit) and '87-4-76' (large fruit). Fruit were from vines with a crop load of 20 fruit/$m^2$.
2. *A. chinensis* fruit were obtained from the HortResearch kiwifruit breeding programme in the Te Puke Research Centre orchard, Te Puke, New Zealand (lat. $37^{o}48'$). Fruit of a wide range of sizes present on vines were obtained from two selections; 'Earligold' (moderate crop load) and '37.6.6a' (high crop load).
3. *A. Arguta* fruit were obtained from 8 year old vines grafted onto Bruno seedling rootstock from Kotunga kiwifruit orchard, Golden Bay, New Zealand (lat 40•51'). Crop load was estimated at 180 fruit/$m^2$.

Relationships were established between seed numbers, seed weight and fruit weight of A. chinensis 'Earligold', *A. chinensis* '37.6.6a' and *A. arguta.* Seeds were separated from fruit pulp, dried to a constant weight in a ventilated oven at 70°C, counted and weighed. Extensive seed number, seed weight and fruit weight data was obtained from *A. deliciosa* 'Hayward' previously (chapter two, section 2.5.1) and the data is presented in this chapter.

For the measurement of fruit cell numbers, well pollinated (according to species) fruit were obtained from each selection. Fresh weight, seed number, seed weight, length, $D_{max}$ and $D_{min}$ were measured as described in chapter two. Fresh transverse sections were taken from each fruit and cell numbers and size were estimated across the minimum equatorial diameter of core, inner pericarp and outer pericarp tissue as described in chapter two.

### 6.2.5 Effect of seed number on cell number and size in transverse and longitudinal sections of 'Hayward' kiwifruit

Experimental design was a randomised complete block design. Two flowers from each of four 'Hayward' kiwifruit vines were selected from well exposed indeterminate fruiting lateral shoots prior to opening. Style numbers were reduced to two styles or not manipulated and immediately hand-pollinated to give either low or high seeded fruits as described in chapter two. Fruit were harvested at maturity in May 1996, permanent sections were made in both the transverse and longitudinal directions and cell numbers and size were estimated as described in chapter two.

## 6.3 Results

### 6.3.1 Inter-fruit competition, girdling and leaf:fruit ratio

The reduction of style numbers to five styles decreased the number of seeds present in fruit, although there was some variation in seed number between fruit with the low style number. It appears possible that some pollination may have occurred prior to de-styling, or that pollen tubes growing down the remaining styles are able to pollinate ovules in several locules when style number is low.

Experiment one: high seeded fruit

Pollination was relatively poor, with high seeded fruit (H-fruit) averaging only 492 seeds. However seed numbers were still high in comparison to low seeded fruit (L-fruit) . There was a significant interaction between girdling and leaf:fruit ratio on the fresh and dry weight of H-fruit. In the absence of a girdle, leaf:fruit ratio did not significantly affect fruit weight (Table 6.1) which averaged 82g. However when a girdle was applied, H-fruit weight was increased to 128g at a leaf:fruit ratio of five, but was decreased to 61g at a leaf:fruit ratio of one (Table 6.1).

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**. Due to the presence of missing values, means presented are population marginal means[1] and are back-transformed from $\log_{10}$.

| Girdle | Leaf:fruit ratio | Fruit fresh weight *(High seed fruit)* (g) | Fruit dry weight *(High seed fruit)* (g) | Fruit seed number *(High seed fruit)*[2] |
|---|---|---|---|---|
| + | one | 61 c | 6.3 c | 546 a |
| + | five | 128 a | 19.1 a | 543 a |
| - | one | 87 b | 13.0 b | 596 a |
| - | five | 77 b | 10.7 b | 467 a |

Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).
[1] For description of population marginal means, see chapter two, section 2.8.3.
[2] ANOVA interaction not significant (P=0.4918) for seed number.

Although there was no significant interaction between inter-fruit competition and girdling or leaf:fruit ratio, there was some evidence that the weight and seed number of H-fruit was affected by the main effect of inter-fruit competition. When H-fruit were in competition with L- fruit, fresh weight averaged 92g. However when competition was with another H-fruit, fresh weight was reduced to 78g (Table 6.2). The decrease in fruit weight due to competition was associated with a significant decrease in seed numbers from 635 to 452 seeds per fruit (Table 6.2).

Table 6.2 The effect of an adjacent, competing fruit (fruit B) containing high or low seed numbers, on the fresh weight, dry weight and seed number of a **high seeded kiwifruit** (fruit A). Due to the presence of missing values, means presented are population marginal means and are back-transformed from $\log_{10}$.

| Competing fruit (fruit B) | Fresh weight of fruit A *(High seed fruit)* (g) | Dry weight of fruit A *(High seed fruit)* (g) | Seed number of fruit A *(High seed fruit)* |
|---|---|---|---|
| Low seed | 92 | 12.4 | 635 |
| High seed | 78 | 10.5 | 452 |
| Contrast (high vs. low) | 0.0678 | 0.1199 | 0.0524 |

Experiment two: low seeded fruit

There was a significant interaction between girdling and leaf:fruit ratio on the weight of L-fruit. At a leaf:fruit ratio of one, there was no difference in fresh or dry weight between fruit from girdled and from non-girdled shoots. However at a leaf:fruit ratio of five on girdled lateral shoots, fresh and dry weight of L-fruit was increased to almost double any other treatment combination (Table 6.3). In addition, the fresh weight of L-fruit from non-girdled shoots with a leaf:fruit ratio of five was over 11g higher than fruit from either girdled or non-girdled shoots with a leaf:fruit ratio of one (Table 6.3). The seed number of L-fruit appeared to be influenced by the leaf:fruit ratio (Table 6.3) and was significantly lower at a leaf:fruit ratio of one (97 seeds), compared with a leaf:fruit ratio of five (185 seeds) (contrast, P=0.0078).

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**. Due to the presence of missing values, means presented are population marginal means and are back-transformed from $\log_{10}$.

| Girdle | Leaf:fruit ratio | Fruit fresh weight *(Low seed fruit)* (g) | Fruit dry weight *(Low seed fruit)* (g) | Fruit seed number *(Low seed fruit)*[1] |
|---|---|---|---|---|
| + | one | 28 c | 3.1 cd | 94 b |
| + | five | 76 a | 11.1 a | 183 a |
| - | one | 30 c | 4.0 bc | 102 ab |
| - | five | 41 b | 5.7 b | 187 a |

Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).
[1] ANOVA interaction not significant (P=0.8989) for seed number.

There was also a significant interaction between girdling and inter-fruit competition on the weight of L-fruit. On non-girdled lateral shoots, competition did not have a significant effect on fruit weights (Table 6.4). However on girdled shoots, fresh and dry weight of L-fruit were reduced by over 50% when they were in competition with H-fruit compared with competition with L-fruit (Table 6.4).

There were also highly significant main effects of inter-fruit competition on fruit weight and seed number, although the significant interaction which occurred between girdling and competition on fruit weight should be held in mind. Both fresh and dry weight of L-fruit was almost doubled when competition was with an L-fruit compared with fruit competing with an H-fruit (Table 6.5). The effect of inter-fruit competition was associated with significantly higher fruit seed numbers in L-fruit when they were competing with L-fruit (193 seeds) than when they were competing with H-fruit ( 94 seeds) (Table 6.5).

Table 6.4 The effect of an interaction between girdling and competition with an adjacent fruit (fruit B) containing high or low seed numbers, on the fresh weight, dry weight and seed number of a **low seeded kiwifruit** (fruit A). Due to the presence of missing values, means presented are population marginal means and are back-transformed from $\log_{10}$.

| Girdle | Competing fruit (fruit B) | Fresh weight of fruit A *(Low seed fruit)* (g) | Dry weight of fruit A *(Low seed fruit)* (g) | Seed number of fruit A *(Low seed fruit)*[1] |
|---|---|---|---|---|
| + | High seed | 32 b | 3.7 b | 78 c |
| + | Low seed | 68 a | 9.2 a | 221 a |
| - | High seed | 31 b | 4.1 b | 113 bc |
| - | Low seed | 40 b | 5.5 b | 168 ab |

Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).
[1] ANOVA interaction not significant (P=0.1699) for seed number.

Table 6.5 The effect of an adjacent competing fruit (fruit B) containing high or low seed numbers, on the fresh weight, dry weight and seed number of a **low seeded kiwifruit** (fruit A). Due to the presence of missing values, means presented are population marginal means and are back-transformed from $\log_{10}$.

| Competing fruit (fruit B) | Fresh weight of fruit A *(Low seed fruit)* (g) | Dry weight of fruit A *(Low seed fruit)* (g) | Seed number of fruit A *(Low seed fruit)* |
|---|---|---|---|
| High seed number | 31 | 3.9 | 94 |
| Low seed number | 52 | 7.1 | 193 |
| Contrast (high vs. low) | 0.0001 | 0.0001 | 0.0031 |

### 6.3.2 IAA diffused from kiwifruit pedicels

IAA was detected in the diffusate of kiwifruit in this experiment. There were no significant interactions between girdling, de-styling or inter-fruit competition. There was

also no significant effects of fruit seed treatment or inter-fruit competition on IAA exuded (Table 6.6). H-fruit had an average IAA flux of 1.32 $pMol.fruit^{-1}.hour^{-1}$ and this was not significantly different to L-fruit which averaged 1.68 $pMol.fruit^{-1}.hour^{-1}$ (contrast, P=0.1833). Although this result cannot be viewed as entirely conclusive as it was not possible to confirm seed numbers at this stage of fruit growth, there is no reason to believe that style manipulation did not result in a reduction in seed number similar to that found in previous experiments. For example, in an experiment conducted in the same season on nearby vines, style manipulation resulted in a reduction in average seed number from 1150 to 600 seeds.

The IAA exuded by fruit was however significantly affected by measurement date and girdling of the lateral shoot. IAA flux was significantly higher on average at 49 DAFB than at either 29 or 22 DAFB (Table 6.7). Although IAA flux was not significantly higher at 29 DAFB than at 22 DAFB, a linear contrast over the three measurement dates was highly significant (P=0.0001), giving evidence of a trend of increasing IAA flux over this period of kiwifruit growth.

Table 6.6 IAA exuded basipetally from kiwifruit with low or high seed numbers (fruit A) through their pedicels when in competition with an adjacent low or high seeded fruit (fruit B). Values are the average from three measurement dates (22, 29 and 49 days after full bloom). Due to the presence of missing values, means presented are population marginal means.

| Competing fruit (fruit B) | Mean IAA flux ($pMol.fruit^{-1}.hour^{-1}$) from a kiwifruit (fruit A) | | |
|---|---|---|---|
| | High seeded fruit | Low seeded fruit | Average |
| High seed | 1.18 a | 1.73 a | 1.44 |
| Low seed | 1.48 a | 1.64 a | 1.56 |

Means with common letters are not significantly different at P=0.05 (lsmeans, SAS).

Fruit from non-girdled shoots had a higher IAA flux than fruit from girdled shoots at all three measurement dates (Table 6.6), although differences were only statistically significant at 49 DAFB (contrast, P=0.0209). Over all the treatment dates, fruit from

non-girdled shoots had an average IAA flux of 1.75 $pMol.fruit^{-1}.hour^{-1}$ which was significantly higher than fruit from girdled shoots which averaged 1.26 $pMol.fruit^{-1}.hour^{-1}$ (contrast, P=0.0464).

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. Due to the presence of missing values, means presented are population marginal means.

| Days after full bloom | Mean IAA flux ($pMol.fruit^{-1}.hour^{-1}$) | | |
|---|---|---|---|
| | Girdled | Non-girdled | Average |
| 22 | 0.68 b | 0.87 b | 0.78 b |
| 29 | 1.03 b | 1.30 b | 1.16 b |
| 49 | 2.06 a | 3.07 a | 2.57 a |

Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS)

### 6.3.3 The effect of seed number on kiwifruit growth

Growth curves of fruit weight and individual fruit dimensions were obtained for fruit which had been separated for presentation into three categories of seed number: 0-400, 400-600 and over 1000 seeds. Data from fruit with between 600 and 1000 seeds are not presented, but showed similar trends to the other seed categories. Fruit from lower seed number categories always had significantly lower fresh and dry weight (Table 6.8). Of the individual fruit dimensions, length was reduced to a greater extent than either maximum or minimum equatorial diameter. Fruit length was reduced by 24% in fruit containing less than 400 seeds compared with fruit containing 1000+ seeds, while diameter was only reduced by between 9 and 11% (Table 6.8).

The increase in fruit weight over the entire growing season was strongly dependent on the fruit seed number (Figure 6.1), with fruit from low seed categories demonstrating lower fruit weight at maturity and throughout development. Fruit growth appeared to approximate a double sigmoid curve although this was most pronounced in lower seed

categories and the double sigmoid was more difficult to visualise when seed number was greater than 1000 (Figure 6.1). The double sigmoid shape was characterised by a period approximating exponential growth which occurred up until 42 DAFB (stage I). This was followed by a 'lag' phase (stage II) between 42 and 63 DAFB during which time growth of fruit slowed. Finally a further period of steady growth occurred until 157 DAFB (stage III) at which time fruit were harvested. The average growth rate (AGR) of fruit with more than 1000 seeds peaked at around 1.8 $g.day^{-1}$ at 42 DAFB and levelled out to an almost consistent growth rate of about 0.5 $g.day^{-1}$ at around 63 DAFB. In fruit with less than 400 seeds, the initial peak of absolute growth rate in stage I was just over half that of fruit containing 1000+ seeds and declined to a steady growth rate in stage III of around 0.3 $g.day^{-1}$ (Figure 6.1). In all three stages of fruit growth, average growth rate (AGR) was significantly lower in fruit from lower seed number categories (Table 6.9). AGR at maturity was still dependent on the seed number category, although differences were not statistically significant between fruit categories with more than 400 seeds (Table 6.9).

Table 6.8 Final weights and dimensions of kiwifruit which had low, medium or high seed numbers resulting from style excision. Due to uneven replication, means presented are population marginal means.

| Number of seeds in the fruit | Fresh weight (g) | Dry weight (g) | Length (mm) | Maximum equatorial diameter (mm) | Minimum equatorial diameter (mm) |
|---|---|---|---|---|---|
| 0-400 (n=15) | 67 c | 8.7 c | 51 c | 51 c | 45 c |
| 400-600 (n=14) | 93 b | 12.8 b | 61 b | 54 b | 49 b |
| >1000 (n=44) | 114 a | 16.5 a | 67 a | 56 a | 51 a |

Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).

Table 6.9 Average growth rate of kiwifruit which had low, medium or high seed numbers resulting from style excision, in stage I (14 to 42 DAFB[1]), stage II (42 to 63 DAFB), stage III (63 to 138 DAFB) and in mature fruit (138-157 DAFB). Due to uneven replication, means presented are population marginal means.

| Number of seeds in fruit | Average growth rate ($g.day^{-1}$) | | | |
|---|---|---|---|---|
| | Stage I | Lag phase (stage II) | Stage III | Mature |
| 0-400 (n=15) | 0.77 c | 0.67 c | 0.32 c | 0.32 b |
| 400-600 (n=14) | 1.02 b | 0.98 b | 0.43 b | 0.40 a |
| 1000 (n=44) | 1.23 a | 1.24 a | 0.49 a | 0.46 a |

Average growth rate was estimated from sequential non-destructive measurements on the same fruits.
Means in a column with common letters are not significantly different at P=0.05 (lsmeans, SAS).
[1] DAFB: Days after full bloom

### 6.3.4 The fruit size and shape of *Actinidia* selections

Vines of the large '87-4-76' and the small '87-9-106' *A. deliciosa* selections were morphologically similar to 'Hayward' vines in respect to canopy growth characteristics, apart from the size of fruits. A number of fruit on the '87-9-106' vines abscised almost immediately after anthesis and crop load was low as a result. For this reason a low crop load was established on the other *A. deliciosa* selections in this study, so that the three selections would be comparable.

The kiwifruit species and selections used in this study represented a wide range in fruit size and this was reflected in the fruit dimensions (Table 6.10). Fruit of the three *A. deliciosa* selections ranged in size from 23g in *A. deliciosa* '87-9-106' up to 176g in *A. deliciosa* '87-4-76' (Table 6.11). *A. deliciosa* '87-9-106' was smaller in all individual dimensions, however the most obvious reduction in size compared with other 'Hayward' selections was due to fruit being extremely short in length (Table 6.10). *A. deliciosa* '87-4-76' was larger than 'Hayward' in both length and diameters, although the diameter to length ratio was 10% lower than 'Hayward' (Table 6.10).

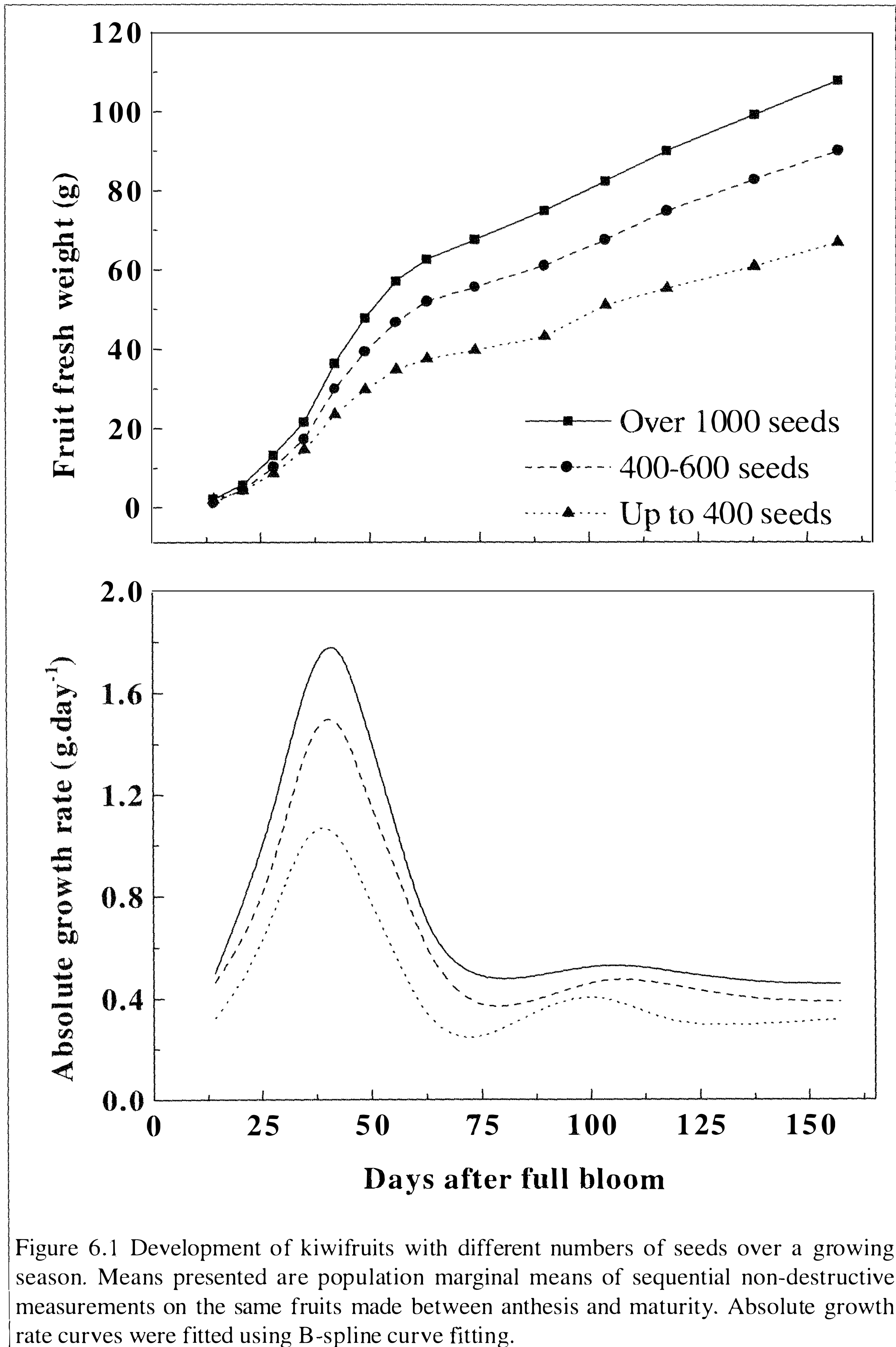


Figure 6.1 Development of kiwifruits with different numbers of seeds over a growing season. Means presented are population marginal means of sequential non-destructive measurements on the same fruits made between anthesis and maturity. Absolute growth rate curves were fitted using B-spline curve fitting.

Table 6.10 Fruit shape characteristics of fruits from different *Actinidia* selections used for determination of cell characteristics (n=10).

| Species | Length (mm) | $D_{max}$ [1] (mm) | $D_{min}$ [2] (mm) | $\frac{D_{min}}{D_{max}}$ | $\frac{D_{min}}{Length}$ |
|---|---|---|---|---|---|
| *A. deliciosa* ''Hayward'' | 63.4 c | 55.1 b | 50.1 b | 0.91 b | 0.79 b |
| *A. deliciosa* '87-4-76' (Large) | 79.9 a | 61.5 a | 56.3 a | 0.92 b | 0.71 c |
| *A. deliciosa* '87-9-106' (Small) | 32.5 e | 35.7 e | 33.1 e | 0.93 ab | 1.02 a |
| *A. chinensis* 'Earligold' | 69.5 b | 49.6 c | 46.2 c | 0.93 ab | 0.66 c |
| *A. chinensis* '37.6.6a' | 51.2 d | 43.7 d | 41.6 d | 0.95 a | 0.81 b |
| *A. arguta* | 18.9 f | 17.2 f | 14.7 f | 0.86 c | 0.79 b |

Means in a column with common letters are not significantly different at P=0.05 (LSD)

[1] $D_{max}$: Maximum equatorial diameter.

[2] $D_{min}$: Minimum equatorial diameter.

Both *A. chinensis* selections had smaller fruit size than 'Hayward' (Table 6.11). However well pollinated 'Earligold' fruit would have been a suitable size for export if the present 'Hayward' size criteria was applied. The larger fruit size of 'Earligold' compared with *A. chinensis* '37.6.6a' was mostly due to differences in fruit length, as diameters were only slightly different (Table 6.10). Both *A. chinensis* selections had a relatively high $D_{min}/D_{max}$ ratio (Table 6.10) indicating roundness of the fruit rather than the flat shape which is often found in 'Hayward' (Plate 6.3). This is likely to be a desirable characteristic, as excessively flattened 'Hayward' kiwifruit are rejected for export. *A. arguta* fruit were comparable in shape to *A. deliciosa* selections, although they appeared to be very flat shaped, which is indicated by a very low $D_{min}/D_{max}$ ratio (Table 6.10). Fruit were very small in size, averaging only 3.4g (Table 6.11).

Table 6.11 Fresh weight, seed number, average weight per seed and number of locules in fruits from different *Actinidia* selections used for determination of cell characteristics (n=10).

| Species | Fruit fresh weight (g) | seed number | seed size (μg) | Locule number |
|---|---|---|---|---|
| *A. deliciosa* 'Hayward' | 107.7 b | 857 a | 1064 c | 42.4 b |
| *A. deliciosa* '87-4-76' (Large) | 176.0 a | 882 a | 1433 a | 45.2 a |
| *A. deliciosa* '87-9-106' (Small) | 22.9 e | 90 c | 478 e | 37.0 c |
| *A. chinensis* 'Earligold' | 95.4 c | 406 b | 1160 b | 25.8 d |
| *A. chinensis* '37.6.6a' | 57.4 d | 353 b | 725 d | 36.5 c |
| *A. arguta* | 3.4 f | 85 c | 68 f | 17.4 e |

Means in a column with common letters are not significantly different at P=0.05 (LSD).

### 6.3.5 The seed number and seed size of *Actinidia* selections

*A. deliciosa* 'Hayward' and '87-4-76' averaged 850 to 900 seeds in a fruit, which is somewhat low for well pollinated 'Hayward' fruit which normally contain 1000-1500 seeds. Although seed number was not significantly different between these two selections, *A. deliciosa* '87-4-76' had seeds which were almost 1.5 times as heavy as 'Hayward' (Table 6.11). *A. deliciosa* '87-9-106' had very low seed numbers and seeds were over 50% smaller than *A. deliciosa* 'Hayward' (Table 6.11).

There were no significant differences in the seed number between the two *A. chinensis* selections, however selections had approximately half the number of seeds present in 'Hayward'. The size of *A. chinensis* 'Earligold' seeds was similar to 'Hayward', although seeds were slightly heavier (Table 6.11). *A. chinensis* '37.6.6a' seeds were approximately 40% smaller than *A. chinensis* 'Earligold' (Table 6.11).

Seed numbers of *A. arguta* were similar to those in the 'Hayward' 87-9-106 selection, however the seeds were particularly small (Table 6.11), although there was obvious variation in size, with many seeds appearing to be empty seed coats.

A linear relationship (Snelgar et al., 1992) was clearly inadequate for the extensive *A. deliciosa* 'Hayward' dataset obtained in chapter two, due to curvature of the relationship at low seed numbers. Inspection of residual plots for the other selections suggested that the relationship may also have been curvilinear at low seed numbers, although there was insufficient data to confirm this.

A power relationship ($Y=ax^b$) has been used to describe the relationship between seed number or weight and fruit weight in 'Hayward' kiwifruit (Pyke and Alspach, 1986) and was fitted to the 'Hayward' dataset (Figure 6.2a). However Hopping (1976a) and Trustrum (1983) found that the relationship changes at high seed numbers, becoming more positively related to seed number again rather than flattening out as in the power relationship. This type of relationship can be approximated by a cubic polynomial equation and was also fitted to the 'Hayward' (Figure 6.2b). Although a cubic relationship did not appear unreasonable once fitted to the data (Figure 6.2b), this shape was not immediately apparent in a scatter plot. In addition, mean residual error was higher from a cubic relationship than a power relationship. It was concluded that a power relationship was more suitable for describing the relationship between seed number or weight and fruit weight.

Non-linear relationships can often be transformed into linear ones with a simple transformation of the data, which facilitates linear regression technique as well as stabilising variance (Bates and Watts, 1988; Edwards, 1976). One advantage of the power relationship is that it can be converted to a linear form, $\log Y=\log_{10}(a) + b.\log_{10}(x)$, by transforming both axes with $\log_{10}$ and this was performed in order to compare the relationships in *A. deliciosa* 'Hayward', *A. chinensis* 'Earligold', *A. chinensis* '37.6.6a' and *A. Arguta*. Positive linear relationships for fruit weight ($\log_{10}$) against both seed number ($\log_{10}$) and seed weight ($\log_{10}$) with high correlation coefficients were found for all selections (Table 6.12). Although fruit weight was more highly correlated to seed weight than to seed number for all selections, the difference in correlation coefficients was only significant for *A. deliciosa* 'Hayward' ($Z_r$ transformation, P=0.05) (Table 6.12).

A scatter plot of fruit weight ($\log_{10}$) against seed number ($\log_{10}$) for the four selections highlighted some overall similarity among the different selections (Figure 6.4) and the correlation coefficient was quite high (0.82) for a single fitted line (Table 6.12). However

a scatter plot of fruit weight ($log_{10}$) against total seed weight ($log_{10}$) for the four selections appeared to be better and suggested that a single line could adequately describe the relationship between seed weight and fruit weight (Figure 6.3). The correlation coefficient for this relationship was very high (0.97) and was significantly better than the fruit weight/seed number correlation (Table 6.12).

Table 6.12 Intercept, slope and correlation coefficient (r) 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 *Actinidia*.

| Species | Seed number | | | Seed weight | | |
|---|---|---|---|---|---|---|
| | intercept | slope | r | intercept | slope | r |
| *A. arguta* | -0.218 | 0.40 | 0.70 a | 1.47 | 0.40 | 0.71 a |
| *A. chinensis* '37.6.6a' | 0.648 | 0.43 | 0.92 a | 2.01 | 0.43 | 0.92 a |
| *A. chinensis* 'Earligold' | 0.534 | 0.55 | 0.92 a | 2.15 | 0.57 | 0.93 a |
| *A. deliciosa* 'Hayward' | 0.916 | 0.38 | 0.90 b | 2.03 | 0.41 | 0.96 a |
| *Actinidia* sp. 'Combined' | -0.885 | 1.02 | 0.82 b | 2.07 | 0.63 | 0.97 a |

Correlation coefficients in the same row with common letters are not significantly different at P=0.05 ($Z_r$ test, see chapter two, section 2.8.4).

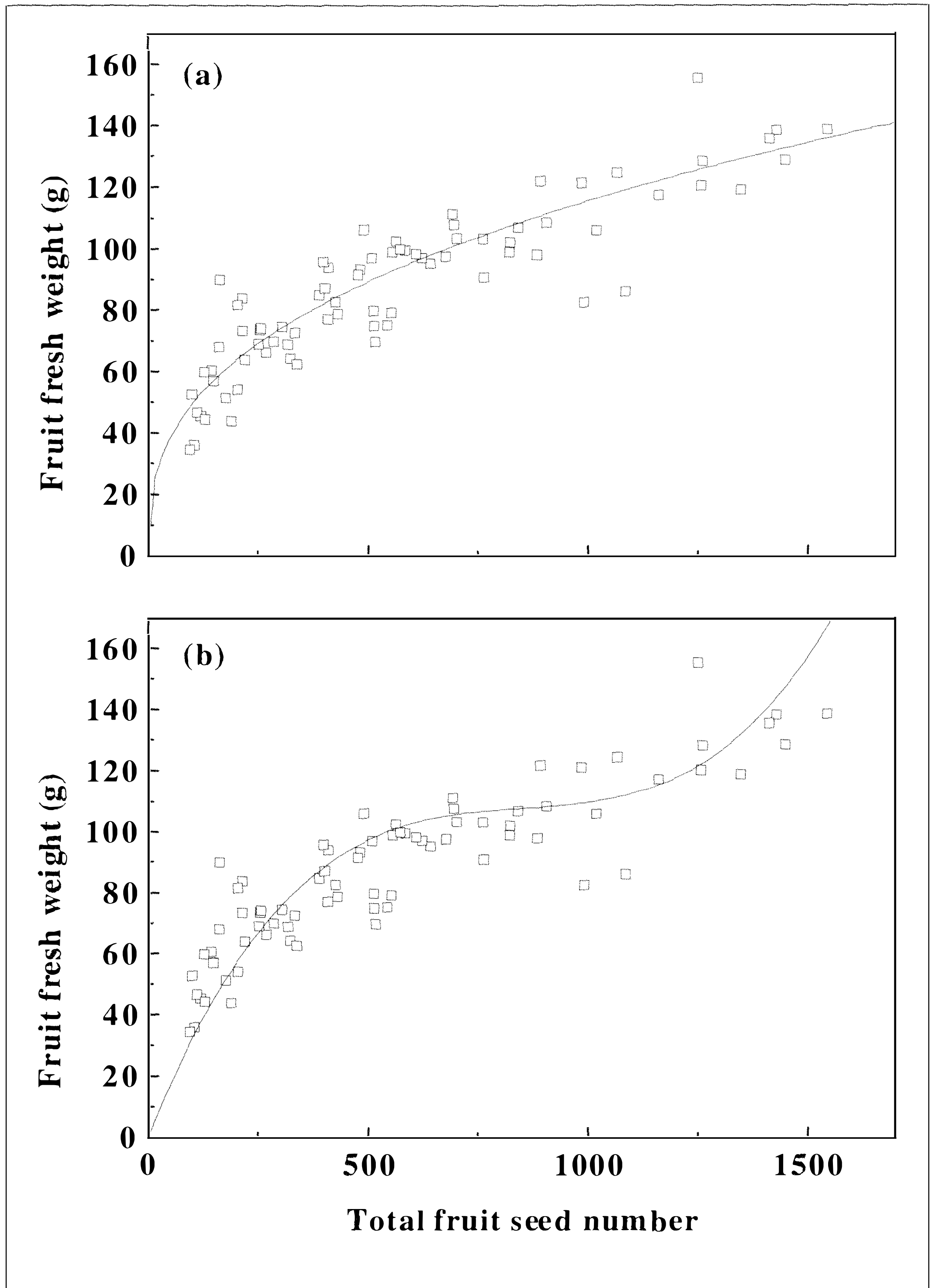


Figure 6.2 Two possible relationships between seed number and fruit fresh weight of a kiwifruit. (a) Power relationship given by the equation $Y = 8.98x^{0.37}$ (MSE=128.4). (b) Cubic relationship given by the equation $Y = 0.36x - 4.1\times10^{-4}x^2 + 1.6\times10^{-7}x^3$ (MSE=181.7). Both equations were fitted with intercept=0, using non-linear least squares regression based on the Levenberg-Marquardt algorithm.

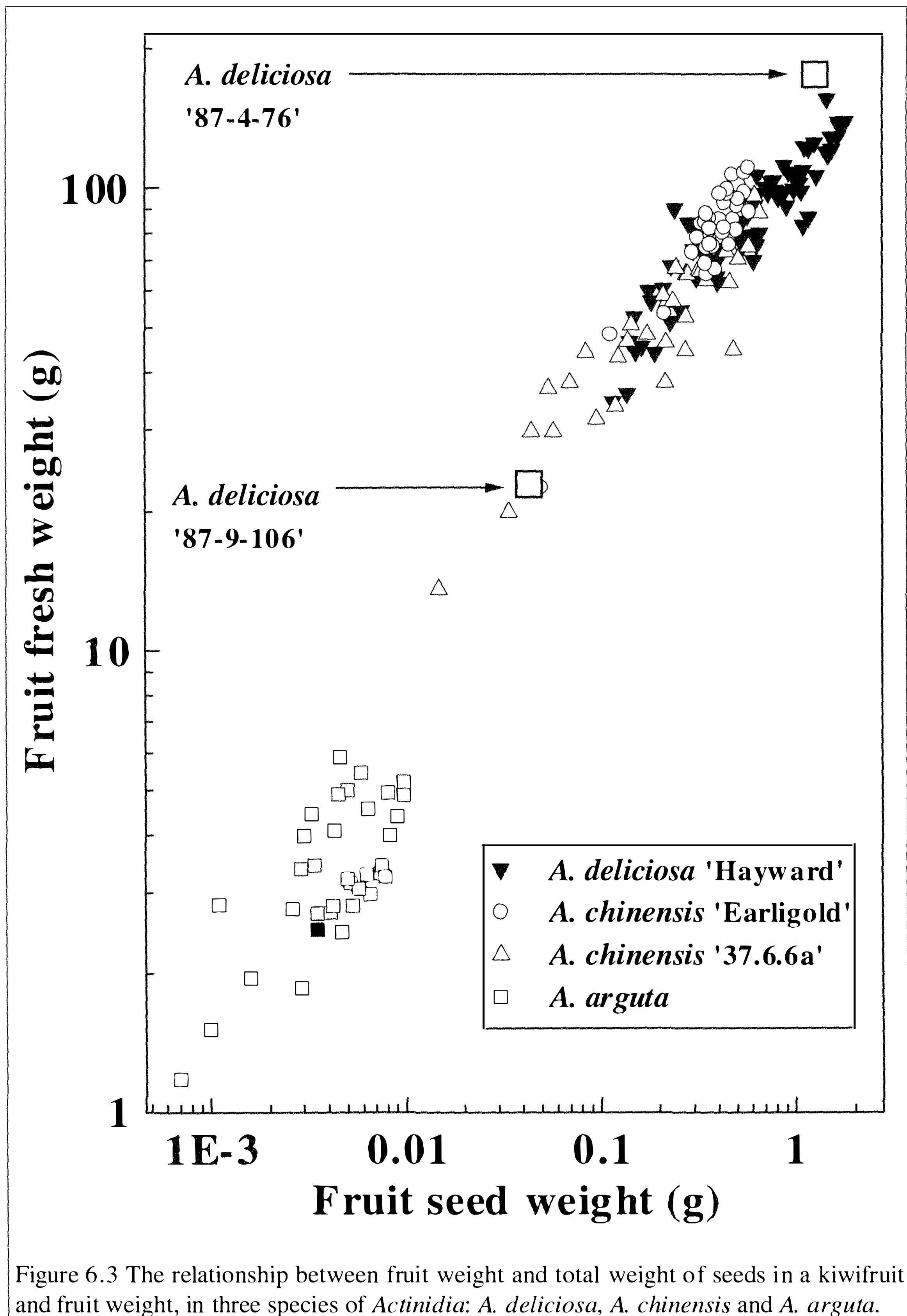


Figure 6.3 The relationship between fruit weight and total weight of seeds in a kiwifruit and fruit weight, in three species of *Actinidia*: *A. deliciosa*, *A. chinensis* and *A. arguta*.

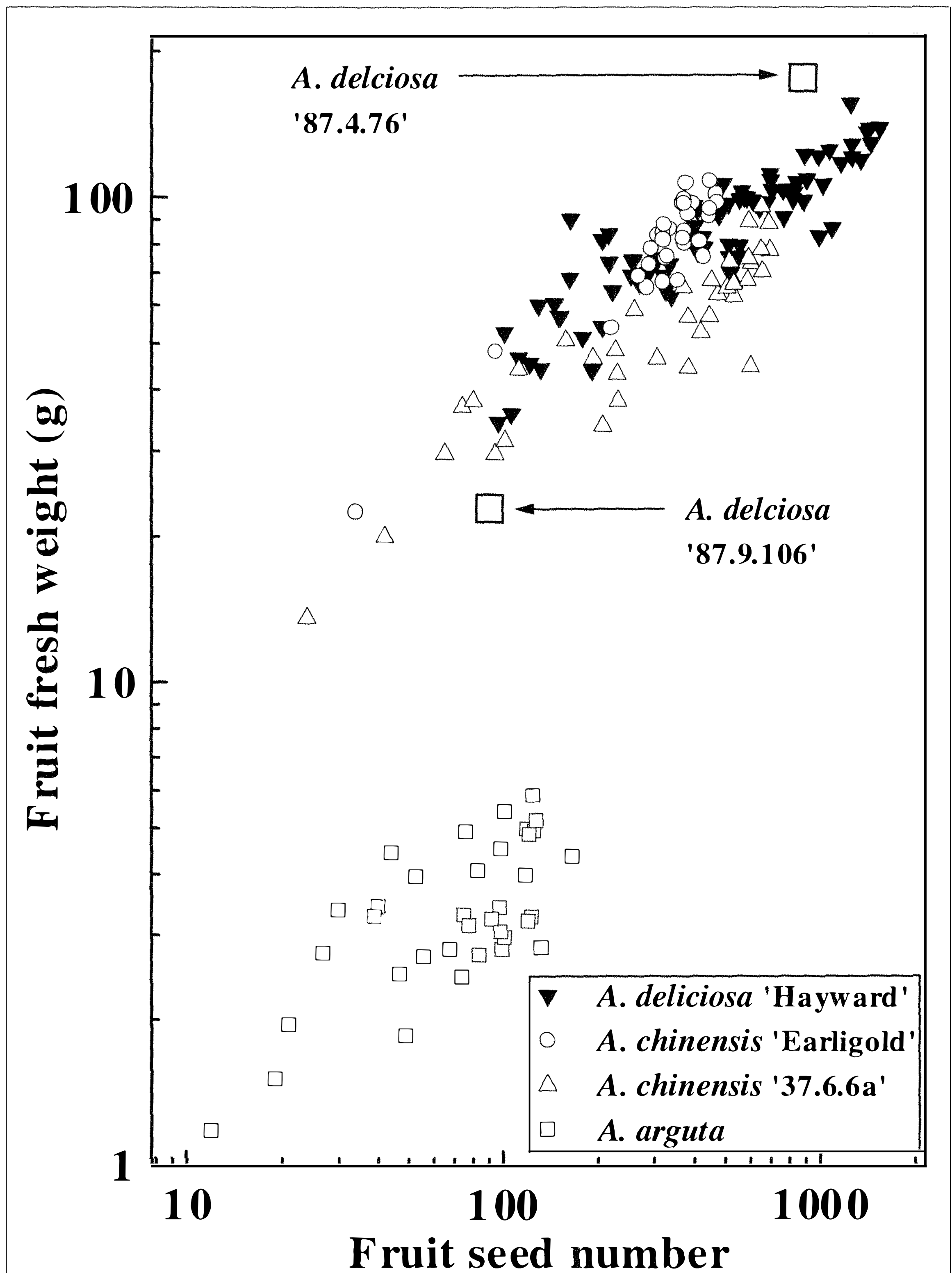


Figure 6.4 The relationship between the total number of seeds in a kiwifruit and fruit weight, in three species of *Actinidia*: *A. deliciosa*, *A. chinensis* and *A. arguta.*

The strong relationship between fruit weight and seed weight (Table 6.12) suggested that a single relationship may be adequate for all of the kiwifruit selections in this study. For a single (joint) relationship to be as good as individual relationships, the mean residual variation about a single line should be the same as the mean residual variation of all of the individual lines and this can be tested using an F-test (S. Ganeshanandam, Personal communication, 1993). However the hypothesis that a single line was better than individual lines was rejected (Table 6.13). The conclusion can therefore be made that although there is a strong relationship over all the species of *Actinidia* tested in this study, it is not as powerful for describing the relationship between fruit weight and seed-weight as individual relationships obtained from each selection.

Table 6.13 ANOVA table of the comparison between fitting a single line or individual lines to each selection, for the relationship between seed weight and fruit weight in different selections of *Actinidia*. If the true relationship is the same for each selection (single line), the mean residual variation (MSR) of a single line should be the same as for individual lines. The ratio of mean square errors (MSR) gives an F value.

| Source | DF | SSR | MSR | F | P>F |
|---|---|---|---|---|---|
| Residual variation about single line | 208 | 3.59 | | | |
| Sum of residual variations about individual lines | 200 | 0.97 | 0.00487 | | |
| Difference (variation of individual lines about single line) | 8 | 2.62 | 0.32711 | 67.1 | <0.005 |

### 6.3.6 The cell number and cell size of *Actinidia* selections

In the three selections of *A. deliciosa* there was no difference in mean cell diameter in any of the individual tissues, except in A. *deliciosa* '87-4-76' outer pericarp which had significantly larger cells than any other selection (Table 6.14). However on average '87-9-106' had significantly smaller cells across a fruit radius than the other two *A. deliciosa* selections (Table 6.14), although there was only 12% difference in average cell diameter between the largest (87-4-76) and smallest (87-9-106) *A. deliciosa* selection.

There were marked differences in cell numbers between the three *A. deliciosa* selections. The small fruited '87-9-106' selection had very low cell numbers in inner and outer pericarp compared with the other two *A. deliciosa* selections (Table 6.15). However the number of core cells in '87-9-106' was similar to the number in 'Hayward' (Table 6.15). *A. deliciosa* '87-4-76' had higher core and inner pericarp cell numbers than 'Hayward', although outer pericarp cell numbers were lower than 'Hayward'. Total cell numbers across a radius were 26% lower in the smallest selection (87-9-106) than in the largest selection (87-4-76) of *A. deliciosa.*

Table 6.14 Average cell diameter (μm) in core, inner pericarp and outer pericarp tissues separately, or average overall, of fruit from six *Actinidia* selections. Measurements of cell diameter were made along a straight line transect in the direction of the minimum equatorial diameter on transverse sections (n=10).

| Species | Core | Inner pericarp | Outer pericarp | Average overall |
|---|---|---|---|---|
| *A. deliciosa* 'Hayward' | 87.2 a | 236.5 a | 111.1 bc | 147.0 a |
| *A. deliciosa* '87-4-76' (Large) | 87.8 a | 216.1 a | 136.3 a | 148.7 a |
| *A. deliciosa* '87-9-106' (Small) | 83.8 a | 212.4 a | 117.8 b | 130.3 b |
| *A. chinensis* 'Earligold' | 57.1 c | nc | 97.2 d | nc |
| *A. chinensis* '37.6.6a' | 71.6 b | 238.7 a | 101.5 cd | 145.3 a |
| *A. arguta* | 80.2 ab | 166.3 b | 85.2 d | 117.9 b |

Means in a column with common letters are not significantly different at P=0.05 (LSD).
nc - not calculated due to inner pericarp tissue disintegration..

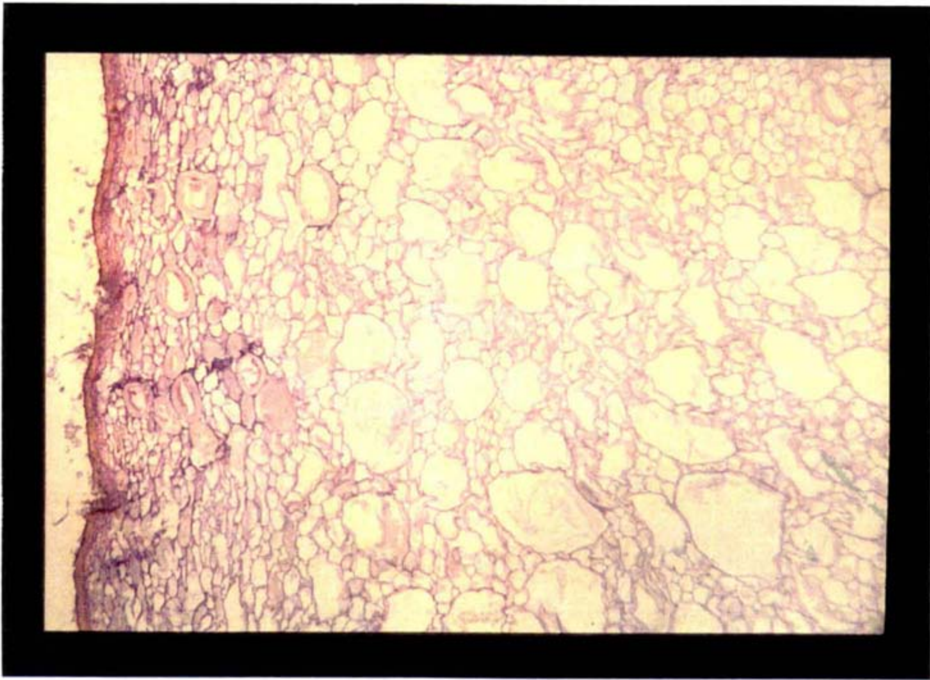

Plate 6.1 Outer pericarp of *A. chinensis* 'Earligold' in transverse section. Magnification=30x.

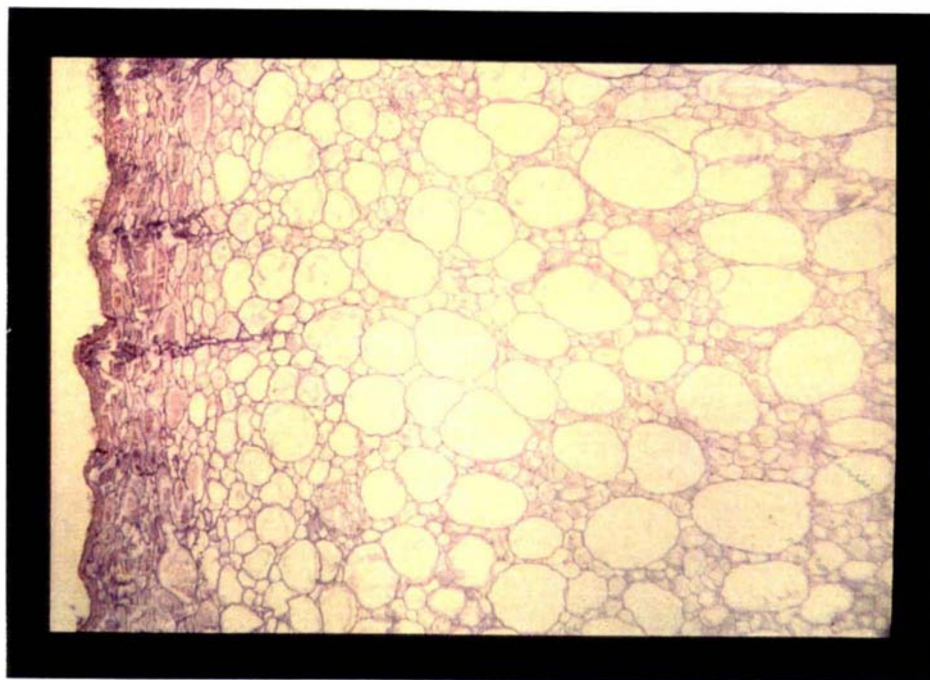

Plate 6.2 Outer pericarp of *A. chinensis* '37.6.6a' in transverse section. Magnification=30x.

Plate 6.3 Five kiwifruit selections investigated in this study. From left: *A. deliciosa* '87-4-76', 'Hayward', '87-9-106', *A. chinensis* 'Earligold' and '37.6.6a'.

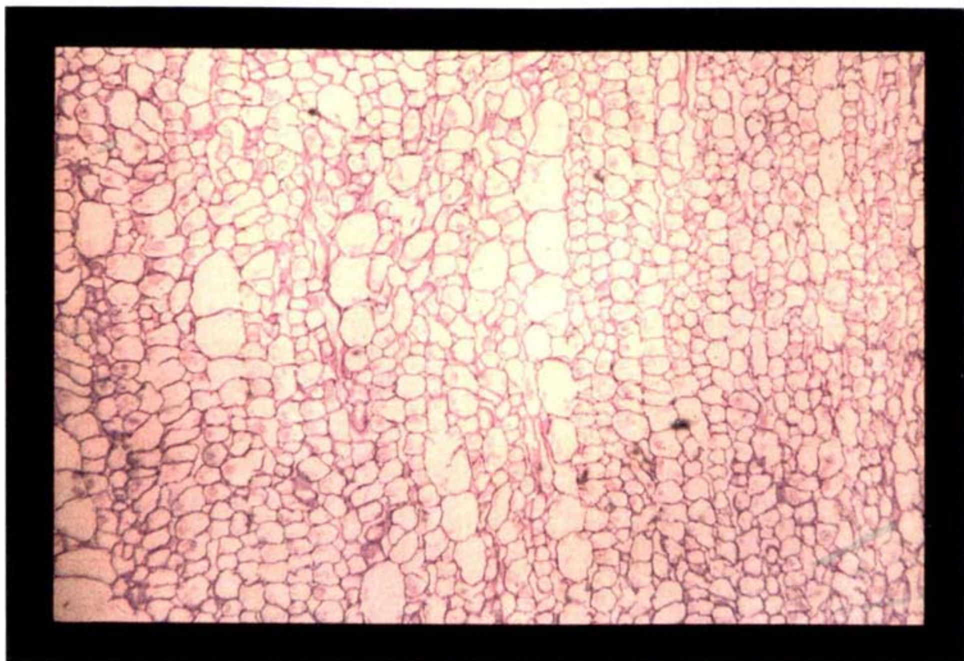

Plate 6.4 Core of a mature, well pollinated 'Hayward' kiwifruit in longitudinal section. The vertical edge of the photograph is parallel to the epidermis. Magnification=30x.

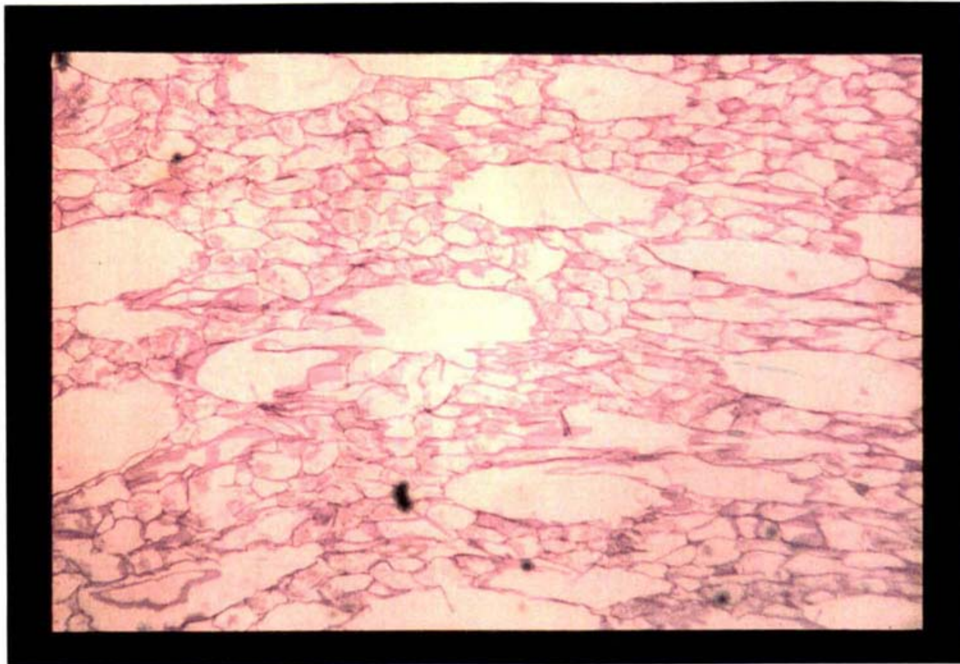

Plate 6.5 Outer edge (left) of the inner pericarp from a mature, well pollinated 'Hayward' kiwifruit in longitudinal section. The vertical edge of the photograph is parallel to the epidermis. Magnification=30x.

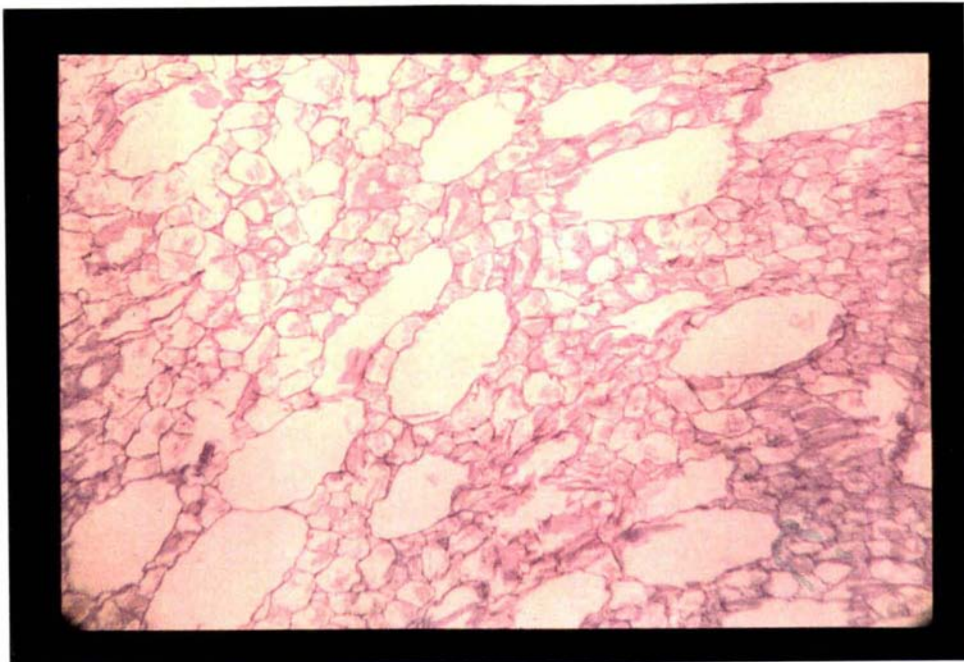

Plate 6.6 Outer edge (right) of inner pericarp and inner edge (left) of outer pericarp from a mature, well pollinated 'Hayward' kiwifruit in longitudinal section. The vertical edge of the photograph is parallel to the epidermis. Magnification=30x.

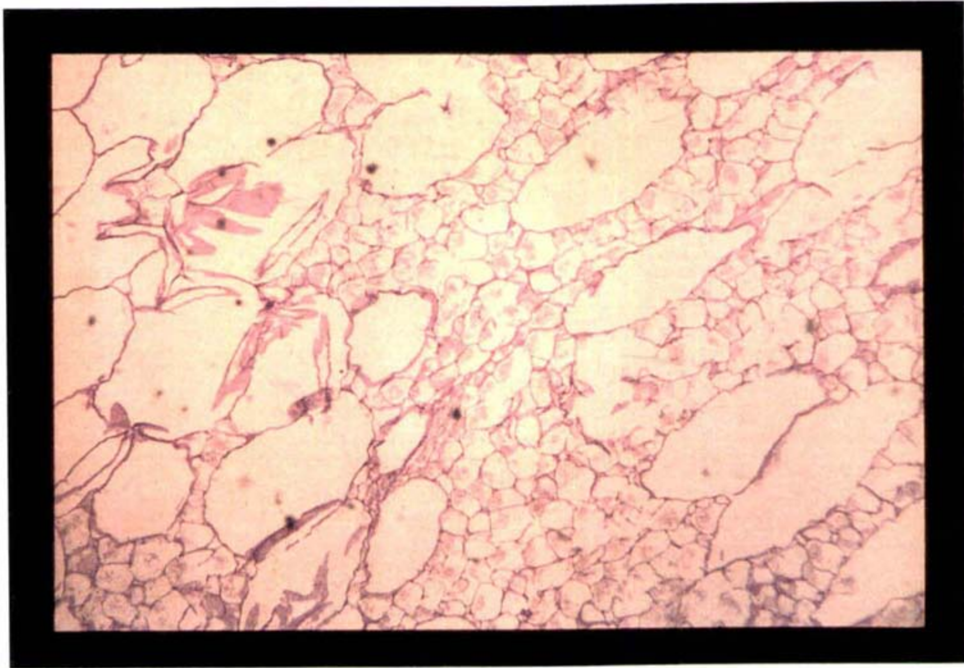

Plate 6.7 Mid point of outer pericarp of a mature, well pollinated 'Hayward' kiwifruit in longitudinal section. The vertical edge of the photograph is parallel to the epidermis. Magnification=30x.

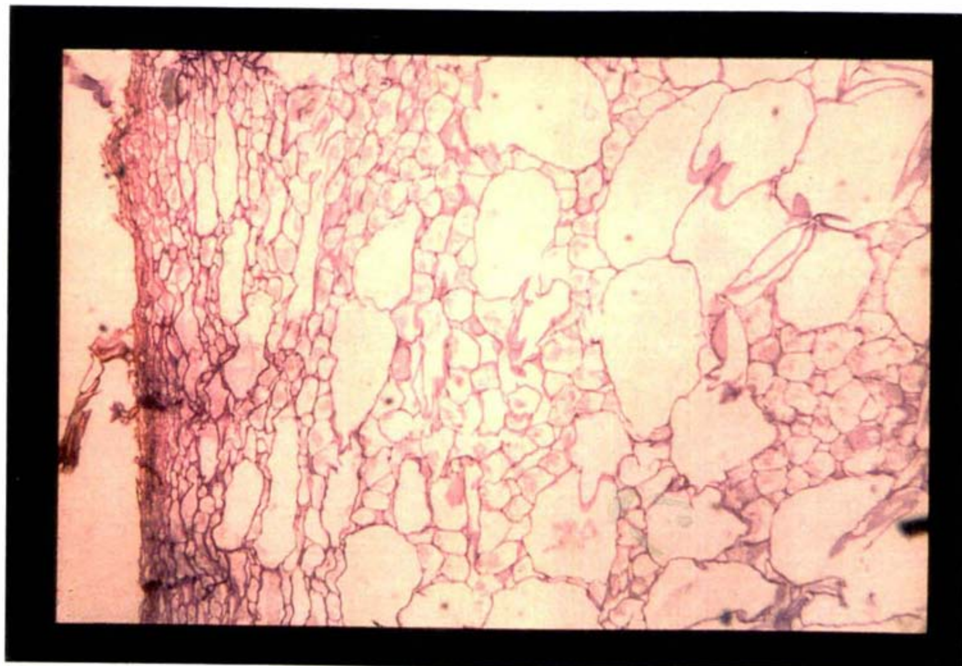

Plate 6.8 Epidermis (left) and outer edge of outer pericarp tissue from a mature well pollinated 'Hayward' kiwifruit in longitudinal section. Magnification=30x.

Both of the *A. chinensis* selections had a lower average cell diameter in the outer pericarp than any of the *A. deliciosa* selections, although the difference was not statistically significant for *A. chinensis* '37.6.6a' (Table 6.14). The outer pericarp cell number was different between the two *A. chinensis* selections and *A. chinensis* 'Earligold' had the highest number of cells in the outer pericarp of any selection in this study (Table 6.15). *A. chinensis* selections had noticeably tougher skin than *A. deliciosa* selections which appeared to be due to an increased number of cells with secondary thickening beneath the epidermis (Plate 6.1, Plate 6.2). Inner pericarp of *A. chinensis* '36.6.6a' was similar to 'Hayward' in both the number and size of cells. However *A. chinensis* 'Earligold' inner pericarp tissue was extremely soft and did not provide sections from which measurements could be made. Both selections of *A. chinensis* had a very small core compared with 'Hayward' kiwifruit due to both low cell number (Table 6.15) and small cells (Table 6.14). Overall, *A. chinensis* '36.6.6a' cell diameter was not significantly different to those of *A. deliciosa* 'Hayward' (Table 6.14), however cell numbers were 11% lower across a fruit radius (Table 6.15). *A. arguta* fruits had very low cell numbers and small cells compared with other selections, in all three tissues. Overall, cell diameter was 20% lower than in 'Hayward' (Table 6.14). However *A. arguta* had 59% less cells than 'Hayward' across a fruit radius (Table 6.15).

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. Counts of cell numbers were made along a straight line transect in the direction of the minimum equatorial diameter on transverse sections (n=10).

| Species | Core (half) | Inner pericarp | Outer pericarp | Total overall |
|---|---|---|---|---|
| *A. deliciosa* 'Hayward' | 46.3 ab | 52.0 b | 53.4 b[1] | 149.7 b |
| *A. deliciosa* '87-4-76' (Large) | 51.6 a | 64.0 a | 45.2 c | 162.4 a |
| *A. deliciosa* '87-9-106' (Small) | 45.7 b | 31.7 c | 33.5 d | 110.9 d |
| *A. chinensis* 'Earligold' | 26.4 d | nc | 68.8 a | nc |
| *A. chinensis* '37.6.6a' | 35.7 c | 50.8 b | 45.7 c | 133.3 c |
| *A. arguta* | 14.1 e | 26.3 c | 21.0 e | 61.4 e |

Means in a column with common letters are not significantly different at P=0.05 (LSD).
nc - not calculated due to tissue disintegration.

### 6.3.7 The effect of seeds on cell numbers and cell size in kiwifruit

Style manipulation was successful in reducing the number of seeds in the fruit to a very low level, however there was no effect of style manipulation on the size of individual seeds (Table 6.16). Although fruit length, minimum and maximum equatorial diameter were reduced by style excision, length was reduced to a greater degree than either diameter (Table 6.16) and L-fruit had the appearance of being short and fat. There was no effect on the number of locules per fruit, however a number of locules in L-fruit did not appear to contain any seeds.

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 (n=8).

| | Two styles intact (low seed) | All styles intact (high seed) |
|---|---|---|
| Fresh weight (g) | 47 | 123 * |
| Seed number per fruit | 137 | 1082 * |
| Individual seed size (μg) | 1181 | 1132 |
| Locules per fruit | 39.3 | 40.5 |
| Length (mm) | 46.8 | 71.0 * |
| Maximum diameter (mm) | 46.6 | 55.3 * |
| Minimum diameter (mm) | 42.5 | 51.7 * |

* Means in the same row are significantly different at P=0.05 (students t)

Transverse sections

In transverse sections there was no evidence that the number of cells across inner pericarp or outer pericarp tissue was affected by seed number (Table 6.17). However H-fruit had a higher number of cells across the core, although total cell number in a fruit radius was not significantly different between seed treatments (Table 6.17). In contrast, cell diameter in both inner and outer pericarp of H-fruit was significantly higher than in

L-fruit and average cell diameter across a fruit radius was significantly higher in H-fruit compared with L-fruit (Table 6.17). In the outer pericarp, the increase in average cell length was due to an increase in diameter of both large and small cells, rather than a change in proportion of these two cell types (Table 6.18).

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. Measurements of cell number and diameter were made along a straight line transect in the direction of the minimum equatorial diameter on **transverse** sections (n=8).

| | Cell number | | Cell diameter (μm) | |
|---|---|---|---|---|
| | two styles (low seed) | all styles (high seed) | two styles (low seed) | all styles (high seed) |
| Core (half) | 43 | 51 * | 86 | 86 |
| Inner pericarp | 48 | 53 | 204 | 259 * |
| Outer pericarp | 47 | 46 | 78 | 113 * |
| Overall (fruit radius) | 139.0 | 149.7 | 124 | 155 * |

* Means for a parameter in the same row are significantly different at P=0.05 (students t).

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. Measurements of cell number and diameter were made along a straight line transect in the direction of the minimum equatorial diameter on **transverse** sections (n=8).

| | Cell number | | Cell diameter (μm) | |
|---|---|---|---|---|
| | two styles (low seed) | all styles (high seed) | two styles (low seed) | all styles (high seed) |
| Large cells | 5.5 | 7.3 | 217 | 273 * |
| Small cells | 41.7 | 38.4 | 60 | 81 * |

* Means for a parameter in the same row are significantly different at P=0.05 (students t).

Longitudinal sections

In longitudinal sections, outer pericarp tissue was similar in appearance to the tissue in transverse section. Near the epidermis, most cells were elongated parallel with the epidermis (Plate 6.8). These elongated cells are probably 'small' cells which appear isodiametric on a transverse section and thus a three dimensional representation would probably be of a tube shaped cell running parallel to the epidermis. In the middle of the outer pericarp, large cells were inter-mingled with smaller isodiametric cells (Plate 6.7, Plate 6.8) and cells in this tissue can be visualised as relatively isodiametric, balloon shaped cells. With increasing distance from the epidermis, cells gradually changed in orientation and shape to become elongated perpendicular to the epidermis (Plate 6.6). These cells correspond to cells which are also elongated perpendicular to the epidermis in transverse sections and can also be visualised as tube shaped cells running perpendicular to the epidermis.

Longitudinal sections of core tissue had the appearance of being composed of ordered columns of similarly shaped and sized cells (Plate 6.4) and gave the impression of cells that had divided from each other relatively recently. This suggests that at least in core tissue, cell division may have been occurring most recently in the longitudinal direction.

In both core tissue and at the edge of the inner pericarp there were no difference in cell length between L-fruit and H-fruit (Table 6.19). However cells measured at the mid point and outer edge of the outer pericarp of H-fruit were larger compared with L-fruit. The differences in cell length at the mid point of outer pericarp tissue were due to both a greater proportion of 'large' cells (Table 6.20) and the increased size of these cells (Table 6.19) in H-fruit compared with L-fruit .

Table 6.19 The effect of seed number in a 'Hayward' kiwifruit on the mean cell length (µm) in individual fruit tissues from **longitudinal sections**. Mean cell lengths were estimated at the mid point of the core, the outer edge of the inner pericarp and at the mid point and outer edge of the outer pericarp. Due to the presence of missing values, means presented are population marginal means.

| | Two styles (low seed) | All styles (high seed) |
|---|---|---|
| Core mean cell length | 81 | 75 |
| Inner Pericarp mean cell length | 97.5 | 92.2 |
| Mid-point Outer Pericarp mean cell length | 113 | 146 * |
| Mid-point Outer Pericarp large cell length | 249 | 308 * |
| Mid-point Outer Pericarp small cell length | 100 | 112 |
| Edge Outer Pericarp mean cell length | 109.7 | 154.9 * |

* Means in the same row are significantly different at P=0.05 (contrast).

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. Due to the presence of missing values, means presented are population marginal means.

| Two styles intact (low seed) | All styles intact (high seed) |
|---|---|
| 8.4 | 17.2 * |

* Means are significantly different at P=0.05 (contrast).

## 6.4 Discussion

### 6.4.1 Dominance and inter-fruit competition in kiwifruit

The results presented in this chapter support the concept of dominance between kiwifruits reported by Lai et al. (1990), although the mechanism by which dominance is exerted in kiwifruit may be different to that proposed by Bangerth (1989). Bangerth (1989) proposed that dominance and fruit competitive ability may be related to the IAA export from a growing organ. In support of this hypothesis, higher diffusible IAA has been found from dominant apple and tomato fruits compared with inhibited fruits and removal of dominant fruit resulted in increased IAA export from the formerly inhibited fruits (Gruber and Bangerth, 1990). The amount of diffusible IAA from kiwifruit from non-girdled shoots ranged between 0.87 and 3.07 pMol.fruit$^{-1}$.hour$^{-1}$ (Table 6.7), which is similar to the amount reported to diffuse from 'Jonagold' apple ranging between 0 and 3.5 pMol.fruit$^{-1}$.hour$^{-1}$ (Gruber and Bangerth, 1990). However in contrast we have found no difference in the rate of IAA export from dominant or inhibited kiwifruit (Table 6.6) or between low and high seeded kiwifruit. This suggests that at least in kiwifruit, dominance may not be associated with IAA export by fruit. As the earliest date from which IAA export was sampled from kiwifruit was 22 DAFB, it is possible that IAA transport prior to this time is important for establishing fruit dominance in kiwifruit.

The inhibitory effect of high seed numbers in adjacent kiwifruit was associated with reduced seed numbers in inhibited fruit (Table 6.2, Table 6.5). This suggests that in kiwifruit, dominance phenomena may be related to increased seed abortion during the early period of growth. Bangerth (1989) points out that in many cited cases, dominance occurs in the early stages of development, while competition operates at later stages. As seed abortion in low seeded kiwifruit was apparently increased at a low leaf:fruit ratio which presumably reduced assimilate availability (Table 6.3), this suggests that effects of inter-fruit competition on seed abortion may be due to resource limitation. Increased seed abortion has been linked to resource limitation in bean (Nakamura, 1988) and zucchini (Stephenson et al., 1988) although to our knowledge there are no other reports of this in kiwifruit. Alternatively, if IAA export is related to seed numbers in kiwifruit

earlier than 22 DAFB, increased IAA export from H-fruit could have inhibited IAA transport (Bangerth, 1989) and increased seed abortion in L-fruit.

Leaf:fruit ratio on a lateral shoot had no effect on fruit weight of H-fruit unless a girdle was applied (Table 6.1), which is consistent with previous reports (Woolley et al., 1992) and results presented in chapter three. High seeded fruit presumably have a threshold level of sink strength and even when a high leaf:fruit ratio is present they are apparently unable to attract the extra carbohydrate required to produce larger fruit unless competition from other sinks on the vine is eliminated by girdling (Woolley et al., 1992). However there was a contrast between the ability of L-fruit and H-fruit to attract carbohydrate. The weight of H-fruit was reduced by a leaf:fruit ratio of one on a girdled shoot compared with non-girdled shoots. In contrast there was no effect of girdling on the weight of L-fruit on lateral shoots which were at a leaf:fruit ratio of one (Table 6.3), but fruit weight was considerably lower than H-fruit on girdled shoots with a leaf:fruit ratio of one (Table 6.1). This suggests that L-fruit have a lower threshold level of sink strength even when competing sinks are eliminated. In addition to the apparent reduced ability of L-fruit to obtain carbohydrate for growth, it appears that increased seed abortion may have occurred in L-fruit at the low leaf:fruit ratio (Table 6.3), which further reduced the sink strength of these fruit.

### 6.4.2 The relationship between seeds and fruit weight in *Actinidia* species

Although a cubic model could be fitted to the relationship between seed number and fruit weight for 'Hayward' kiwifruit, visual appraisal and residual errors suggested that a power model was more suitable (Figure 6.2). In this study, seed number was reduced in fruit by excision of styles and fruit were selected at anthesis from equivalent lateral shoots, while in studies where a cubic relationship was found (Hopping, 1976a; Trustrum, 1983; Lai, 1987), fruit were selected *post-hoc* at maturity. Thus differences between these studies may be due to additional factors correlated to the number of seeds in the fruit such as canopy position, proximity to pollinisor and time of anthesis. D. Woolley (personal communication, 1996) suggests that the increase in fruit weight at

high seed numbers may be due to these fruit being in canopy positions which are both well exposed for pollination and light penetration enabling enhanced photosynthesis of nearby leaves which predominantly supply the fruit with carbohydrates. This hypothesis is supported by the results of Grant and Ryugo (1984) who noted a tendency to higher seed numbers in fruits from exposed shoots compared with those arising on shaded shoots.

Another alternative was highlighted by Trustrum (1983) who demonstrated a positive relationship between locule number and seed number, which was more pronounced at high seed number. This suggests that fruit with higher numbers of locules, not only have the potential for very high seed numbers, but due to tissue associated with the additional locules they may higher potential for enlargement (Trustrum, 1983).

### 6.4.3 The relationship between seed number, seed size and fruit size in *Actinidia* selections

In this study, different selections of *Actinidia* have been shown to differ in both the number of seeds per fruit and the weight of individual seeds. Fruit weight in the five selections was able to be well described by a single relationship between total seed weight and fruit weight (Figure 6.3). This is somewhat surprising considering the smallest selection, *A. arguta* averaged only 3.4g while the largest selection *A. chinensis* '87-4-76' averaged 176g. Pyke and Alspach (1986) commented that kiwifruit weight was correlated more strongly with total seed weight rather than with seed number. This has been confirmed in this study over the five selections together and for the 'Hayward' selection individually. The stronger relationship between fruit weight and seed weight may be partly due to the inability of a seed count to distinguish between viable and non-viable seeds. A number of seeds in fruit from all selections appeared to be empty, although *A. arguta* appeared to have a relatively high proportion of empty seeds. Empty seeds are likely to have been non-viable and may not have contributed to seed associated fruit development, particularly if they abort early on. There was however considerable variation in the size of fully developed seeds between different selections. Kiwifruit seed size has been found to be related to some vine factors such as shading (Grant and Ryugo, 1984) and assimilate supply (Lai et al., 1989). Therefore differences in crop load

between *A. deliciosa* and *A. chinensis* selections could have influenced both seed size and fruit size, resulting in increased variation about a common line of fruit weight against seed number.

It is possible however that large seeds provide a greater source of growth regulators for stimulation of fruit growth. Although a causal effect of seed number on the size and growth of a kiwifruit can be easily demonstrated via inhibition of pollination, establishing a causal effect of seed size is difficult. As it is not possible to generate experimental treatments to alter the size of seeds, it is difficult to establish whether the size of seeds affects fruit size or *vice versa*. Seeds of *A. arguta* were particularly small compared with the other selections (Table 6.11) and *A. deliciosa* 'Bruno' males were used for pollination of the *A. arguta* fruits. Being a different species to it's pollinisor, it is possible the pollen may not be fully compatible, leading to low seed numbers and/or smaller seeds. In other fruit species, it has been shown that the pollen parent can sometimes influence both fruit growth, seed numbers and size of individual seeds (Kahn et al., 1994; Stern et al., 1993), as well as many other fruit characters (Denney, 1992). There are few reports on the effect of different pollen parents on seed and fruit growth in *Actinidia* species. *A. deliciosa* fruit which were cross pollinated with *A. chinensis* pollen were smaller than fruit pollinated by males from their own species and ovules failed to develop properly (Harvey et al., 1992). The fruit size of hand pollinated 'Hayward' flowers was found to be dependent on the parental source of pollen, although it was concluded that this may have been due to the inability of some pollinisors to fertilise ovules furthest from the styles, leading to lower seed numbers (McNeilage et al., 1992). These studies highlight the possibility that selection of appropriate pollinisors for different A*ctinidia* species on the basis of resulting seed number, size and viability may be important to obtain the maximum fruit size and quality in new *Actinidia* selections.

### 6.4.4 The relationship between cell number and fruit size in *Actinidia* selections

Fruit weight was closely related to the fruit minimum equatorial diameter ($D_{min}$) in the different selections (Table 6.10, Table 6.11). This suggests that estimates of cell numbers

and cell diameter in straight line transects across $D_{min}$ may be representative of fruit weight differences. Although there was some variation in fruit length to diameter ratio and $D_{max}$ to $D_{min}$, the variation was small and fruit selections with higher weight always had a higher minimum equatorial diameter.

The results presented suggested that differences in fruit size were more closely related to cell number than to cell diameter. Average cell diameter was not significantly different between *A. deliciosa* 'Hayward', *A. deliciosa* '87-4-76' and *A. chinensis* '36.6.6a' (Table 6.14), although the two smallest selections A. *deliciosa* '87-9-106' and *A. arguta* had smaller cells. However selections with higher fruit size always had higher average cell numbers (Table 6.15). This is in contrast to measurements of cell cross sectional area in *A. chinensis* '37-3-18a' reported recently by Clayton (1996), who suggested that cell size was between 16 and 19 times smaller in *A. chinensis* '37-3-18a' than those reported for 'Hayward' by Patterson et al. (1993). However these studies may not be comparable as measurements of cell size in *A. chinensis* '37-3-18a' were on the entire outer pericarp (Clayton, 1996), while those in 'Hayward' were only on the 20 outer most layers of mesocarp (Patterson et al., 1993).

The close relation that we have found between cell number and fruit size in different *Actinidia* selections is in agreement with other studies which have correlated cell numbers and fruit size among different cultivars in fruit species. For example Bohner and Bangerth (1988a) found that the pericarps of large fruit tomato mutants had significantly higher cell numbers than smaller wild tomato fruits. Scorza et al. (1991) found that large fruited peach cultivars had significantly higher fruit cell numbers than small fruited cultivars. In addition Cheng and Breen (1992) found that cell count per receptacle was the predominant component of variation in receptacle size between four cultivars of strawberry. In all of these studies, cell number differences between cultivars were established prior to anthesis, although the rate of cell division between cultivars did not remain static after anthesis in tomato (Bohner and Bangerth, 1988a). Similarly, 'Hayward' kiwifruit ovaries arising from early flowers are larger than those from late arising flowers (Cruz-Castillo, 1994) and have been found to produce larger fruit (Lai et al., 1990). Differences in ovary size were associated with significantly higher numbers of core cells at anthesis, while at maturity there were higher cell numbers in all three fruit tissues of fruit from early flowers though this was only significant in outer pericarp tissue

(Cruz-Castillo, 1994). In addition, there were no significant differences in cell sizes at anthesis or maturity (Cruz-Castillo, 1994). Although no pre-anthesis cell counts were made on the different *Actinidia* selections, it seems very likely that cell number in different *Actinidia* selections is also largely determined prior to anthesis.

### 6.4.5 Influence of seeds on post-anthesis cell division in kiwifruit

Large differences in seed numbers and/or seed weight existed between all the selections and these were strongly correlated with fruit size differences (Figure 6.3). As fruit size differences were also associated with differences in cell numbers across transverse sections (Table 6.15), this suggested that some of the differences between selections may be due to the influence of seeds on cell division. If this were the case, it would follow that the function of seeds in stimulating growth of kiwifruit is by stimulation of post-anthesis cell division, rather than attraction of carbohydrate and cell expansion. However this hypothesis is rejected for the following reasons:

1. Style manipulation was performed on equivalent 'Hayward' kiwifruit flowers from the same vines in order to acquire fruit with low and high seed number/weight. However in transverse sections, seed number only significantly affected cell numbers across the core and not overall (Table 6.17). The reduction in fruit diameter was almost entirely due to smaller cell diameter in L-fruit (Table 6.17). This confirms cell counts in transverse sections from low and high seeded kiwifruit made at 40 DAFB in a similar study by Lewis (1994).
2. There were no differences in the proportion of cells in the cell division phase G2 (double DNA content in nuclei), in low seeded kiwifruit compared with high seeded kiwifruit over the period 0 to 40 DAFB (Lewis, 1994).
3. The average growth rate of kiwifruit with low seed numbers was lower than from well pollinated kiwifruit throughout the entire period of fruit development (Figure 6.1, Table 6.9). In addition, growth of parthenocarpic kiwifruit can be induced by repeated applications of naphthalene acetic acid plus benzyladenine plus gibberellic acid (100 + 50 + 50 $mg.litre^{-1}$ respectively) up until 115 days after flowering (Costa

and Ryugo, 1978) cited in Hopping (1990). This suggests that fruit growth may be dependent on seed formation for fruit expansion past the period of cell division, which concludes in pericarp tissue at around 40 to 60 DAFB (Chapter three).

As fruit size was dependent on seed number or seed weight both within a selection and between selections, this suggests that the genetic differences in cell number of the selections may not be of major importance in directly influencing fruit size. Instead it is possible that fruit cell number at anthesis was associated with the formation of a limited number and size of ovules which would be available for fertilisation. In the work of Lai et al. (1990), early kiwifruit flowers grew to a higher weight, but also had significantly higher seed and locule numbers than late flowers, although differences in seed number did not fully explain fruit size differences. Similarly, 84% of the variation in receptacle size of four different strawberry cultivars was accounted for by the number of achenes which developed after anthesis (Cheng and Breen, 1992). As the number of cells per achene was not different between strawberry cultivars (Cheng and Breen, 1992) and strawberry receptacle growth post anthesis is strongly correlated with the number of achenes which develop (Nitsch, 1950), this strongly suggests that the potential number of achenes which can be forrned is related to pre-anthesis cell division in different strawberry cultivars. In contrast, the weight of fruit from tomato plants which had been treated with the gibberelin $GA_3$, was independently related to both seed number and locule number in the different trusses (Sawhney and Dabbs, 1978). Kiwifruit weight was loosely related to locule number among the kiwifruit selections (Table 6.11) and in 'Hayward' fruits (Burge et al., 1990). However the strong relationship between fruit weight and seed number or seed weight within 'Hayward' and between selections suggests that the main contribution of locule number to fruit weight was in providing ovules available for fertilisation. Unlike tomato where application of $GA_3$ resulted in increased locule numbers in fruit (Sawhney and Dabbs, 1978), pre-anthesis applications of $GA_3$ to kiwifruit canes had no effect on locule numbers and fruit size was reduced slightly when the application was made near to the time of flowering (Burge et al., 1990).

In other fruits it has been found that the requirement for continued seed development varies considerably. For example, removal of seeds from 'Cox's Orange Pippin' apple

fruit prior to 'June drop' (40 to 60 days after fruit set) resulted in abscission of all fruits, but after the 'June drop' removal of seeds did not affect abscission (Abbott, 1959). In contrast, continued growth of strawberies was dependent on the presence of achenes throughout development (Nitsch, 1950). Although kiwifruit growth appears to be related to seed number for the entire period of fruit development (Figure 6.1, Table 6.9), this does not necessarily imply that continued development of seeds themselves is essential. The requirement for growth regulator applications up to 115 days after flowering to stimulate parthencarpic kiwifruit development (Costa and Ryugo, 1978) cited in Hopping (1990), coincides with the period of embryo development (Harvey and Fraser, 1988; Hopping, 1976b). This suggests that development of the embryo may be important for seed related sink strength in kiwifruit. However in crosses of *A. chinensis* and *A. deliciosa*, fruit growth was initially dependent on the fertilisation of ovules, but fruit growth continued when embryos failed to develop (Harvey et al., 1992). Although this questions the requirement for viable embryo development, it is still certain that initial successful fertilisation and subsequent ovule development is the essential stimulus which largely determines kiwifruit sink strength.

There was a non-equivalence between *A. deliciosa* '87-9-106' and experimentally induced low seeded 'Hayward' fruits in terms of cell numbers. Both of these selections had similar seed numbers, although '87-9-106' seeds were particularly small (Table 6.11, Table 6.16) and therefore '87-9-106' had lower total seed weight. Differences in cell numbers between the naturally low seeded fruit of '87-9-106' and those experimentally induced on 'Hayward' may have been due to genetic factors, which determined the initial number of cells in the ovaries of these fruit. However many fruit abscised from '87-9-106' vines, suggesting that the small seeds may largely be non-viable in fruit from this selection. Completely unpollinated 'Hayward' kiwifruit ovaries were found to have fewer cells in the G2 cell division phases after anthesis, although fruit were not retained long enough to allow histological examination (Lewis, 1994). Thus the possibility cannot be excluded that the few, small seeds present in *A. deliciosa* '87-9-106' fruit were insufficient to stimulate commencement of normal post-anthesis cell division in the transverse plane. As *A. deliciosa* '87-9-106' fruit were not in competition with any H-fruit, this may have enhanced the ability of fruit to obtain carbohydrates, thus allowing a

reduced number of cells to grow to a comparatively larger size compared with the low seeded 'Hayward' fruit which would have been in competition with H-fruits in the same vines.

Although our work and that of Lewis (1994) found almost no effect of seed number on cell division in the transverse plane, the effect of seeds on fruit expansion is most evident in the longitudinal direction. For this reason, some longitudinal sections were made of 'Hayward' fruit with experimentally induced low or high seed number. At low seed numbers, outer pericarp cell length in the longitudinal axis was reduced (Table 6.19), as was the case in the transverse plane (Table 6.17). Thus it can be concluded that kiwifruit seed number has a major influence on outer pericarp longitudinal as well as radial cell expansion and probably little or no effect on outer pericarp cell division in either plane. There was a gradient of cell shape from epidermis inwards through the pericarp, which meant that cells changed from elongate parallel to the epidermis to being elongate perpendicular to the epidermis. In the inner pericarp, longitudinal cell length was not different between L-fruit and H-fruit. It is therefore possible that decreased longitudinal cell division occurred in the inner pericarp of L-fruit. Similarly in core tissue, L-fruit had a significantly lower numbers of cells in the transverse plane, but transverse cell diameter and longitudinal cell length were not different to H-fruit. This suggests that core cell division may also have been reduced in the longitudinal direction in L-fruit.

It can be speculated that if seeds have some influence on cell division in kiwifruit, this may be greatest on cells in close proximity to seeds. This hypothesis is supported by the following evidence:

1. Both inner pericarp and core tissue are in close proximity to the seeds and the number of cells across a transverse plane of the core was significantly lower in L-fruit. The lack of difference in core and inner pericarp cell length between seed number treatments suggests that seeds may also have increased longitudinal cell division in both of these tissues.
2. In H-fruit, longitudinal cell length became significantly longer at the mid-point of the outer pericarp and longer still near the outer edge of the fruit furthest from seeds. As

fruit grow, outer pericarp cells become more distant from the seed limiting cell division and cells could become limited to expansion.

3. Cell division in the transverse plane ceases first in outer pericarp tissue (Chapter three), which also becomes further away from seeds as fruit grow larger. However cell division continues for over 100 days in the core (Hopping, 1976b; Chapter three) which remains in close proximity to seeds. Core cells in the longitudinal direction appeared to be in ordered columns, which suggests that cell division may have been recent enough that orientation of divided cells had not been altered by subsequent uneven expansion rates (Plate 6.4).

## 6.5 Summary

The dominance of kiwifruit with high seed numbers over the growth of kiwifruit with low seed numbers has been confirmed in this chapter. However unlike other fruit species such as apple and tomato, dominance in kiwifruit may not be related to IAA export from fruit. It seems likely that the long term inhibitory effect of high seeded kiwifruit on growth of low seeded fruit was by increasing seed abortion in low seeded fruit during the first three weeks after fruit set, probably due to a reduction in assimilate supply to inhibited fruit.

A strong relationship was found between the weight or number of seeds in a kiwifruit and fruit weight and the relationship extended over five selections. As seed weight correlated better than seed number this suggests that larger seeds may be a better source of growth regulators for stimulating fruit growth than small seeds. Alternatively it is possible that seed abortion and the effects of crop load on seed size are the factors which improve the relationship of fruit weight to seed weight.

As fruit weight differences between the selections were mostly attributable to cell number rather than cell size, an initial hypothesis was raised that the function of seeds was in stimulating post-anthesis cell division in kiwifruit. However this hypothesis was rejected following further experimental work in which cell numbers were counted in low and high seeded 'Hayward' kiwifruit. Seed formation appears to be essential for kiwifruit growth throughout the entire season, but it uncertain whether viable ovules must continue growth for fruit development to continue.

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# 7. The Role of Auxin Transport in Kiwifruit Growth

## 7.1 Introduction

Transport of the plant auxin indole-3-acetic acid (IAA) occurs primarily from apical tissues to basal tissues (Kaufman et al., 1995). This basipetal transport is thought to be essential for a variety of plant processes including apical and correlative dominance (Bangerth, 1989), tropism's (Kaufman et al., 1995), and vascular development (Aloni, 1995). Basipetal transport is thought to occur via chemiosmotic polar diffusion mediated by three main modes of IAA transfer across cell plasma membranes (Rubery and Sheldrake, 1974). Firstly, un-dissociated (un-charged) IAA molecules are able to diffuse from the lower pH cell wall across the plasma membrane, followed by dissociation in the higher pH cytoplasm, where the now charged IAA anions have a reduced ability to traverse the plasma membrane (Raven, 1975; Rubery and Sheldrake, 1974). Secondly, there is evidence of a specific IAA uptake carrier protein which is saturable by a high concentration of auxin (Rubery and Sheldrake, 1974). Evidence for carrier mediated uptake includes the observation that auxin can accumulate in sealed zucchini membrane vesicles to five times that which would be predicted by diffusion alone (Lomax, 1986). Thirdly, efflux of IAA from the cells involved in auxin transport is widely accepted to occur via an efflux carrier protein (Rubery, 1990; Morris et al., 1991; Lomax et al., 1995; Bernasconi, 1996), which is saturable by auxin (Sussman and Goldsmith, 1981a; Delbarre et al., 1996). The efflux carrier is non-competitively inhibited by a variety of synthetic compounds including phytotropins such as N-1-naphthylphthalamic acid (NPA, see below), benzoic acids, morphactins (Katekar and Geissler, 1980; Rubery, 1990), and also some endogenous flavonoid compounds such as quercetin (Rubery, 1990). The polarity of auxin transport is thought to be mediated by the localisation of the influx carrier on the apical end, and the efflux carrier on the basal end of cells involved in auxin transport (Lomax et al., 1995). Location of the NPA binding protein at the basal end of IAA transporting starch sheath cells of pea stems has been demonstrated by immunofluorescence studies using monoclonal antibodies generated against the NPA binding protein (Jacobs and Gilbert, 1983; Jacobs and Short,

1986) which is closely associated with the efflux carrier (Jacobs and Short, 1986; Morris et al., 1991) .

In an investigation of N-aryl phthalamic acids, Hoffman and Smith (1949) found that one compound they had synthesised, NPA, demonstrated marked inhibition of fruit growth when applied as a spray to tomato flowers. Since then it has been demonstrated that NPA is a potent inhibitor of the basipetal auxin transport process (Thomson et al., 1973; Katekar and Geissler, 1977), by inhibiting the auxin efflux carrier (Lomax et al., 1995). This may also result in the accumulation of IAA in cells due to continued uptake carrier activity (Delbarre et al., 1996). NPA is widely used in plant physiology research to characterise the mechanisms involved in polar auxin transport (Katekar and Geissler, 1977; Goldsmith, 1977; Rubery, 1990; Lomax et al., 1995), as a pre-emergence herbicide inhibiting seed germination and root growth of seedlings (Ashton and Crafts, 1981). Following investigation of structure-activity relationships, (Katekar and Geissler, 1980) assigned NPA and several closely related compounds to a group known as the 'phytotropins'. Phytotropins have a structure based on a 2-carboxyphenyl group separated from a second phenol group, and a mode of action that appears to be solely due to non-competitive inhibition of the auxin efflux carrier (Katekar and Geissler, 1980). The NPA binding site was initially thought to be a subunit of the auxin efflux carrier itself (Lembi et al., 1971; Rubery, 1987), but more recent evidence has suggested that a separate NPA receptor exists which is closely linked to the efflux carrier possibly by a third rapidly turned over protein (Morris et al., 1991; Wilkinson and Morris, 1994; Cox and Muday, 1994). The function of the NPA receptor is considered likely to be endogenous regulation of auxin transport by naturally occurring compounds such as quercetin and lunularic acids which show phytotropin-like activity such as competitive inhibition of NPA binding, stimulation of IAA uptake into membrane vesicles and tissue segments, and abolition of gravitropism in seedlings (Jacobs and Rubery, 1988; Katekar et al., 1993).

Application of polar auxin transport inhibitors to fruit have usually led to a decrease in fruit yield. NPA applied to tomato plants as a spray severely inhibited fruit set (Hoffman

and Smith, 1949). When nectarine trees were sprayed with 100 $mg.l^{-1}$ 2,3,5-triiodo benzoic acid (TIBA) at 30 days after full bloom, fruit size was reduced compared with fruit from control trees (Wand et al., 1991a). However application of NPA to cucumbers induces parthenocarpic development of normal fruit due to an accumulation of IAA (Beyer and Quebedeaux, 1974; Kim et al., 1994).

The aim of experimental work described in this chapter was to investigate the effect of auxin transport on kiwifruit growth and dry matter accumulation, as auxin transport out of fruit pedicels appears to have a role in the determination of correlative dominance and sink strength of fruits (Bangerth, 1989). For this work, the polar auxin transport inhibitor 1-N-naphthylpthalamic acid (NPA) was applied solely to the fruit pedicel in order to inhibit transport of IAA out of the fruit, as opposed to application to the fruit where direct inhibitory effects might be expected. NPA was used because it has been reported to be a highly potent inhibitor of auxin transport (Katekar and Geissler, 1980), have limited mobility (Thomson et al., 1973; Ashton and Crafts, 1981) and negligible phytotoxicity (Katekar and Geissler, 1980). At the same time as this work was undertaken, the effect of pedicel applied NPA on calcium accumulation of kiwifruit was being investigated (G.S.Lawes and D.J. Woolley Department of Plant Science, Massey University), and this aspect was not investigated.

## 7.2 Materials and Methods

### 7.2.1 Synthesis of 1-N-Naphthylpthalamic acid (NPA)

NPA was synthesised in our laboratory using standard methods (Thomson et al., 1973; Tweedy and Houseworth, 1976). Reportedly, the reagents 1-Naphthylamine and toluene have mammalian toxicity and 1-Naphthylamine has carcinogenic properties (Budavari et al., 1989). Extreme caution was used when synthesising NPA, with all operations carried out in a fume hood, while wearing gloves and a dust mask. 1-Naphthylamine (500mg) was dissolved in 50ml of toluene, and Phthalic anhydride (500mg) was dissolved in a further 50ml toluene. The two toluene solutions were mixed, and placed overnight in the dark at 4°C. NPA, a purple powder is insoluble in toluene and can be separated from the reagents by filtration. Precipitated NPA was filtered through Whatman filter paper, and the precipitate washed five times with clean toluene to remove any un-reacted reagents. Toluene was removed from the NPA formed by evaporation, and NPA was stored at 0-2°C.

For application, NPA was dissolved in a small volume of ethanol, and mixed into warm, fully hydrated lanolin as described in chapter five. Where plain lanolin was used as a control, it was also fully hydrated, and had the same volume of ethanol added.

### 7.2.2 The effect of NPA on transport of $^{14}$C-indole-3-acetic acid by kiwifruit pedicels

Indole-3-[1-$^{14}$C]-acetic acid ($^{14}$C-IAA), 46 mCi/mMol (Amersham) in 80% ethanol was diluted to 160 000 radioactive disintegrations per minute (DPM) per 100 µl with 0.2 M Phosphate buffer pH 6.0 (Thomson et al., 1973) and mixed with an equal volume of 3% water agar, resulting in a 1.5% water agar containing 80 000 DPM (780 pmol IAA) per 100 µl. While still molten, 100 µl aliquots of the $^{14}$C-IAA agar were transferred to glass receptacles for use as $^{14}$C-IAA donor agar.

Well pollinated, evenly sized kiwifruit in exposed positions were selected from three vines at the Massey University fruit crops unit at seven weeks after full bloom (WAFB). Fully hydrated lanolin containing 3.4 mM NPA was applied in a narrow band (<10 mm) to the pedicel of 18 fruit, and two control treatments of plain fully hydrated lanolin in a narrow band or no pedicel treatment were applied to a further 18 fruit each.

All fruit were harvested from the orchard 48 hours after application of treatments and immediately taken to the laboratory. Pedicels were cut to exactly 20 mm in length encompassing the NPA/lanolin bands. Pedicels from 12 fruit per treatment were placed with the proximal (shoot) end into 1.5% agar receiver blocks (prepared as for donor blocks but with no $^{14}$C-IAA), and a $^{14}$C-IAA donor block placed firmly on the distal (fruit) end of the pedicel. This orientation simulated normal basipetal IAA transport. The remaining six pedicels were oriented in the reverse direction to check for any non-specific transport of $^{14}$C-IAA. Auxin transport was allowed to proceed for 24 hours at 20°C, and 100% relative humidity to avoid uptake of moisture by the pedicels. Receiver blocks were placed into 5 ml methanol and shaken for one hour, 15 ml scintillation fluid added and radioactivity measured by liquid scintillation counting.

### 7.2.3 The persistence of NPA activity on kiwifruit pedicels in the field

Fruit were selected as in experiment 7.2.2 at eight WAFB, and randomly labelled with the treatment to be applied. Fully hydrated lanolin containing 3.4 mM NPA was applied in a narrow band to pedicels of 12 fruit per time zero, seven, 14, 17 and 19 days after selection. Two days after the final NPA treatment, a plain hydrated lanolin band was applied to the twelve remaining fruit, and all fruit and pedicels were harvested. Measurement of $^{14}$C-IAA transport through a 20 mm length of pedicel was made as described in experiment 7.2.2.

### 7.2.4 The translocation of NPA activity along kiwifruit pedicels

Fruit were selected as in experiment 7.2.2 at five WAFB, but in the following season. Fully hydrated lanolin containing 3.4 mM NPA or plain fully hydrated lanolin were applied in a narrow band close to the shoot (proximal) end of the pedicel. One week after application of the lanolin, all fruit were harvested and taken to the laboratory. Each pedicel was separated into the 'proximal' portion with the NPA/lanolin band, and the 'distal' portion which had not had NPA/lanolin applied directly (Figure 7.1). $^{14}$C-IAA transport was measured through a 20 mm length of both portions of each pedicel as described in experiment 7.2.2.

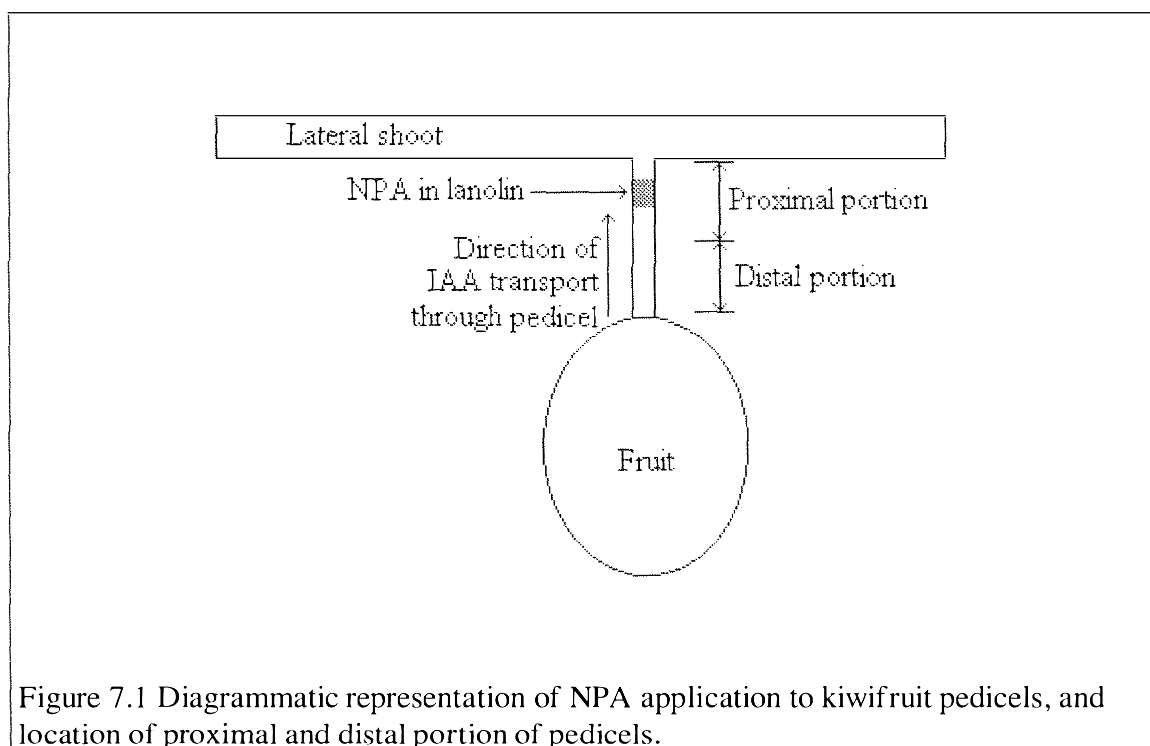


Figure 7.1 Diagrammatic representation of NPA application to kiwifruit pedicels, and location of proximal and distal portion of pedicels.

### 7.2.5 The effect of NPA applied to pedicels on kiwifruit weight and seed viability

Fruit were selected as in experiment 7.2.2 at three WAFB. Two treatments of either fully hydrated lanolin containing 3.4 mM NPA applied in a narrow band around the centre of kiwifruit pedicels (NPA) or no treatment (control) at this time. Each treatment was

replicated six times on each of four vines. Fruit were harvested at commercial maturity, and fresh weight, dry weight and seed numbers estimated.

Germination tests were conducted on seeds from characteristic fruit from each treatment which were non-randomly selected after harvest. After extraction and air drying, seeds were stored in a refrigerator for four months prior to germination testing. A random sample of 50 seeds from each fruit were obtained and soaked for 24 hours in 2500 PPM $GA_3$ to overcome seed dormancy (Lawes and Anderson, 1980), surface sterilised for 5 minutes in 3% orthocide fungicide, and placed on moistened absorbent paper at 20°C. Germination was considered to have occurred once the radical was 1mm in length (Lawes and Anderson, 1980).

In a separate experiment, fully hydrated lanolin containing 3.4 mM NPA, or plain fully hydrated lanolin were applied to kiwifruit pedicels six WAFB. Controls were completely undisturbed pedicels. Treatments were applied in a randomised block design, and were replicated three times on each of three vines.

### 7.2.6 Timing and concentration of NPA applied to pedicels

Fruit were selected as in experiment 7.2.2 at full bloom. The experimental design used was a randomised complete block design with a factorial treatment structure. Four levels of application time in combination with four levels of NPA concentration were applied. All flowers were randomly selected and allocated to treatment combinations at full bloom just prior to fruit set, and each treatment combination was replicated twice on each of three vines. The NPA concentrations used were 0, 0.34 mM, 1.7 mM and 3.4 mM in fully hydrated lanolin. NPA was applied in a narrow band towards the top of the pedicel, in order to minimise the possible translocation into the fruit. Fruit were harvested at commercial maturity and fresh weight, dry weight and seed numbers estimated using a surface seed correlation as described in chapter two. Average seed weight and total seed weight per fruit were estimated in fruit which had pedicels treated with 0, 1.7 or 3.4 mM NPA. In these fruit, seeds were removed from the cut surfaces following surface seed

counting. Seeds were then washed, and dried for 48 hours at 70°C after which a dry weight was taken and the seeds in the sample counted.

In a separate experiment, the effect of a short term application of NPA on fruit growth at different stages of development was investigated. The experimental design used was a completely randomised block design with eight levels of NPA application time. Each treatment was replicated three times on each of three vines in December 1993. Treatments of 3.4 mM NPA applied in a narrow band near the top of pedicels were applied at 0, 1, 2, 3, 4, 5, 6 and 8 WAFB. A hydrated lanolin control treatment was applied at three WAFB. All lanolin bands were removed from pedicels with moist tissues, three days following application.

### 7.2.7 Direct application of NPA to kiwifruit ovaries

A saturated solution of NPA was made by dissolving 1g NPA in 10 ml ethanol and diluting to 100 ml with de-ionised water containing 0.25 ml/l citowett spreader-sticker (O'Connor, 1984), and decanting the solution off precipitated NPA. A control solution was made up by diluting 10 ml ethanol to 100 ml with de-ionised water containing 0.25 ml/l citowett. At three WAFB, fruit were dipped in the appropriate solution for ten seconds up to 5 mm below the point of attachment of the pedicel , and harvested at commercial maturity.

### 7.2.8 Application of NPA to stems of lateral fruiting shoots

Experimental design was a randomised complete block design with a factorial treatment structure. Two levels of NPA treatment were applied in combination with two levels of leaf:fruit ratio. Each treatment combination was replicated five times on each of two vines at three WAFB. NPA treatments were either 0 or 3.4 mM NPA in lanolin applied in a wide (30 mm) band at the base of the lateral shoot. The leaf:fruit ratio treatments were eight leaves and two fruit (four) which is sufficient for normal fruit growth, or zero

leaves and two fruit (zero) which would require fruit to import carbohydrates from neighbouring lateral shoots. The fruit fresh and dry weight were measured at commercial maturity.

### 7.2.9 The interaction between NPA and exogenous growth regulators

The experimental design used was a randomised complete block design with a factorial treatment structure. Two levels of NPA treatment were applied in combination with five levels of plant growth regulator treatment. Each treatment combination was applied to fruit on six lateral shoots at three WAFB in 1994/95. Treatments of 0 or 3.4 mM NPA were applied in a narrow band near the top of pedicels. Growth regulator applications were as follows:

- Control
- $GA_3$ 50 PPM
- 2,4-D (sodium salt) 25 PPM
- CPPU (e.c.) 10 PPM
- $GA_3$ + 2,4-D + CPPU (at above concentrations)

All growth regulators were diluted in de-ionised water containing 0.25 ml/l citowett spreader-sticker (O'Connor, 1984). Control fruit were dipped in de-ionised water containing 0.25 ml/l citowett at the same time as other fruit. Fruit were harvested at commercial maturity and fresh and dry weight measured.

## 7.3 Results

### 7.3.1 The effects of NPA on transport of $^{14}$C-IAA in kiwifruit pedicels

Radioactivity in receiver agar blocks from all treatments with pedicels oriented in the basipetal direction was significantly above background (Students t, P<0.05). However radioactivity in receiver agar from reverse orientation pedicels from all treatments was not significantly above background (students-t P>0.05), and did not exceed a net count above the background of 10 disintegrations per minute (DPM) in any one receiver block. This indicated preferential basipetal transport of $^{14}$C-IAA in the kiwifruit pedicels, and that contamination of receiver blocks by non-specific transport was negligible. The presence of a hydrated lanolin band had no significant effect on $^{14}$C-IAA transport compared with control pedicels (contrast, P=0.23). NPA treatment of pedicels almost entirely inhibited the transport of IAA through the pedicel to approximately 2.5% of controls (Table 7.1).

Table 7.1. The effect of applying 3.4 mM NPA in lanolin to kiwifruit pedicels 48 hours prior to harvesting on $^{14}$C-IAA transport through 20 mm segments of kiwifruit pedicels over a period of 24 hours. Values are radioactive disintegrations per minute (DPM) in receiver agar blocks, and are back-transformed from $\log_{10}$.

| Treatment | Basipetal transport (n=12) | Acropetal transport (n=6) |
|---|---|---|
| Control (untreated) | 736.3 a | 12.6[1] a |
| Lanolin only | 882.6 a | 14.0[1] a |
| Lanolin containing 3.4 mM NPA | 20.8 b | 15.7[1] a |

Means in a column with common letters are not significantly different at P=0.05 (LSD).
[1] Not significantly above background (12.8 DPM) at P=0.05 (Students t).

Transport of $^{14}$C-IAA through kiwifruit pedicels was found to be severely inhibited for at least 21 days after the application of NPA (Table 7.2). Inhibition was strongest between 7 and 14 days after NPA application, and a significant (P=0.0001) quadratic trend was found

using polynomial contrasts. By 21 days following NPA application, IAA transport was still low compared with control pedicels, however it was significantly higher than at 4, 7 and 14 days after NPA application, whcih indicates that the transport inhibition was beginning to diminish.

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 $^{14}C$-IAA. Values are mean radioactive disintegrations per minute (DPM) in receiver agar blocks, and are back-transformed from $\log_{10}$ (n=12).

| Time of NPA application prior to test | DPM |
|---|---|
| Control (lanolin only) | 1271.7 a |
| 2 days | 75.5 bc |
| 4 days | 55.8 c |
| 7 days | 30.1 d |
| 14 days | 28.8 d |
| 21 days | 132.3 b |

Means with common letters are not significantly different at P=0.05 (LSD).
All treatment means are significantly above background (14.3 DPM).

In order to determine whether NPA affected auxin transport away from the point of application, the transport of $^{14}C$-IAA was measured on the proximal (closest to shoot) and distal end (closest to fruit) of pedicels where NPA was applied only to the proximal end (Figure 7.1). It was clear that auxin transport in control pedicels was similar along the length of the pedicel (contrast, P=0.85). Auxin transport through the proximal end of NPA treated pedicels was reduced similarly to that found in the previous experiments. Transport in the non-treated section (distal) of NPA treated pedicels was reduced, but not to the same extent in the proximal section (Table 7.3), although it was significantly lower (contrast P=0.0213) than in the corresponding section of control pedicels. Variation was comparatively high in the distal section of the NPA treated pedicels, as some of the pedicels had very low $^{14}C$-IAA transport (below 100 DPM) while others had auxin transport levels similar to controls.

Table 7.3 The effect of applying 3.4 mM NPA or lanolin to the proximal portion (closest to shoot) of kiwifruit pedicels on $^{14}C$-IAA transport through either the proximal portion or the distal (closest to fruit) portion of the pedicel. Values are radioactive disintegrations per minute (DPM), back-transformed from $log_{10}$ (n=12).

| Treatment | Section of pedicel | DPM |
|---|---|---|
| Control (lanolin) | Proximal | 1301.5 a |
| Control (lanolin) | Distal | 1202.2 a |
| NPA | Proximal | 529.9 b |
| NPA | Distal | 29.8 c |

Means with the same letter are not significantly different at P=0.05 (LSD).
All treatment means are significantly above background (14.8 DPM) .

### 7.3.2 The effect of NPA on fruit size and seed viability

From as early as two weeks after application of NPA to kiwifruit pedicels, there was a noticeable decrease in fruit size compared with the control fruit. Fruit with NPA treated pedicels appeared to be growing normally in terms of fruit shape, however the fruit were noticeably smaller. NPA application to fruit pedicels at three WAFB resulted in reductions in fruit fresh and dry weight of almost 50% compared with control fruits (Table 7.4). In addition, the percentage dry matter of fruit was reduced in fruit with NPA treated pedicels. High numbers of seeds were present in NPA treated fruits, however many seeds did not appear to be fully mature as a number of seeds were light brown or white in colour and appeared to be smaller in size than those from the control fruit.

When NPA was applied to fruit pedicels at six WAFB, no significant model or treatment effects on fruit weight were found (Table 7.5).

The method used to germinate seeds was successful in obtaining over 90% germination of control seeds. Seeds from both treatments began to germinate two weeks following GA treatment. Seeds extracted from NPA fruit had significantly lower germination at all times, and had a significantly lower final percentage germination (Figure 7.2). Radicles arising from control fruit appeared healthy and very vigorous, with abundant root hairs. Radicles arising

from seeds extracted from NPA fruit were slow growing, with few root hairs and some had a stunted appearance (Plate 7.1).

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. Due to the presence of missing data, values presented are population marginal means[1].

| | Control | NPA | Contrast P>F (control vs. NPA) |
|---|---|---|---|
| Fresh weight (g) | 111.5 | 65.2 | 0.0001 |
| Dry weight (g) | 17.7 | 9.7 | 0.0001 |
| Percent dry matter | 16.2 | 15.0 | 0.0147 |
| Seed number | 1138 | 1279 | 0.0504 |

[1] For description of population marginal means, see chapter two, section 2.8.3.

Table 7.5 The effect of applying 3.4 mM NPA to kiwifruit pedicels at six weeks after full bloom on fruit weight at maturity. Values are arithmetic means (n=9).

| | Control | Lanolin only | NPA in lanolin |
|---|---|---|---|
| Fresh weight (g) | 107.9 (2.8) | 107.5 (2.3) | 103.2 (4.6) |
| Dry weight (g) | 17.6 (0.4) | 17.0 (0.5) | 16.3 (0.9) |
| Percent dry matter | 16.3 (0.2) | 15.9 (0.3) | 15.7 (0.3) |

Standard error of the mean in parentheses.

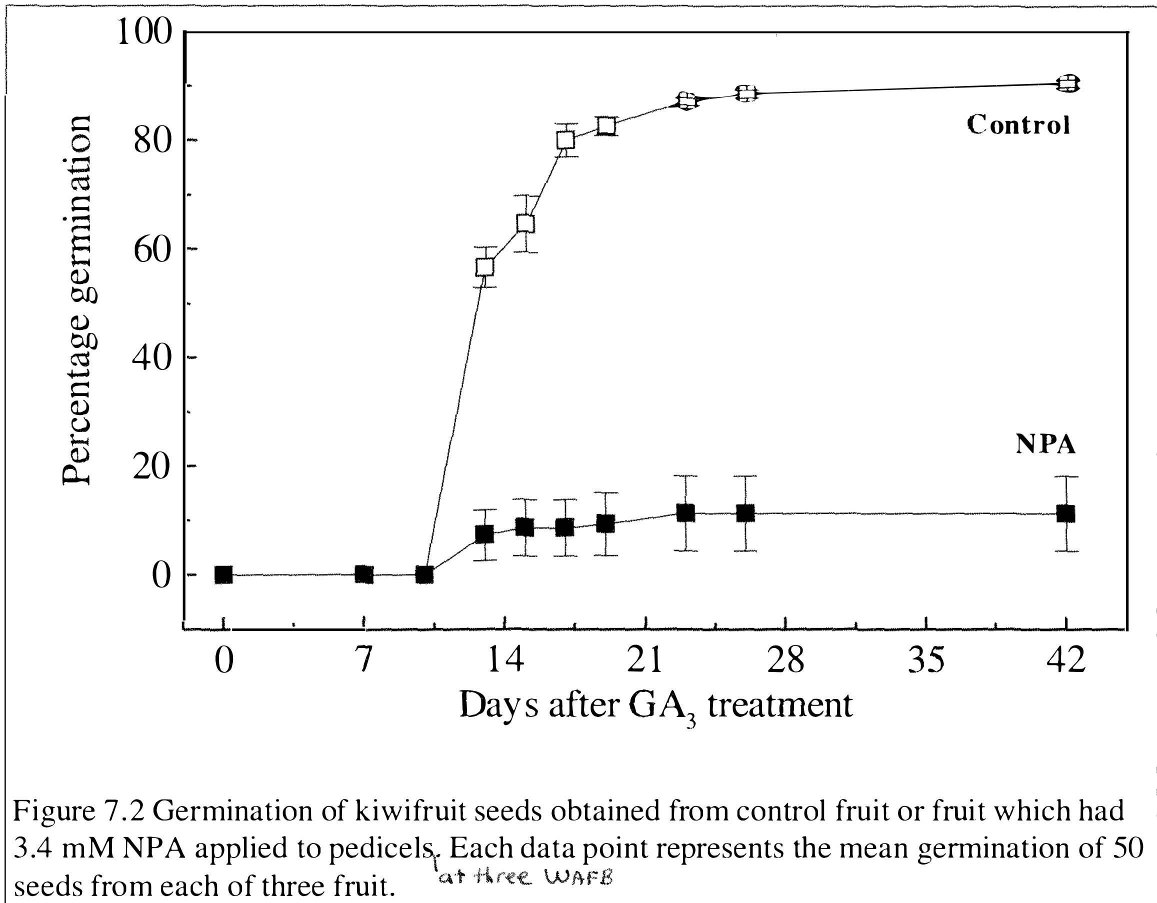


Figure 7.2 Germination of kiwifruit seeds obtained from control fruit or fruit which had 3.4 mM NPA applied to pedicels at three WAFB. Each data point represents the mean germination of 50 seeds from each of three fruit.

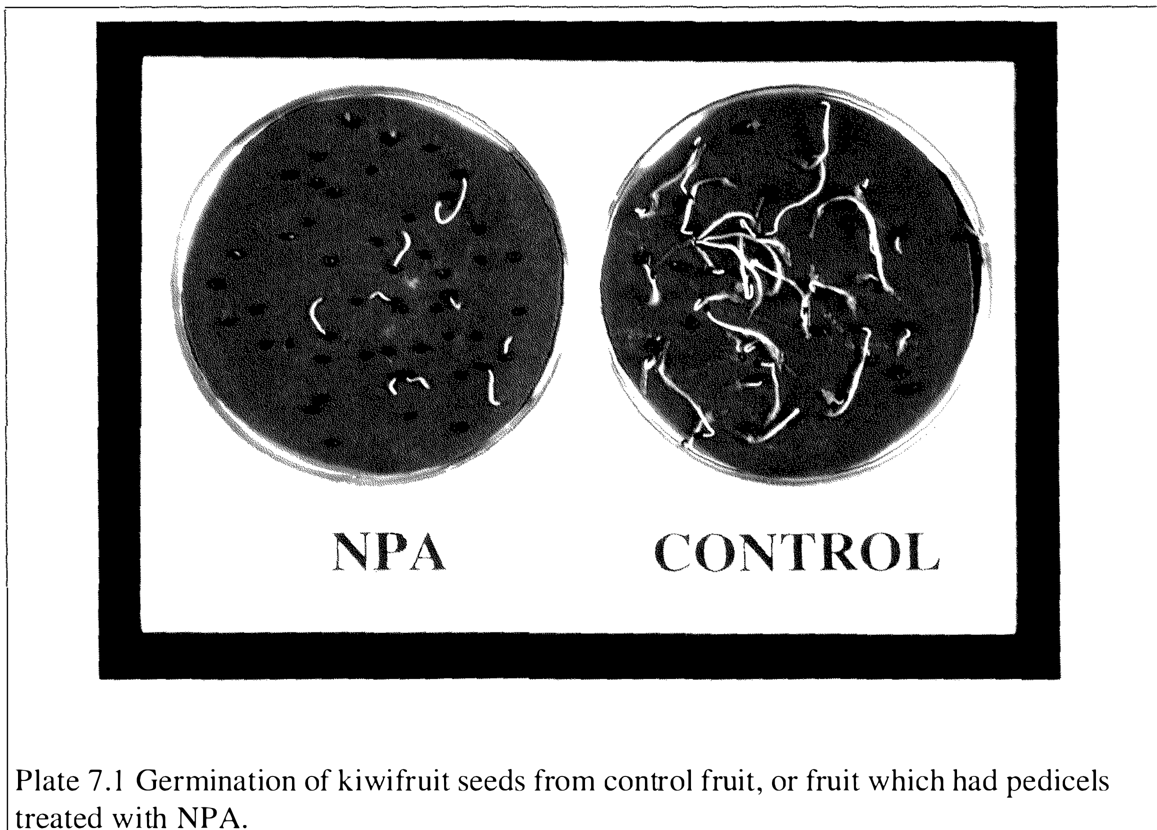


Plate 7.1 Germination of kiwifruit seeds from control fruit, or fruit which had pedicels treated with NPA.

### 7.3.3 Application of different NPA concentrations during early kiwifruit growth

There was a significant interaction between the timing and concentration of NPA application to kiwifruit pedicels. Between full bloom and two WAFB, fruit weight was reduced to a similar extent by any one NPA concentration, however at three WAFB, there was no significant effect of any NPA concentration on fruit weight (Figure 7.3). This is in contrast to results obtained in the previous year, where application of NPA at three WAFB was particularly effective (Table 7.4).The effect of the lowest concentration of NPA, 0.34 mM, was not influenced by treatment date (Figure 7.3), and the main effect was a reduction in fruit fresh weight of 19.6g per fruit (contrast, P=0.0001) and dry weight of 2.8g per fruit (contrast, P=0.0023) compared with control fruit. The response of fruit size to 0.34 mM NPA was less than at the two higher concentrations. There were no significant differences between 1.7 and 3.4 mM NPA at any time between any of the treatment dates.

The fruit percentage dry matter averaged 15.9% over all treatment combinations including the control, but was not significantly affected by the time of application or concentration of NPA. Estimated seed numbers were significantly affected by NPA treatment (ANOVA, P=0.0479). Fruit from NPA treated pedicels had significantly higher estimated seed numbers overall than control fruit (contrast, P=0.0064). This confirmed that treatment of fruit with NPA did not cause visible seed abortion. There were significant main and interaction effects on the average seed size (weight). NPA treatments resulted in significantly smaller seeds than control treatments overall (contrast P=0.0001), although the effect of NPA treatments on seed size was diminished at later treatment dates (Figure 7.3). To obtain an estimate of total seed weight for any fruit, seed size was multiplied by estimated seed number. Fruit fresh and dry weight from the 16 treatment combinations were found to be well correlated to total fruit seed weight (Figure 7.4). Correlation of fruit weight to seed number of the sample of fruit used was poor (F-test slope≠0: P=0.32).

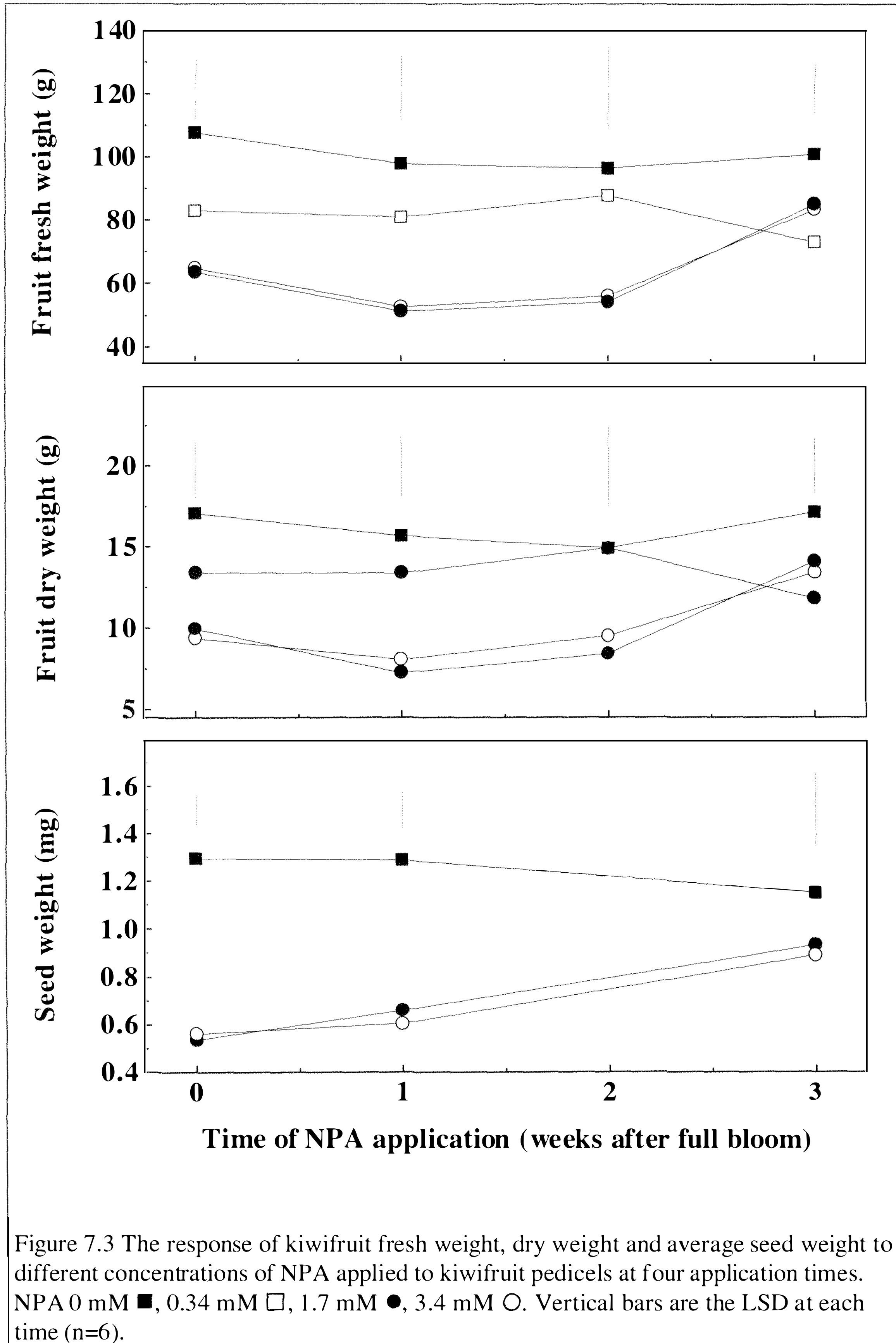


Figure 7.3 The response of kiwifruit fresh weight, dry weight and average seed weight to different concentrations of NPA applied to kiwifruit pedicels at four application times. NPA 0 mM ■, 0.34 mM □, 1.7 mM ●, 3.4 mM ○. Vertical bars are the LSD at each time (n=6).

### 7.3.4 Short-term application of NPA

Short-term application of NPA to kiwifruit pedicels for only three days (Table 7.6) had a similar effect to previous applications where NPA had remained in contact with the pedicel (Table 7.1). Short-term treatment of kiwifruit pedicels with 3.4 mM NPA significantly reduced fruit dry weight below control fruit when applied between zero and four WAFB, however there was no significant difference to control fruit when NPA was applied at five and six WAFB (Table 7.6). Fruit fresh weight was influenced similarly to dry weight by the timing of NPA application, except that fresh weight was not significantly lower than controls at four WAFB (Table 7.6). Fruit fresh and dry weight were most sensitive to NPA when pedicels were treated at two WAFB (Table 7.6). There were no significant model effects on estimated seed number (P=0.64) in this experiment.

Table 7.6 The effect of applying NPA kiwifruit pedicels between 0 and 6 weeks after full bloom on final fruit weight. NPA was removed from pedicels three days after application. Pedicels of control fruit were treated with hydrated lanolin at 3 weeks after full bloom. Due to the presence of missing data, means presented are population marginal means.

| Time of NPA application | Fruit fresh weight (g) | | Fruit dry weight (g) | |
|---|---|---|---|---|
| (Weeks after full bloom) | Mean | Contrast: P>F (NPA *vs.* control ) | Mean | Contrast: P>F (NPA *vs.* control ) |
| *Control* | *100.5* | - | *15.6* | - |
| 0 | 68.9 | **0.0002** | 8.7 | **0.0004** |
| 1 | 68.5 | **0.0001** | 8.4 | **0.0001** |
| 2 | 54.0 | **0.0001** | 7.5 | **0.0001** |
| 3 | 80.1 | **0.0054** | 10.7 | **0.0042** |
| 4 | 93.2 | 0.3074 | 12.3 | **0.0451** |
| 5 | 98.0 | 0.7255 | 14.4 | 0.4850 |
| 6 | 95.7 | 0.4946 | 14.3 | 0.4438 |

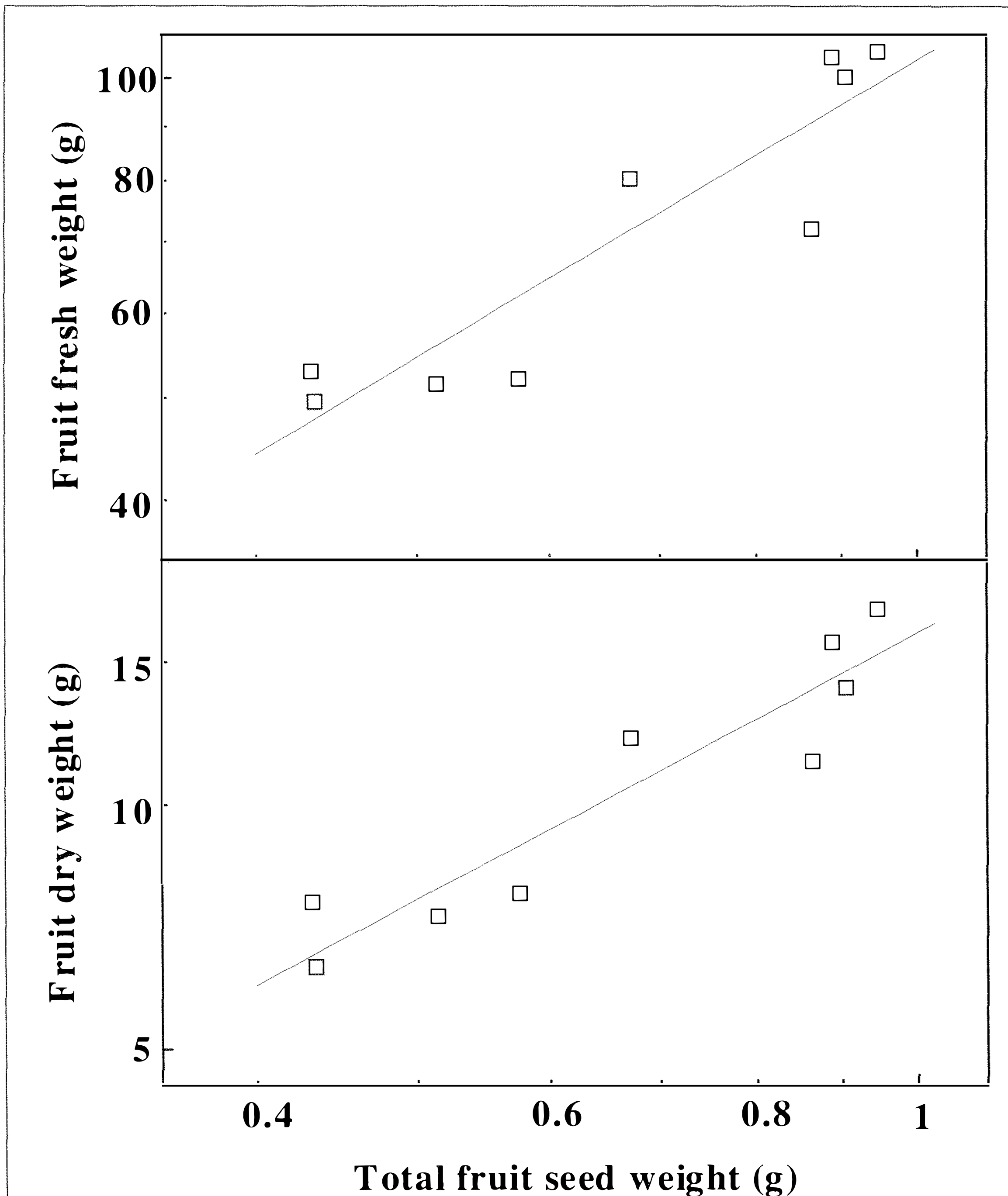


Figure 7.4 The relationship between seed weight and fruit fresh or dry weight for fruit which had NPA applied to pedicels. Each data point represents the mean seed weight and fruit weight of a treatment combination between three NPA concentrations (0, 1.7, 3.4 mM) and three times of application to kiwifruit pedicels (0, 1, 3 weeks after full bloom). Equations of fitted lines Fresh weight Y=2.02+0.93X; $R^2$=0.84. Dry weight Y=1.21+1.09X $R^2$=0.88.

### 7.3.5 Direct application of NPA to kiwifruit ovaries

Application of NPA to fruit as a ten second dip was particularly effective at inhibiting kiwifruit growth. The dry weight of fruit dipped in NPA was reduced by almost 50% (Table 7.7). There was also a significant decrease (ANOVA. P=0.0414) in percentage dry matter of over 2% in fruit which had been dipped in NPA (Table 7.7).

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.

| | NPA treated fruit | Control fruit |
|---|---|---|
| Fresh weight (g) | 67.5 | 117.3 * |
| Dry weight (g) | 10.1 | 19.5 * |
| Percentage dry matter | 14.6 | 16.6 * |

* Means in the same row are significantly different at P=0.05 (LSD).

### 7.3.6 Application of NPA to shoots

There was some evidence of a significant interaction between the leaf:fruit ratio and application of NPA to the stem of fruiting lateral shoots on fruit dry weight (ANOVA P=0.078), although not fruit fresh weight (ANOVA, P=0.210). Fruit weight on control treated (0 ppm NPA) lateral shoots was not significantly affected by the leaf:fruit ratio (Table 7.8). However fruit fresh weight and dry weight on lateral shoots with no leaves which were treated with 3.4 ppm NPA were significantly lower (contrast P=0.0007, 0.0054 respectively) than all of the other treatment combinations overall (Table 7.8). Fruit weight on lateral shoots which had a leaf:fruit ratio of four did not respond to application of NPA to the stem overall (Table 7.8).

Table 7.8 The interaction between leaf:fruit ratio and application of NPA to the base of fruiting lateral shoots. Values are means of fruit fresh and dry weights from 10 replicate lateral shoots.

| Leaf:fruit ratio | Concentration of NPA (mM) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|
| 0:1 | 0 | 104.4 (3.9) | 17.2 (0.8) |
| 0:1 | 3.4 | 91.8 (4.5) | 14.6 (1.1) |
| 4:1 | 0 | 109.9 (4.5) | 17.1 (0.6) |
| 4:1 | 3.4 | 106.1 (3.5) | 17.3 (0.7) |

Standard error of the mean in parentheses.

### 7.3.7 The interaction between NPA and exogenous growth regulator application

The effects of the application of NPA were consistent with the results obtained in the previous experiments (sections 7.3.2 to 7.3.6). On otherwise untreated (growth regulator control) fruit, the application of 3.4 mM NPA to kiwifruit pedicels resulted in a significant (contrast P=0.0002) reduction in both fresh and dry weight (Table 7.9).

Fruit responded positively to the application of some of the exogenous growth regulators. Compared with the appropriate control fruit, there were no significant effects of 2,4-D or $GA_3$ application on fruit weight either on fruit with untreated pedicels or fruit with NPA treated pedicels (Table 7.9). However application of CPPU to fruit with untreated pedicels significantly increased fresh weight by 74g and dry weight by 8.9g above control fruit (Table 7.9). Application of CPPU to fruit with NPA treated pedicels resulted in a similar increase in fresh weight (84g), and dry weight (10.3g), although the fruit were still significantly smaller than when CPPU was applied to fruit with untreated pedicels (Table 7.9). There was no additional response to $GA_3$ and 2,4-D when these were applied to fruit in combination with CPPU.

Table 7.9 Interactions between NPA applied to kiwifruit pedicels and growth regulators applied exogenously as a five second dip to the fruit. Due to the presence of missing values, means presented are population marginal means from up to six lateral shoots.

| Growth regulator | Fruit fresh weight (g) | | Fruit dry weight (g) | |
|---|---|---|---|---|
| | Untreated pedicels | NPA treated pedicels | Untreated pedicels | NPA treated pedicels |
| Control | 104 c | 68 d | 14.7 d | 9.1 e |
| 2,4-D | 111 c | 69 d | 16.3 cd | 9.3 e |
| $GA_3$ | 102 c | 82 d | 13.9 d | 11.9 de |
| CPPU | 178 a | 152 b | 23.6 a | 19.4 b |
| CPPU + 2,4-D + $GA_3$ | 176 a | 147 b | 24.7 a | 18.8 bc |

Means of a parameter with common letters are not significantly different at P=0.05 (lsmeans, SAS).

## 7.4 Discussion

### 7.4.1 The transport of $^{14}$C-IAA in kiwifruit pedicels

The system used for measurement of auxin transport capacity of kiwifruit pedicels only allowed basipetal transport of $^{14}$C-IAA, as demonstrated by the nil IAA measured in receiver agar of pedicels oriented in the reversed orientation. This confirms results obtained in chapter six that kiwifruit pedicels have polar auxin transport capacity. Basipetal export of endogenous IAA out of fruits has also been demonstrated in apple (Gruber and Bangerth, 1990), tomato (Banuelos et al., 1987), avocado (Cutting and Bower, 1990), and peach (Wand et al., 1991b).

Application of 3.4 mM NPA to the pedicels of kiwifruit almost entirely inhibited their ability to tranport $^{14}$C-IAA. Auxin transport through NPA treated pedicels was reduced to 6% of that in control pedicels within 48 hours of application and to less than 2.5% of that in control pedicels within 7 days of application (Table 7.2). In wheat coleoptile sections, the transport of $^{14}$C-IAA was almost completely eliminated at a concentration of 0.01 mM NPA (Thomson et al., 1973). However in intact fruit pedicels, uptake of NPA is likely to be poor, and thus higher concentrations are probably required to eliminate IAA transport. Application of 5.4 mM NPA in lanolin to pedicels of tomato fruit was found to only reduce transport of endogenous IAA from attached fruits to approximately 70% of control pedicels 24 hours after application (Banuelos et al., 1987). This suggests that uptake of NPA by tomato pedicels may be poorer than in kiwifruit.

The amount of radioactivity present in the receiver agar of control kiwifruit pedicels was around 1300 DPM, equivalent to 12.7 pmol IAA. This is similar to the endogenous level (17.5 pMol, estimated visually from graph) of IAA found to be exuded from tomato pedicels over a 24 hour period (Banuelos et al., 1987), and from apple fruits (Gruber and Bangerth, 1990). However the amount of endogenous IAA diffusing from kiwifruits ranged from 21 to 74 pMol.fruit$^{-1}$ over a 24 hour period (Chapter six). Therefore the

IAA transport rates observed in the experimental system used in this study probably under-represents the levels of endogenous IAA transport from kiwifruit normally transported through pedicels. However as NPA binding to the specific membrane protein responsible for inhibition of IAA transport is non-competitive with IAA (Jacobs and Rubery, 1988), the results obtained from $^{14}C$-IAA studies were probably still representative of the effect that NPA has on endogenous IAA transport from kiwifruit.

Within seven days of the application of NPA to kiwifruit pedicels, there appeared to be significant movement of NPA activity along a kiwifruit pedicel, although some transport of $^{14}C$-IAA was able to occur on the portion of the pedicels not treated with NPA. This suggests that NPA itself may have been translocated within the pedicel within a seven day period, but that the rate of translocation was quite low. Alternatively, high concentrations of accumulated auxin in the cells responsible for basipetal auxin transport may have competitively reduced $^{14}C$-IAA movement through the pedicels, as both influx and efflux carriers are known to be saturable by IAA (Goldsmith, 1982; Delbarre et al., 1996). It is possible the accidental contamination of the lower part, or cut surface of the pedicel may have occurred on two pedicels where IAA transport was severely inhibited. Polar and acropetal movement of $^{3}H$-NPA through 2mm long corn coleoptiles has been found to be minimal over a period of three hours (Thomson et al., 1973). This is in agreement with the results we have obtained here, and suggests that NPA may be translocated to fruit slowly. However translocation of NPA from roots to shoots has been found from wheat plants growing in aerated nutrient solution containing 90 $\mu$M NPA (Devlin and Karczmarczyk, 1977). Thus it is possible that NPA applied to pedicels could enter fruits by diffusion into xylem, and subsequent translocation in the transpiration stream. In addition, cell to cell transport of NPA in the symplast can probably also occur, as NPA must enter the cytoplasm of auxin transporting cells in order to bind to the NPA binding protein (Hertel et al., 1983; Cox and Muday, 1994). Translocation is likely to have been enhanced when NPA was in contact with pedicels for a long period of time, as continued uptake could have occurred, leading to saturation of NPA binding sites in cells close to the point of application, and increased levels of unbound NPA available to be translocated.

### 7.4.2 The inhibition of fruit growth by NPA

Application of NPA to kiwifruit pedicels at between full bloom and three WAFB inhibited the ability of fruit to assimilate carbohydrate, as demonstrated by decreases in fruit dry weight of over 50% (Table 7.6). However this effect was mimicked by direct application of NPA to kiwifruit themselves, where care was taken that NPA did not contact the pedicel (Table 7.7). As significant $^{14}$C-IAA transport inhibition activity was translocated along pedicels, it seems likely that some of the NPA applied to kiwifruit pedicels was also translocated into fruit. Although the short term application trial where the NPA in lanolin band was removed from the kiwifruit pedicel after three days should have minimised this possibility, the fruit response was similar to the other experiments (Table 7.6). If significant quantities of NPA were translocated into fruit, it is likely that a more direct inhibition of cell growth or seed development could have resulted, rather than an indirect effect elicited by the blocking of IAA transport out of fruits. The effect on fruit growth of auxin transport inhibitors on fruit growth has been ascertained in a number of crops, however the response has been variable. TIBA applied to 'Golden Delicious' apple pedicels caused fruit to abscise (Stahly and Williams, 1972). Final fruit dry weight of nectarines was significantly lower when trees were sprayed with 100 ppm TIBA at 30 days after full bloom, which corresponds with a period of cell enlargement in the fruit (Wand et al., 1991a). However TIBA sprays at 16 days after full bloom, corresponding with the period of cell division, temporarily inhibited fruit growth, but final fruit weight was not affected (Wand et al., 1991a). NPA sprayed onto whole tomato plants at 20 to 2000 ppm completely inhibited fruit set (Hoffman and Smith, 1949), however NPA sprayed onto whole tomato plants at 25 ppm resulted in a 1.5 times increase in yield (Moore, 1957). Similarly, NPA applied at concentrations of between 120 and 1200 ppm to whole cucumber plants induces parthenocarpic fruit development (Quebedeaux and Beyer, 1974; Kim et al., 1994).

It is generally thought that the effects of NPA on plant growth and development are solely attributable to the inhibition of auxin transport. NPA was not found to be phytotoxic to pea stem sections, and did not inhibit the response of these to exogenous auxin (Katekar and Geissler, 1980). In addition, NPA does not inhibit auxin transport by

competing for auxin binding sites (Sussman and Goldsmith, 1981b; Depta et al., 1983) but appears to bind to a protein peripherally associated with the internal face of the plasma membrane (Cox and Muday, 1994), but not the auxin efflux carrier itself (Morris et al., 1991; Cox and Muday, 1994; Wilkinson and Morris, 1994). This would suggest that if NPA binding proteins are present in kiwifruit tissue, NPA is unlikely to either directly inhibit the action of auxin or cause direct cellular damage. Therefore, the inhibitory effect of NPA on kiwifruit growth is likely to be entirely due to the inhibition of the auxin efflux carrier in fruit and/or seed cells. Although some authors have speculated that the NPA binding protein could regulate cellular processes involving auxin binding other than the efflux carrier (Katekar et al., 1987; Morris et al., 1991), there is no evidence to confirm such a role. The majority of auxin binding sites, other than the efflux carrier, appear to be located on endoplasmic reticulum (Rubery, 1990) whereas NPA binding sites are located on the plasma membrane (Cox and Muday, 1994), therefore this hypothesis seems relatively unlikely.

Some inhibitory effects of NPA on kiwifruit growth may be due to an increase in the cellular concentration of auxin in response to inhibition of the auxin efflux carrier. NPA has been shown to exhibit slight auxin activity (Keitt and Baker, 1966; Katekar and Geissler, 1980), although it seems unlikely that this could be responsible for the inhibition of plant growth, as this activity is only barely detectable (Katekar and Geissler, 1980). However there are numerous reports of NPA stimulating net auxin uptake in cultured cells (Delbarre et al., 1996), sealed membrane vesicle preparations (Sabater and Rubery, 1987) and stem sections (Sussman and Goldsmith, 1981b), due to continued influx carrier mediated uptake while the efflux carrier is inhibited. There is also evidence that NPA causes elevated auxin levels in intact plant organs. IAA content in NPA induced parthenocarpic cucumber ovaries was 3.4 times higher than in un-pollinated ovaries at anthesis (Kim et al., 1992). As stimulation of processes such as assimilate transport and cell elongation by auxin is often dose dependent with an optimum concentration (Patrick and Woolley, 1973; Bouchet et al., 1983) the effects of NPA on the inhibition of kiwifruit growth could be due to stimulated uptake of auxin by kiwifruit cells leading to super-optimum cellular concentrations, and subsequent inhibition of cell division or expansion processes or seed development. This would be dependent on the

presence of auxin efflux carriers and the NPA binding protein in cells that were not associated with polar auxin transport.

Alternatively the effects of NPA on fruit growth could be due to localised auxin deficiency in fruit cells. Cells which are more distant from biosynthetic source of auxin in the fruit could have become deficient in auxin due to inhibition of an influx/efflux carrier mediated distribution system. Such a mechanism depends on the presence of the NPA binding proteins, and the auxin efflux carrier in cells that are not associated with polar auxin transport. If this is the case, this would imply that auxin is actively distributed rather than diffusing in fruit tissue on a concentration gradient. NPA binding has been characterised in a variety of tissues such as cultured tobacco cells (Delbarre et al., 1996), coleoptile, leaf, stem and root tissue in maize (Katekar and Geissler, 1989), hypocotyl tissue from lupin and zuchini (Sabater and Sabater, 1986; Bernasconi, 1996), cauliflower curd (Firn, 1987), and pea stem tissue (Jacobs and Gilbert, 1983). However with the exception of the cultured cells, all of these tissues contain phloem tissue, and therefore probably polar auxin transporting cells. Lomax et al. (1995) points out that in coleoptiles, all cells may be able to transport auxin. However in coleus leaves fed with $^{14}$C-IAA, microautoradiography demonstrated that IAA transport was confined to the region of the phloem (Goldsmith et al., 1974), and in pea stems, polar auxin transport was confined to cambial tissue surrounding the phloem elements (Morris and Thomas, 1978). In contrast, no significant binding of NPA was found in the inner tissues of cucumber fruit (Firn, 1987), which suggests that NPA binding and consequently auxin efflux carriers may not be present in the bulk of cells in fruits which are not associated with phloem, and involved in polar auxin transport. An extension of studies of NPA binding and the presence of influx/efflux carriers in non phloem-associated fruit cells would be worthwhile to ascertain if auxin distribution in fruit tissues is carrier mediated or by simple diffusion.

Although inhibition of auxin transport from fruit by NPA up until three WAFB was found to severely inhibit fruit growth, the effects of auxin transport inhibition were diminished after this time (Table 7.6), and were non-significant when NPA was applied at six WAFB (Table 7.5, Table 7.6). Although some tissues lose the ability to transport IAA later in development (Suttle, 1991), this does not appear to be the case in kiwifruit

pedicels, as high levels of diffusible auxin from kiwifruit pedicels were measured at seven WAFB (Chapter six), and the capacity for the transport of $^{14}C$-IAA has been demonstrated in kiwifruit pedicels as old as 11 WAFB (Table 7.2). Similarly, NPA applied to *Phaseolis vulgaris* stems eliminated basipetal IAA transport, but had no effect on the total amount of $^{14}C$-sucrose translocated to tissue above the point of NPA application (Morris, 1989). It has been suggested that auxin transport is essential for a growing organ (Bangerth, 1989), however these results show that this is not the case for growth of kiwifruit, at least after six WAFB.

### 7.4.3 The effects of NPA on seed development

It appears that seed development was negatively affected by the application of NPA to fruit pedicels. In addition to the general immature appearance of many seeds from NPA treated fruit, seeds were found to be significantly smaller, and had poor germination and radicals were not as vigorous as seeds from control fruits. NPA has been reported to inhibit early root growth of seedlings (Brunn et al., 1992; Katekar and Geissler, 1980), as well as seed germination in some species (Netien and Conillat (1951) cited in Ashton and Crafts (1981)). This suggests that NPA translocated into fruits may have directly inhibited seed development. This suggestion is supported by the effects NPA concentration and time of application have on the average seed weight (Figure 7.3), and the strong correlation between total seed weight per fruit and fruit fresh or dry weight (Figure 7.4). Kiwifruit weight is strongly dependent on the presence of high numbers of viable seeds (Hopping, 1976; Pyke and Alspach, 1986), although a better correlation has been found to total weight of seeds in a fruit both within 'Hayward' kiwifruit (Lai, 1987), and over several different kiwifruit selections (Chapter six). Thus effects of NPA on seed development are consistent with the fruit growth responses which were recorded in the experiments described here. Inhibition of seed development could conceivably reduce the fruits ability to produce and distribute the growth regulators necessary for fruit growth.

The absolute response of kiwifruit to CPPU application was almost identical on fruit with untreated pedicels to fruit which had pedicels treated with NPA (Table 7.9). This is similar to the interaction between CPPU application and fruit seed number (Woolley et al., 1992). Application of CPPU to kiwifruit with (an average of) 1140 or 350 seeds resulted in an increase in fruit weight of 56.8g or 53.0g respectively, although fruit with very low seed counts did not respond to CPPU to the same degree (Woolley et al., 1992). The additive effects of CPPU on the growth of kiwifruit limited by both low seed number or NPA treatment, strongly suggests that the effect of NPA on fruit growth is via viable seed number or seed weight.

### 7.4.4 Vascular development

An attractive mechanism for the role of auxin transport in enhancing sink strength is that it may induce differentiation of phloem elements. The long term result of reduced auxin transport from a dominated organ, or one where auxin transport has been inhibited by NPA could be associated with a limited ability to form phloem elements. The polar flow of auxin is known to be important for cambial growth and vascular development (Aloni, 1995; Uggla et al., 1996), thus inhibition of vascular development in pedicels could have occurred in NPA treated kiwifruit, limiting the carbohydrate import rate. A positive relationship between pedicel weight and fruit weight has been found in kiwifruit which extends to fruit with NPA treated pedicels (G.S. Lawes, personal communication, 1994). Kiwifruit weight has also been found to be positively correlated to pedicel diameter (Lai, 1987). It has been shown that there is relatively little expansion of the cross sectional area of kiwifruit pedicels between anthesis and 30 days after anthesis, while major expansion occurs after this time (Antognozzi et al., 1991), which corresponds to an increase in diffusible auxin from kiwifruit pedicels (Chapter six). Expansion of pedicels between 30 days after anthesis (approximately 4 WAFB) and fruit maturity was shown to be composed of increases in both xylem (6 fold increase) and phloem (3 fold increase) (Antognozzi et al., 1991), which could be important for allowing increased transport of watrer, nutrients and photo-assimilates into fruit. In contrast, the effect of NPA applied to kiwifruit pedicels has been demonstrated to be inhibitory to kiwifruit growth only up

until four to five WAFB (Table 7.6), while applications after this time had no effect on the assimilation of carbohydrate (dry weight) and water (fresh weight). If inhibition of vascular differentiation by NPA was a contributing factor to inhibition of fruit development, it would be expected that applications after four WAFB would be effective in reducing fruit weight.

Nevertheless results presented here suggest that a reduction of auxin transport in the stem could affect the ability of lateral shoots to import carbohydrate. It has been demonstrated that kiwifruit are able to readily import carbohydrate from nearby shoots to meet any shortage in the local supply of carbohydrate within the shoot (Lai et al., 1989). However the application of NPA to the base of lateral shoots appeared to reduce the ability of fruit on these shoots to obtain carbohydrate from nearby shoots (Table 7.8). It seems unlikely that NPA would have been preferentially translocated to fruit on lateral shoots with a low leaf:fruit ratio, as fruit on lateral shoots with a high leaf:fruit ratio were not affected by application of NPA to the shoot. The most likely explanation is that NPA applied to the base of lateral shoots inhibited the development of vascular traces at the base of the shoot. Conceivably this could limit development of vascular connections of the lateral shoot to phloem in the main stem. At a low leaf:fruit ratio, this could reduce the ability of lateal shoots to import carbohydrate from nearby shoots. Although fruit at the high leaf:fruit ratio might have been expected to respond positively to shoot NPA application in a similar manner to fruit on girdled shoots, this was not the case, possibly because of the low competitive ability of kiwifruit for the additional carbohydrate to produce large fruit (Woolley et al., 1992; Chapter six).

### 7.4.5 The involvement of calcium in NPA inhibition of fruit growth

It has been demonstrated that polar auxin transport is closely linked to acropetal transport of calcium (De Guzman and Dela Fuente, 1984). NPA applied to pedicels of tomato (Banuelos et al., 1987) and avocado fruitlets (Cutting and Bower, 1990) has been demonstrated to reduce the non-transpirational uptake of calcium into the fruit. Kiwifruit with NPA treated pedicels have been shown to have calcium concentrations

approximately half that of normal fruit, within three weeks of NPA application (G.S. Lawes, Personal communication 1994). Calcium has a wide range of functions in plants including signal transduction, mitosis, cell division and cytoplasmic streaming (Hepler and Wayne, 1985). This suggests that a reduction in fruit calcium induced by the effect of NPA inhibition of auxin transport could affect kiwifruit growth.

## 7.5 Summary

The aim of experimental work described in this chapter was to determine the effect of auxin transport out of kiwifruit pedicels on kiwifruit growth. NPA synthesised in our laboratory was able to inhibit up to 97% of $^{14}$C-IAA transport through 20 mm segments of kiwifruit pedicels, which suggested that endogenous IAA export would be similarly affected. When NPA was applied to kiwifruit pedicels prior to three WAFB, fresh and dry weight of fruit was reduced by up to 50%. Although it is possible that the effects of NPA on fruit growth were due to a restriction on auxin transport out of pedicels, we were unable to confirm this, as results suggested that some NPA may have been translocated to fruit and therefore could have had a direct effect on cell growth or seed development. The effect of NPA on fruit growth was strongly correlated to effects on seed development as seeds from fruit with NPA treated pedicels were comparatively small and demonstrated poor germination. Although all of the effects of NPA on fruit growth could be explained by effects on seed development, it is also possible that NPA translocated to fruit could have resulted in localised excess or deficiency of auxin in fruit cells which could have inhibited fruit growth. Application of radiolabelled NPA would be useful to determine if NPA is translocated into fruits, or if the effects are instead due to inhibition of transport out of fruits. In addition, location of NPA binding activity in fruit tissue would be useful to determine if NPA affects fruit development by inhibiting translocation of auxin throughout fruit.

As treatment of pedicels at six WAFB had no effect on fruit development, the role of auxin transport from kiwifruit after this time is not clear. However it demonstrates that IAA transport is not crucial for kiwifruit growth after this time.

Although it is possible that NPA had a direct effect on vascular development of the pedicels, it seems relatively unlikely that this would have had the major effect on fruit growth that was found. However it was concluded that NPA applied to stems may have reduced vascular development in the stems and this limited carbohydrate movement between lateral shoots.

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# 8. Effects of Cytokinins and Abscisic Acid on Kiwifruit

## 8.1 Introduction

Application of adenine based cytokinins to developing kiwifruit have not been found to enhance fruit growth unless they are applied in combination with auxin and gibberellin (Hopping, 1976; Cruz-Castillo, 1994). However a pilot study indicated that there may be an interaction between adenine based cytokinins and the synthetic cytokinin compound N-(2-chloro-4-pyridyl_-N'-phenylurea (CPPU) (D.J.Woolley, unpublished results). Although CPPU has been found to produce a strong stimulation to the fruit growth of kiwifruit (Iwahori et al., 1988; Lawes et al., 1992), the mechanism of action is not understood. There are indications that phenylurea cytokinins interact with endogenous cytokinins, perhaps by protecting them from metabolism (Burch and Horgan, 1989). Thidiazuron, a phenylurea cytokinin structurally similar to CPPU has been found to increase the concentration of endogenous cytokinins in soybean callus (Thomas and Katterman, 1986). Endogenous cytokinins in kiwifruit occur at high concentrations at anthesis, but drop to low levels soon after, before rising to very high levels as the fruit mature (Lewis et al., 1996b), but their role in controlling fruit growth is not clear (Lewis et al., 1996a). Levels of abscisic acid (ABA) in kiwifruit decrease after anthesis, before levelling off at low levels, however high concentrations in fruit were correlated with populations of fruit within a vine of larger size (Smith et al., 1995).

The experiments described in this chapter were designed to investigate the interactions between CPPU and exogenous cytokinin application, and effects of CPPU on endogenous cytokinins and abscisic acid (ABA). The response of endogenous cytokinins in kiwifruit ovaries to CPPU treatment has been investigated recently (Lewis et al., 1996a), however these authors used CPPU to induce parthenocarpic development in unpollinated fruit or applied CPPU to fruit immediately following pollination which appeared to increase seed abortion.

## 8.2 Materials and Methods

### 8.2.1 The Interaction between CPPU, exogenous cytokinins and genotype

This experiment was designed to investigate the possible interactive effects of CPPU and adenine based cytokinins on kiwifruit size in four kiwifruit selections.

**Experiment one:** The experimental design used was a completely randomised block design with a factorial treatment structure. Two levels of CPPU in combination with seven levels of cytokinins were applied to developing kiwifruit ovaries at three weeks after full bloom (WAFB). CPPU treatments were 0 ppm (CPPU-control) and 10 ppm CPPU. Cytokinin treatments were no cytokinin (cytokinin-control), zeatin (Z) zeatin-riboside (ZR) dihydrozeatin (DHZ), dihydrozeatin-riboside (DHZR), isopentenyl-adenine (2iP), and isopentenyladenine riboside (IPA), all at 0.05 mM. Treatments were applied to up to three fruit per shoot on three shoots per vine. All fruit were treated by dipping fruit in the appropriate CPPU plus cytokinin mixture (including control treatment combinations) containing Tween-20 surfactant as a wetting agent. Fruit were harvested at commercial maturity and weighed immediately.

Treatment combinations were applied separately to four different kiwifruit selections as follows:

| | | |
|---|---|---|
| 1. *A. deliciosa* 'Hayward' | MUFCU, Palmerston North | 4 vines |
| 2. *A. deliciosa* 'Skelton' | Private Orchard, Te Puke | 4 vines |
| 3. *A. deliciosa* 'Hermaphrodite' | Te Puke research centre, Te Puke | 2 vines |
| 4. *A. chinensis* 'F6L3 CCH4' | Te Puke research centre, Te Puke | 4 vines |

Vines from the Te Puke research centre were part of the HortResearch kiwifruit breeding program.

**Experiment two:** A second experiment was set up using the same kiwifruit vines to compare the response of different kiwifruit selections to different concentrations of CPPU. Experimental design was a randomised complete block design. Treatments were 0, 0.1, 1 and 10 ppm CPPU applied to kiwifruit ovaries at three WAFB. Fruit were harvested at commercial maturity, as in experiment one.

### 8.2.2 The effect of CPPU on endogenous cytokinins and abscisic acid (ABA) in kiwifruit

**Experiment one:**

Kiwifruit flowers on a mature vine at MUFCU were hand pollinated on two consecutive days at full bloom, and were tagged with treatment details at this time. For hormone analysis prior to CPPU treatment, 20 ovaries were harvested at full bloom and one week after full bloom (WAFB), 10 ovaries at two WAFB, and six ovaries at three WAFB. CPPU (10 ppm) or nil (control) treatments were each applied to 15 ovaries at three WAFB. Harvests of six ovaries were made at three days after treatment (3.3 WAFB), and two ovaries per treatment at seven, 14 and 21 days after treatment (four, five and six WAFB). At each harvest time, ovaries were dissected into core, inner pericarp and outer pericarp tissue, and tissue was immediately snap frozen in liquid nitrogen and lyophilised. Prior to three WAFB, separated tissue from all fruits was pooled as the quantity of tissue was very small, while after this time fruit were divided into two replications. Tissue was analysed for cytokinins and ABA as described in chapter two.

**Experiment two:**

In a second experiment with increased replication, the response of fruit ABA concentrations to CPPU application was investigated further. Fruit from two adjacent vines were either treated with CPPU (10 ppm) or not treated (control) at five WAFB. For ABA analysis, harvests of two fruit per treatment from each vine were made at 2, 7, 14, 21 and 35 days after treatment as described above.

## 8.3 Results

### 8.3.1 The Interaction between CPPU and exogenous cytokinins

There was a significant response to CPPU application in all four kiwifruit selections tested. The lowest concentration applied, 0.1 ppm, resulted in a higher fruit weight in all selections except *A. chinensis*, however the increase was not statistically significant in 'Skelton' at this concentration (Table 8.1). In general, higher concentrations of CPPU resulted in an increased fruit weight in all selections although *A. chinensis* did not appear to respond to CPPU below 10 ppm (Table 8.1).

Table 8.1 The effect of CPPU applied to fruit from four kiwifruit selections on final fruit fresh weight (g). Due to the presence of missing values, means presented are population marginal means[1].

| Selection | CPPU concentration (ppm) | | | |
|---|---|---|---|---|
| | 0 | 0.1 | 1 | 10 |
| *A. deliciosa* 'Hayward' | 84.5 d | 99.6 c | 120.8 b | 136.4 a |
| *A. deliciosa* 'Skelton' | 105.4 d | 116.6 cd | 123.8 bc | 145.7 a |
| *A. chinensis* 'F6L3 CCH4' | 51.3 b | 50.3 b | 56.0 b | 70.5 a |
| *A. deliciosa* 'Hermaphrodite' | 56.6 b | 74.5 a | 69.7 a | 78.2 a |

Means in a row with common letters are not significantly different at P=0.05 (lsmeans, SAS).
[1] For description of population marginal means, see chapter two, section 2.8.3.

There were no significant treatment effects of the different cytokinins applied to any of the kiwifruit selections either alone, or in combination with CPPU. In addition there were no significant effects of free base cytokinins compared with ribosyl cytokinins, or between the different cytokinin types (zeatin- dihydrozeatin- or isopentenyl-) in any of the selections (e.g. Table 8.3). For this reason, and to simplify presentation, the experiment was further analysed for interactions between CPPU and cytokinin by

reducing the seven cytokinin treatments to two levels: 1. Nil cytokinin (control), 2. 0.05 mM cytokinin (Z, ZR, DZ, DZR, 2iP, IPA treatments pooled).

Table 8.2 The interaction between CPPU and adenine based cytokinin applied to fruit from four kiwifruit selections on fruit fresh weight (g). CPPU was applied at 1 PPM, while cytokinins were applied at 0.05 mM. There were no significant differences between individual cytokinins (zeatin, dihydrozeatin, isopentenyl-adenine and their ribosides) (see Table 8.3), thus results for all cytokinins were pooled. Due to the resulting unbalanced replication, values presented are population marginal means.

| CPPU treatment | Nil | Nil | CPPU | CPPU | ANOVA, P>F |
|---|---|---|---|---|---|
| Cytokinin treatment | Nil | Cytokinin | Nil | Cytokinin | (CPPUxCytokinin) |
| *A. deliciosa* 'Hayward' | 79.6 c | 84.2 c | 117.5 b | 138.3 a | 0.0001 |
| *A. deliciosa* 'Skelton' | 105.4 c | 106.3 c | 123.8 b | 136.2 a | 0.0025 |
| *A. chinensis* 'F6L3 CCH4' | 50.9 bc | 49.8 c | 55.3 b | 66.4 a | 0.0002 |
| *A. deliciosa* 'Hermaphrodite' | 56.6 b | 58.7 b | 69.7 a | 74.1 a | 0.2843 |

Means in a row with common letters are not significantly different at P=0.05 (lsmeans, SAS).

Table 8.3 The effect of an interaction between CPPU and individual naturally occurring cytokinins applied to 'Hayward' kiwifruit on fruit fresh weight (g). Due to the presence of missing values, means presented are population marginal means.

| CPPU | Cytokinin | | | | | | |
|---|---|---|---|---|---|---|---|
| | Nil | Z | ZR | DZ | DZR | 2iP | IPA |
| Nil | 79.6 c | 83.3 c | 86.2 c | 86.9 c | 79.0 c | 83.1 c | 87.0 c |
| CPPU | 117.5 b | 141.0 a | 132.9 a | 136.9 a | 136.1 a | 142.8 a | 140.4 a |

Means with the same letter are not significantly different at P=0.05 (lsmeans, SAS).

A significant interaction was found between the application of CPPU and application of cytokinins in all selections except for the hermaphrodite kiwifruit (Table 8.2). In all selections there was no significant effects from cytokinins alone. However application of

cytokinins in combination with CPPU increased the mean fruit weight by 20% in *A. chinensis*, 10% in 'Skelton' and 18% in 'Hayward', compared to fruit treated with CPPU alone (Table 8.2).

### 8.3.2 Effects of CPPU on endogenous cytokinins and ABA

Free bases and riboside cytokinins were putatively identified following HPLC and radioimmunoassay (chapter two). Abscisic acid was putatively identified following ELISA using extremely high specificity monoclonal antibodies (chapter two). In general, the cytokinins present in fruit declined from very high concentrations at flowering, to a low and relatively constant concentration by approximately three WAFB. ZR was present in the greatest amount (Figure 8.2) while Z, 2iP and IPA were present at fairly low concentrations (Figure 8.2, Figure 8.3). All cytokinins tended to have the highest levels in inner pericarp tissue which contained the developing seeds. ABA levels also declined from a high level at flowering to a relatively constant level by one or two WAFB, and was at the highest concentration in outer pericarp tissue (Figure 8.1). There were no significant differences in cytokinin concentrations between control and CPPU treated fruits in the two weeks following treatment. However in experiment one, the concentration of ABA was lower in core, inner pericarp and outer pericarp tissues from fruit treated with CPPU than in control fruit (Figure 8.1).

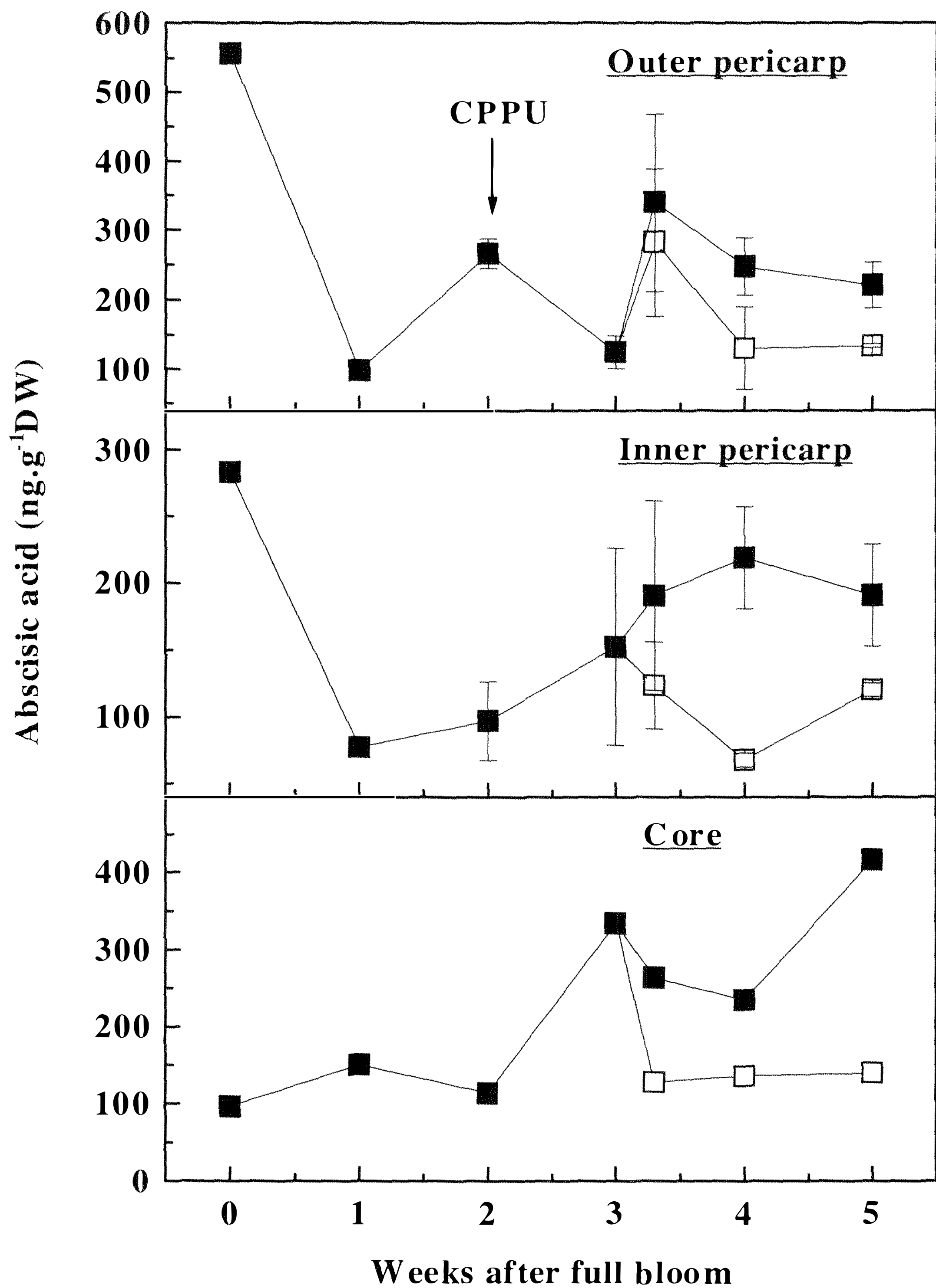


Figure 8.1 The abscisic acid concentrations from untreated (■) or CPPU treated (□) kiwifruit separated into core, inner pericarp containing seeds, and outer pericarp tissue. (Inner and outer pericarp n=2, except 0 and 1 WAFB n=1. Core tissue at all dates n=1).

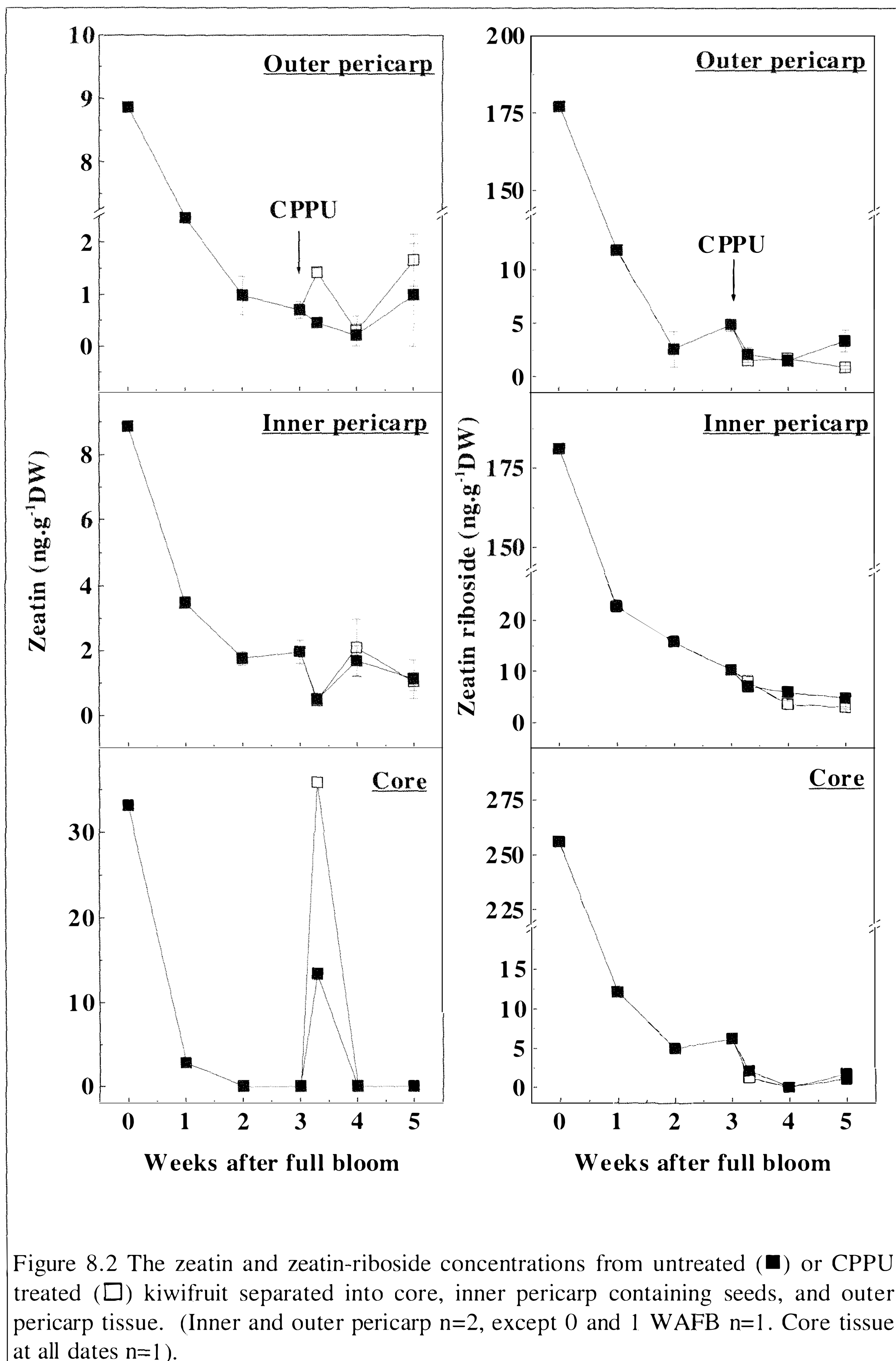


Figure 8.2 The zeatin and zeatin-riboside concentrations from untreated (■) or CPPU treated (□) kiwifruit separated into core, inner pericarp containing seeds, and outer pericarp tissue. (Inner and outer pericarp n=2, except 0 and 1 WAFB n=1. Core tissue at all dates n=1).

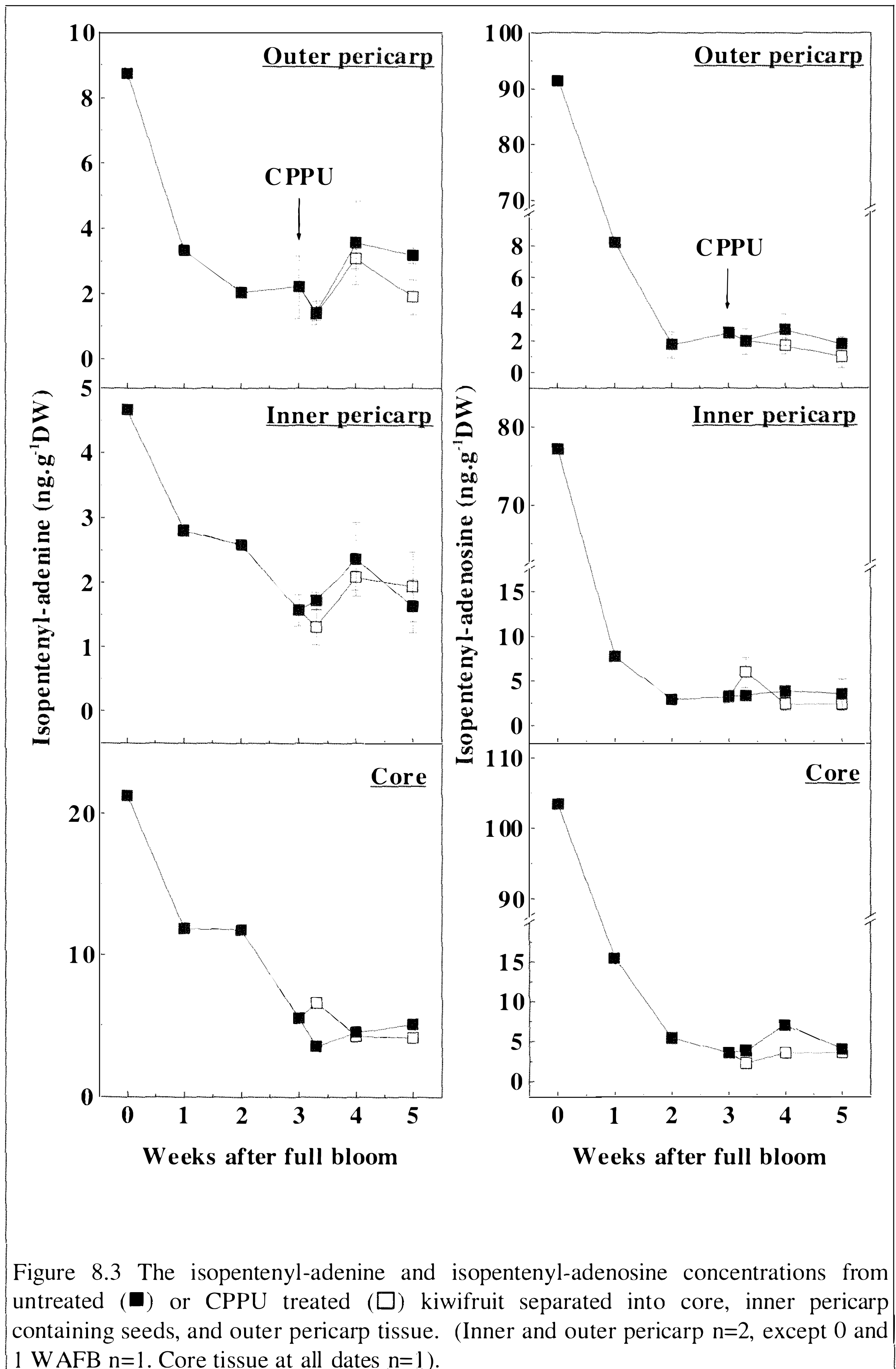


Figure 8.3 The isopentenyl-adenine and isopentenyl-adenosine concentrations from untreated (■) or CPPU treated (□) kiwifruit separated into core, inner pericarp containing seeds, and outer pericarp tissue. (Inner and outer pericarp n=2, except 0 and 1 WAFB n=1. Core tissue at all dates n=1).

The effect of CPPU application on ABA concentrations was further investigated in experiment two, which had higher replication and allowed significance testing. ABA levels in inner and outer pericarp of control fruit fluctuated between approximately 100 to 300 $ng.g^{-1}DW$ over the five weeks following treatment, but appeared to demonstrate no obvious pattern over this time (Table 8.4, Table 8.5). Within 7 days of treatment, the ABA concentration in both inner and outer pericarp from CPPU treated fruit had decreased to below 50% of that in control fruit (Table 8.4, Table 8.5). Although ABA levels remained lower in CPPU treated fruit tissues, they showed a steady increase over the period of measurements, and by 35 days after treatment there were no significant differences between CPPU and control fruit in both inner and outer pericarp tissues.

Table 8.4 Inner pericarp tissue concentration of ABA ($ng.g^{-1}.DW$) from control or CPPU treated kiwifruit (experiment two). Treatments were applied to fruit at five weeks after full bloom (n=4).

| CPPU treatment | Days after treatment | | | | |
|---|---|---|---|---|---|
| | 2 | 7 | 14 | 21 | 35 |
| Control | 214 a[1] | 219 a | 173 a | 318 a | 280 a |
| CPPU | 267 a | 104 b | 106 a | 199 b | 243 a |

Means in the same column with the same letter are not significantly different at P=0.05 (LSD)

Table 8.5 Outer pericarp tissue concentration of ABA ($ng.g^{-1}.DW$) from control or CPPU treated kiwifruit (experiment two). Treatments were applied to fruit five weeks after full bloom (n=4).

| CPPU treatment | Days after treatment | | | | |
|---|---|---|---|---|---|
| | 2 | 7 | 14 | 21 | 35 |
| Control | 260 a | 278 a | 240 a | 170 a | 214 a |
| CPPU | 264 a | 117 b | 133 b | 183 a | 150 a |

Means in the same column with the same letter are not significantly different at P=0.05 (LSD)

## 8.4 Discussion

### 8.4.1 The response of kiwifruit to CPPU

The effect of CPPU on the growth of 'Hayward' kiwifruit has been well documented. However the effect of CPPU on the growth of other species of kiwifruit other than *A. deliciosa* have not been reported before. As the result of CPPU application, an increase in fruit weight of about 1.4 times was reported in *A. deliciosa* 'Monty' (Kurosaki and Mochizuki, 1990), while a 30g increase in fruit weight of *A. deliciosa* 'Kramer Hayward' was reported (Cruz-Castillo, 1994). Application of 2.5 ppm CPPU at three WAFB to 'Hayward' kiwifruit resulted in an increase in fruit size of 41g, while increases of up to 73g were achieved with repeated applications of 10 ppm CPPU (Lawes et al., 1992). This is similar to the 36g increase which we have found at 1 ppm CPPU in 'Hayward' (Table 8.1). Increases in size of between 40 and 60g have been found following the application of 10 ppm CPPU to 'Hayward' kiwifruit when it was applied at three WAFB (Iwahori et al., 1988; Patterson et al., 1993; Woolley et al., 1992; Lawes et al., 1992). Again we have found a similar response to 10 ppm CPPU (Table 8.1). There are immediate potential benefits if CPPU could be used to increase the size of kiwifruit varieties which may have desirable characteristics, but are limited by small fruit size. It has been demonstrated here that the application of CPPU is able to improve the fruit size of two other *A. deliciosa* selections which are substantially different to 'Hayward', and *A. chinensis*, although CPPU was not as effective as in 'Hayward'. It appears that there is an interaction between CPPU application and fruit seed number in 'Hayward' kiwifruit (Woolley et al., 1992). At high and moderate seed numbers, the absolute increase in fruit size as a result of CPPU application was not found to differ, however at low seed numbers the response was approximately halved (Woolley et al., 1992). Although seed numbers were not recorded in this experiment, both hermaphrodite kiwifruit and *A. chinensis* kiwifruit have been found to contain lower seed numbers than 'Hayward' (McNeilage, 1992; Chapter six). Thus the ability of other kiwifruit varieties to respond to CPPU may be limited by their ability to form seeds. However histological examination of two other *A. chinensis* selections has revealed that these appear to have a wider band of the relatively thick walled cells at the outer edge of the pericarp underneath the epidermis

(Chapter six). This could possibly limit the uptake of CPPU by some kiwifruit selections. As the response to CPPU has been found to be increased at higher application rates (Table 8.1), reduced uptake or translocation would diminish the response, as a lower concentration would be present in the fruit. Unlike the other selections, the *A. chinensis* selection did not respond to the application of concentrations of CPPU below 10 ppm, which may be indicative of poorer uptake by these fruits. Studies of the uptake of $^{14}C$-CPPU by 'Hayward kiwifruit revealed that CPPU has extremely poor mobility (Cruz-Castillo, 1994). For example, 21 days after application, over 88% of $^{14}C$-CPPU which was absorbed by fruits was retained in the 'skin' , while only 4% had been translocated to the inner pericarp and core (Cruz-Castillo, 1994). As the 'skin' in the study of Cruz-Castillo (1994) presumably included the outer band of thick walled cells, and this was where most of the CPPU was recovered, this suggests that the mobility of CPPU could be further inhibited in kiwifruit selections such as *A. chinensis* which have a thicker band of these type of cells.

### 8.4.2 Interactions between CPPU, cytokinins and ABA

There appears to be a positive interaction between CPPU and adenine based cytokinins on fruit size of kiwifruit, which extended over three different selections. Application of adenine based cytokinins on their own was found to have no effect whatsoever on fruit size which is in agreement with the results obtained by (Hopping, 1976). Although CPPU on it's own was found to increase fruit size, application of CPPU plus adenine based cytokinins increased the fruit size further. To our knowledge, interactions between CPPU and naturally occurring adenine cytokinins have not been reported previously, although an additional effect of CPPU on kiwifruit size was found when it was applied in combination with the proprietary mixture promalin, which contains the adenine cytokinin benzylaminopurine plus gibberellin $A_{4+7}$ (Cruz-Castillo, 1994). In this case it is difficult to know whether the additional effect was due to an interaction of CPPU with the cytokinin, the gibberellin, both, or due solely to the promalin (no promalin only control was reported). However positive interactions between CPPU and gibberellin on fruit size have been reported in both kiwifruit (Cruz-Castillo, 1994) and apple (Bangerth and Schroder, 1994). It was suggested that the interaction between

CPPU and gibberellins could be due either to a deficiency in cytokinins in gibberellin induced fruit, or protection of gibberellins from metabolism by CPPU (Bangerth and Schroder, 1994). Although these hypotheses were related to fruit set of seedless fruits, similar mechanisms could be operating in pollinated fruits.

Of additional interest was the lack of any differences between any of the different adenine cytokinins when applied with CPPU. This suggests that either the individual adenine cytokinins are equally effective at stimulating growth, or that inter-conversion of the cytokinins occured readily, metabolising the different cytokinins to the active forms.

There is evidence in the literature that phenylurea cytokinins such as CPPU may act by increasing levels of adenine cytokinins. Adenine cytokinins were found to accumulate in soybean callus treated with the phenylurea thidiazuron (Thomas and Katterman, 1986), which is closely related to CPPU structurally, and has many similar cytokinin-like properties (Takahashi et al., 1978). It was suggested that cytokinin accumulation in thidiazuron treated callus tissue was due to increased synthesis or reduced breakdown of adenine cytokinins (Thomas and Katterman, 1986). Thidiazuron enhances conversion of inactive cytokinin nucleotides to nucleosides (Capelle et al., 1983), as well as inhibiting the degradation of IPA to adenine by the enzyme cytokinin oxidase (Chatfield and Armstrong, 1986). Some inhibition of cytokinin oxidase by CPPU in kiwifruit tissue has also been found (Lennard, 1994), and along with the interaction which occurs between CPPU and exogenously applied adenine cytokinins, this would suggest that in kiwifruit CPPU may be acting to protect cytokinins from oxidation. Although we have not found a general increase in cytokinin levels as a result of CPPU application in this experiment, this may be due to a dilution of the effect by sampling the entire outer pericarp. As both Lewis et al. (1996a) and Woolley and Bangerth (unpublished) have recorded lower cytokinin levels in CPPU treated kiwifruit, this suggests that there may be a dilution effect of increased fruit size in CPPU treated fruit on cytokinin concentration. CPPU has limited mobility into kiwifruit by 21 days after application (Cruz-Castillo, 1994), and effects of CPPU on adenine cytokinins could therefore have been confined to the outer edge of outer pericarp tissue, and not detected by separation into the entire outer pericarp tissue. This suggestion is also consistent with the main effect of CPPU on cell division in kiwifruit which is in the outer pericarp (Woolley et al., 1992). There may be

some indications that zeatin, which is thought to be the active cytokinin in kiwifruit (Lewis et al., 1996a), were higher in the outer pericarp of CPPU treated fruit than in untreated fruit over the two weeks after treatment (Figure 8.2). However this is in contrast to results obtained by Lewis et al. (1996a) who were unable to detect zeatin in kiwifruit treated with CPPU at anthesis, or in CPPU induced parthenocarpic kiwifruit. However the application of CPPU to fruit at anthesis, resulted in a reduction in seed number of over 50% compared to control fruit (Lewis et al., 1996a). As levels of zeatin were correlated to the number of pollinated ovules (Lewis et al., 1996a), it is possible that CPPU applied to kiwifruit at anthesis also interferes with development of seeds which may control the ability of fruit to metabolise cytokinins.

One further possible explanation for interactions between CPPU and cytokinins or gibberellins could be that these growth regulators may enhance the uptake of CPPU. Treatment of beetroot discs with kinetin has been found to increase membrane permeability (Naik et al., 1980), although cytokinins have also been found to maintain low membrane permeability in some tissues (Beckman and Ingram, 1994). Gibberellin treatment has been found to enhance uptake of 2,4,5-T in poison ivy (Devlin et al., 1971) cited in Devlin and Karczmarczyk (1977) and NPA in wheat (Devlin and Karczmarczyk, 1977). Higher concentrations of CPPU applied to fruit have been found to result in increased fruit weight in three kiwifruit selections (Table 8.1), while $^{14}C$ tracer studies demonstrated that a large portion of $^{14}C$-CPPU applied to 'Hayward' kiwifruit does not permeate the skin (Cruz-Castillo, 1994). This suggests that improved uptake of CPPU could result in an additional fruit growth response.

Although the effects of CPPU on cytokinins were inconclusive, CPPU application to kiwifruit at three WAFB was found to decrease the concentration of ABA throughout the fruit (Figure 8.1). The effect of CPPU on the reduction in kiwifruit ABA levels was confirmed in a second experiment (Table 8.4, Table 8.5). Levels of ABA measured in this experiment appeared to fluctuate between 100 and 300 $ng.g^{-1}$.DW, which is similar to ABA concentrations in kiwifruit reported by Smith et al. (1995). It is not certain whether reduction of fruit ABA levels as a result of CPPU application acts to improve fruit sink strength. ABA has more commonly been linked to improved sink strength by

enhancing the unloading of photoassimilates (Tietz et al., 1981; Clifford et al., 1986; Ross et al., 1987). However in the experiment described in this chapter, the improvement of sink strength by CPPU resulted in lower ABA concentration. Lower ABA concentrations have also been found in kiwifruit skin and pericarp tissues of fruit from the upper part of the canopy which also had a higher dry weight, compared to fruit from the lower part of the canopy (Smith et al., 1995). Although Smith et al. (1995) found that upper canopy fruit had higher total ABA content, and correlated this to the larger fruit dry weight, tissue concentration rather than total amount is probably more important in determining plant responses to hormones. Also as fruit in the upper canopy are more exposed, they are more likely to be under water stress, which could increase ABA levels in the fruit. In addition, the growth of kiwifruit inner pericarp callus treated with ABA was reduced compared to control callus, unless seeds or exogenous cytokinin were also present (Cruz-Castillo et al., 1992). This would suggest that reduced ABA concentrations caused by CPPU could be at least partly responsible for the stimulation of fruit growth which occurs.

The highest concentrations of cytokinins was found in the inner pericarp of kiwifruit in this study. Higher concentrations of endogenous cytokinins in kiwifruit inner pericarp tissue were also reported by Lewis et al. (1996a). Kiwifruit inner pericarp tissue is distinguished as containing developing seeds, which appears to suggest that the seeds are a biosynthetic source of cytokinins in kiwifruit. Parthenocarpic ovaries have been found to contain low cytokinin concentrations compared to pollinated ones (Mapelli, 1981; Nagar and Rao, 1981). In contrast, unpollinated kiwifruit ovaries were found to contain higher concentrations of cytokinins at 10 and 20 days after anthesis and a similar total amount of cytokinins as pollinated ovaries 10 days after anthesis, although total amount was lower in unpollinated ovaries by 20 days after anthesis (Lewis et al., 1996a). However zeatin levels were correlated with the number of pollinated ovules at both dates (Lewis et al., 1996a). This may suggest that external sources of cytokinins are imported into kiwifruit at least initially, but that seeds are required either for synthesis of zeatin, for conversion of the pathway intermediates into zeatin, or to inhibit zeatin metabolism. In tomato, metabolism of $^{14}C$-zeatin was actually higher in seeded tomato fruits

compared to gibberellin-induced parthenocarpic fruits (Palmer et al., 1982). This would tend to suggest that seeds do not inhibit the metabolism of zeatin, although cytokinin metabolism could be different in kiwifruit.

## 8.5 Summary

The cytokinin compound CPPU is known to stimulate fruit growth in *A. deliciosa,* however we have found that CPPU also stimulates fruit growth in a hermaphrodite kiwifruit, and *A. chinensis*, although fruit did not respond to the same degree as 'Hayward' kiwifruit. The lower fruit response of the hermaphrodite and *A. chinensis* may be due to interactions with seeds which have been found in 'Hayward', or to reduced uptake of CPPU. Evidence was also presented of a positive interaction between CPPU and adenine based cytokinins in stimulating kiwifruit growth. Although this suggested that CPPU may also interact with endogenous cytokinins, perhaps by protecting them from metabolism as has been suggested elsewhere, we were unable to detect any increase in endogenous cytokinins as a result of CPPU application. However it is possible that the response of endogenous cytokinins were undetected due to the effects of CPPU being localised in the outer pericarp near the fruit surface, due to the low mobility of CPPU in kiwifruit. However in two separate experiments, CPPU application significantly reduced ABA levels in fruit, which suggests that reduction of ABA levels may have been stimulatory for fruit growth.

## 8.6 References

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# 9. General Discussion and Conclusions

## 9.1 Thesis Objectives

The increase in world production of kiwifruit in recent years has seen New Zealand move from a dominant position in the international market to become just one of many countries in an over-supplied market. Consequently prices have slumped, and returns to growers have plummeted to levels which have forced many to leave the industry. In 1990 a move was made by the New Zealand Kiwifruit Marketing Board to encourage production of large fruit to maximise overall returns and reduce fruit numbers to levels which could be easily marketed. Payments were made to growers which favoured fruits in the large fruit size counts, and discouraged production of small fruit by providing low returns. For example in 1993 after costs of packaging were removed, New Zealand kiwifruit growers received $0.40 for a 170g fruit, $0.18 for a 150g fruit, $0.07 for a 100g fruit, and $0.03 for a 80g fruit.

With a policy encouraging production of large fruit in place, research was initiated into means of increasing kiwifruit size. While some success has been obtained with new rootstocks which may provide increased yield and/or improved fruit size (Lowe et al., 1992; Cruz-Castillo, 1994), this is of limited value for growers with existing vines who are financially unable to re-plant their orchards in the short term. Research in this thesis was focused into three main areas with the potential to yield information on factors which limit kiwifruit growth which could have potential to improve fruit size on both existing cultivars and in new cultivars:

1. Alteration of source-sink relationships by girdling.
2. The mechanism by which seeds influence kiwifruit sink strength.
3. The role of plant growth hormones in kiwifruit sink strength.

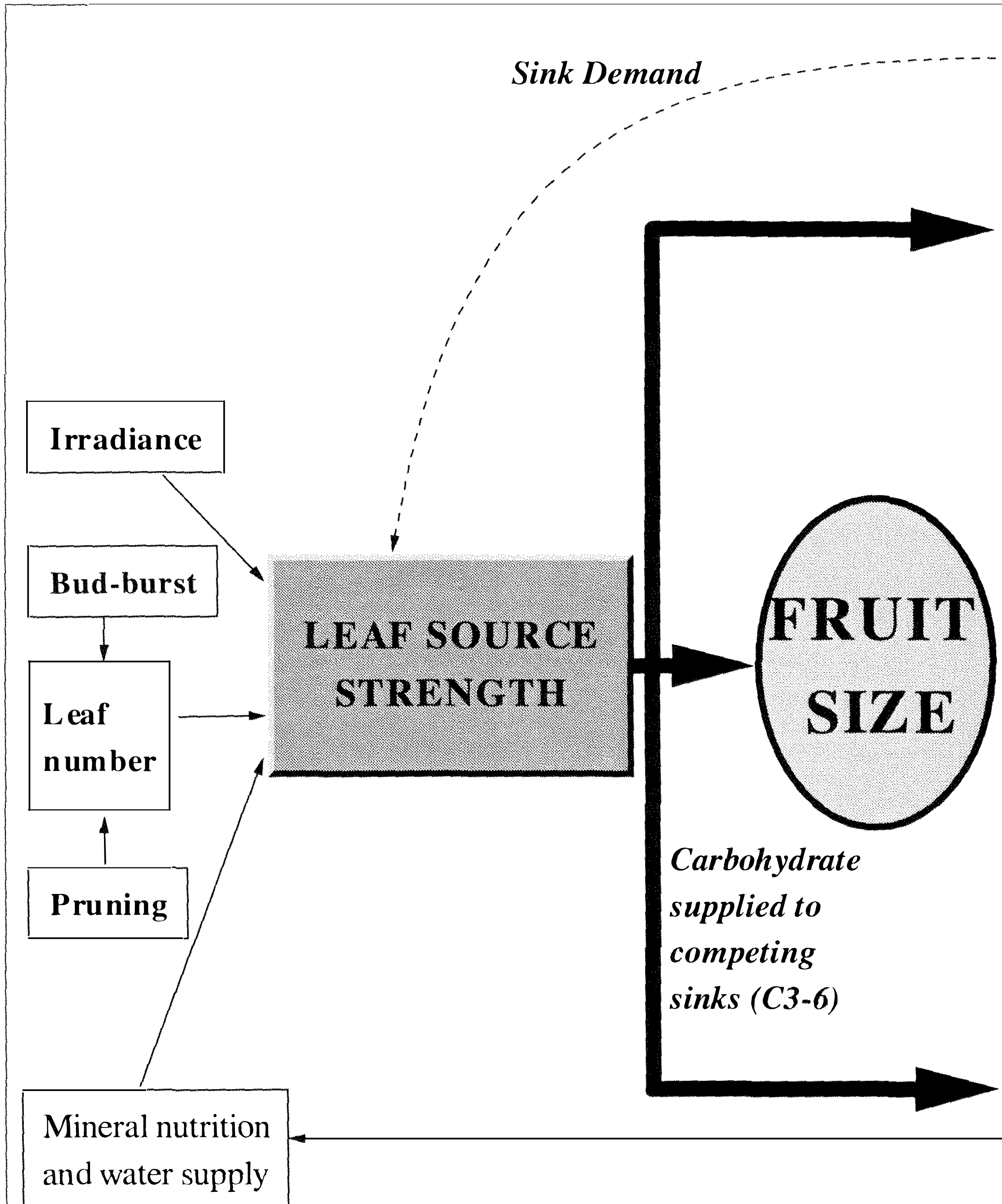


Figure 9.1 A diagrammatic representation of how source strength may impose a limitation to fruit size of kiwifruit. The photosynthetic rate of leaves may be limited by irradiance, mineral nutrition, water deficit, and sink demand. Leaf area is mostly limited by bud-burst and pruning in winter and summer. Where aspects were investigated in this thesis, chapter numbers are indicated (C3-C6).

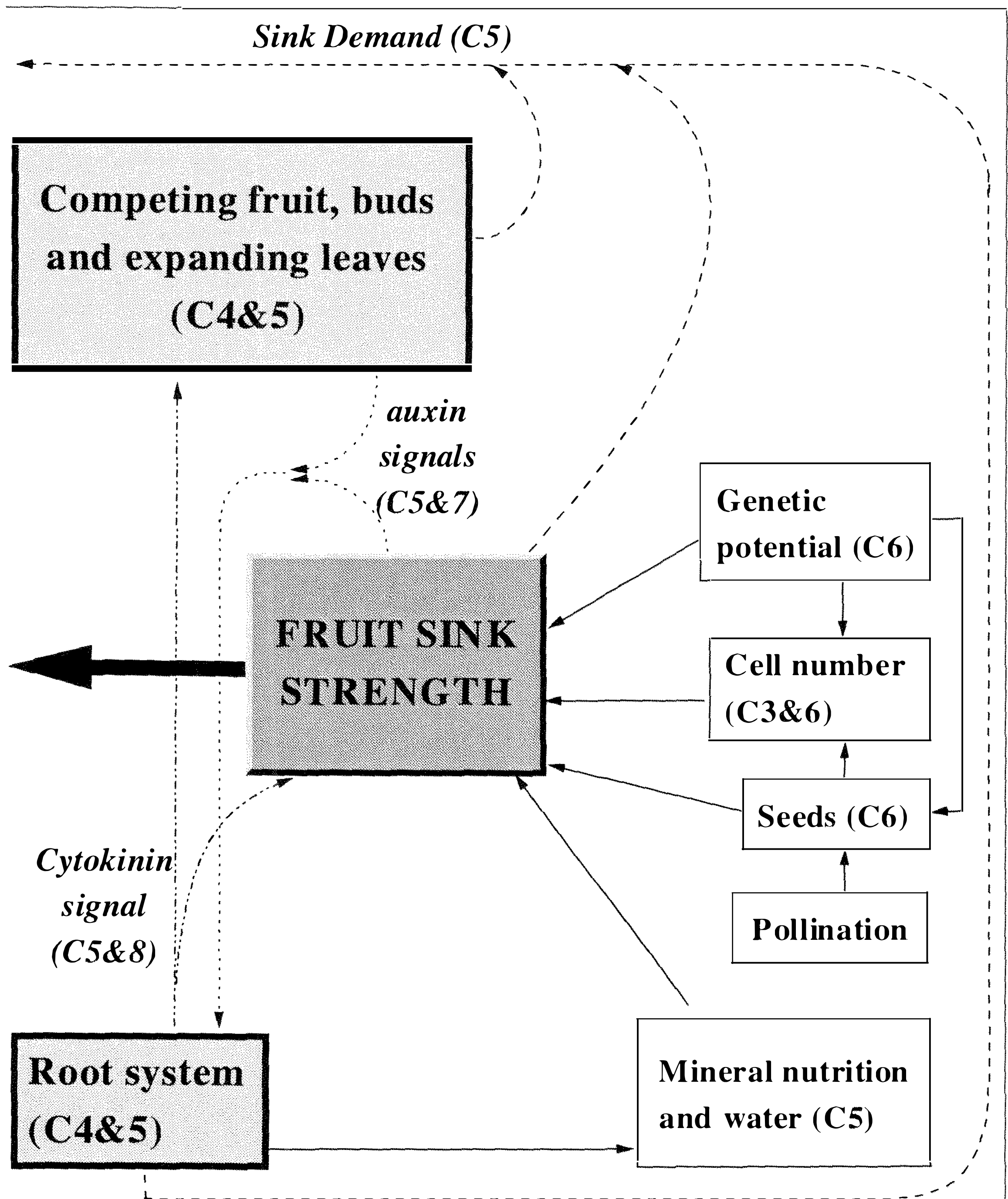


Figure 9.2 A diagrammatic representation of the main factors which interact to determine sink strength of an individual kiwifruit. Sink strength is largely determined by total weight of seeds, cultivar, cell number at anthesis, mineral nutrition and water supply. Auxin signals from fruits may suppress development of competing sinks, but the overall auxin signal from shoots to roots may affects sink strength of shoot organs by regulating cytokinin export from roots. Where aspects were investigated in this thesis, chapter numbers are indicated (C3-C8).

## 9.2 Girdling and the Effect of Competing Sinks on Kiwifruit Growth

The effects of competing sinks on the growth of kiwifruit can be separated into two distinct areas. Firstly, there is competition for the limited supply of carbohydrate produced by leaves. Secondly, it appears that competing sinks may emit inhibitory signals which decrease the competitive ability of nearby sinks. It has been clearly demonstrated in this thesis and elsewhere (Snelgar et al., 1986; Lai et al., 1989a) that kiwifruit growth responds to elimination of sink competition by girdling. Fruit from girdled lateral shoots or canes with high leaf:fruit ratios grow to a larger size, have a higher dry weight, and increased percentage dry matter than control fruit (chapter three).

Girdling of entire canes was found to produce similar effects to lateral shoot girdling in terms of increasing fruit size (chapter four). Increases in average fruit size of between 20 and 30g were found on girdled canes versus non-girdled canes on a vine (chapter four). However when a higher proportion of canes on any one vine were girdled, there was a general negative effect on fruit size of fruit from both girdled and non-girdled parts of the vine (chapter four). In more extensive investigations of these results (chapter five), there was no discernible effect of increased cane girdling on a number of physiological processes which might have been expected to be altered if carbohydrate had become limited on vines. These included root growth, water relations, fruit mineral content, stomatal resistance, and photosynthetic rate. Although return bloom may have been negatively affected by increased cane girdling, this was unable to be confirmed.

The role of root-shoot signals in determining the negative response to girdling was investigated as girdling has been found to result in a decreased basipetal auxin signal from the shoots towards roots (Dann et al., 1985). Girdling or decapitation of rooted kiwifruit cuttings resulted in an increase in the concentration of cytokinins in exuded xylem sap, but this was able to be partially inhibited by the application of the auxin NAA to decapitated surface. This suggested that cytokinins, either produced by roots or released from bark into xylem sap, is influenced by the basipetal auxin signal. Likewise, increased percentage cane girdling on a vine resulted in an increase in cytokinins in xylem sap extracted from girdled canes. Thus evidence has been found that girdling of

kiwifruit influences hormonal signals from shoots to roots (decapitation experiment), and in return from roots to shoots (decapitation experiment, cane girdling) as was hypothesised. Bud-burst of buds on lateral shoots and subsequent vegetative growth was correlated with the increase in cytokinin concentration in xylem sap, and it has been hypothesised that increased vegetative growth may partially inhibit fruit growth relative to that on other girdled canes with less vegetative growth. While this may explain the situation on girdled canes, no satisfactory conclusion was able to be reached regarding the situation on non-girdled parts of vines, where fruit growth was also inhibited.

In addition to the inhibitory cane girdling effect which was found, a further inhibitory effect of girdling on fruit growth was found on lateral shoots which were girdled at approximately 14 days after anthesis (chapter three). In two seasons, fruit on lateral shoots with a high leaf:fruit ratio, which had been girdled at this time had a depressed fruit fresh weight, dry weight and percentage dry matter compared to fruit on lateral shoots girdled either prior to, or after this time. Evidence presented in chapter six has indicated that the inhibitory effect of kiwifruit with high seed numbers on the growth of adjacent fruit which was reported by Lai et al. (1990) was due to increased abortion of seeds in the inhibited fruit. There is also an increasing body of evidence that dominant fruit inhibit the growth of other fruits by emitting an inhibitory auxin signal (Bangerth, 1989), and that auxin transport may be correlated to seed numbers (Sjut and Bangerth, 1984; Bangerth et al., 1989). The auxin transport inhibitors NPA (chapter seven) or TIBA (Lawes and Woolley, unpublished) applied to kiwifruit pedicels during the first three weeks after full bloom severely limited fruit growth. There was evidence that inhibition of fruit growth by NPA was possibly by damage to seeds, although it was not ascertained whether the damage was indirect by inhibition of auxin transport, or by a direct toxic effect of NPA. However as the only reported properties of NPA are in regard to inhibition of auxin transport rather than direct toxic effects (Katekar and Geissler, 1980), there is a high possibility that auxin efflux from seeds or out of the fruit is important for development of seeds. Although studies of auxin exudation from fruit pedicels have failed to confirm differences between high and low seeded kiwifruit, auxin was able to be measured in the exudate (chapter six). A temporary build up of auxin in the bark of girdled peach branches (Dann et al., 1985), and the effect of a girdle on

increasing exuded xylem cytokinins from decapitated kiwifruit cuttings (chapter five) is consistent with the notion that girdling inhibits basipetal auxin transport. Bangerth (Personal communication 1996) has found that inhibition of auxin transport from dominated organs leads to a build up of IAA metabolites followed by a decrease in auxin production in the organ. Girdling reduced diffusible IAA from kiwifruit (chapter six), which may also be due to a build up of IAA metabolites in fruit. If the dominance effect found in kiwifruit is due to an inhibition of auxin transport from dominated ovaries, and the result of this is increased seed abortion, then a girdle placed on a lateral shoot at a critical time of seed development such as 14 days after full bloom (DAFB) could have a similar effect on seed development. In fact shoot girdling up to as late as 84 DAFB reduced seed numbers compared to control fruit (chapter three), which supports the hypothesis that continued IAA transport is required for seed development.

If girdling results in altered hormonal signals from roots to the shoot (Cutting and Lyne, 1993; Chapter five) this raises the question as to what effect an alteration in xylem hormones caused by girdling has on fruit growth *per se*, independent of the effect of elimination of competing sinks. An increase in the supply of root cytokinins could enhance fruit growth, although there appears to be only uncertain evidence which supports the hypothesis that endogenous cytokinins enhance kiwifruit growth. Although seed number, and CPPU application do not appear to influence fruit growth by an increase in levels of endogenous cytokinins (Lewis et al., 1996b; Chapter eight), interactions between exogenously applied adenine cytokinins and auxin, auxin plus gibberellin (Hopping, 1976a) or CPPU (chapter eight) have been found, which suggests that endogenous cytokinins could be important in determining kiwifruit sink strength.

## 9.3 How do Seeds Influence Kiwifruit Growth?

Kiwifruit growth rate is largely determined by the number of seeds formed in the fruit (Chapter six) Experiments where the formation of seeds is inhibited by excision of styles (Lai et al., 1990; Chapter six) or by inhibiting access of pollinating insects (Hopping, 1976a) have resulted in stunted growth of fruit, indicating a causal relationship. However there is an improved relationship between the total weight of seeds and fruit weight (Lai, 1987), which extends to a variety of different kiwifruit selections (chapter six). This may be due to a seed weight measure being sensitive to empty and small, seeds which may have low viability and may be expected to not contribute to stimulation of growth .

Well pollinated 'Hayward' kiwifruit average around 1000 seeds per fruit (chapter three, chapter six). Although seeds appeared to influence cell division in kiwifruit, the effect was minimal at very low seed numbers of approximately 140 seeds per fruit (Chapter six) although cell division activity was reduced in completely un-pollinated fruit (Lewis, 1994). However no effect on fruit cell division was found in fruit averaging 270 seeds by (Lewis, 1994). It can be concluded from this evidence that kiwifruit seeds are required for cell division, however the seed derived cell division factor(s) can be supplied by a relatively small number or weight of seeds. Total cytokinin content of kiwifruit in the early cell division stage is not affected by reducing the number of seeds, and in fact cytokinin concentrations in fruit are increased at low seed number (Lewis et al., 1996a; Woolley and Bangerth, unpublished), which suggests that seed produced cytokinin may not be the cell division factor. In contrast there is some indirect evidence that kiwifruit seeds may be a source of cytokinins. Application of naturally occurring growth regulators to kiwifruit does not substitute for the presence of seeds unless both auxin and cytokinin are applied (Hopping, 1976a), although CPPU applied to ovaries prior to fruit set can induce parthenocarpic fruit growth (Lewis et al., 1996a). Parthenocarpic development induced by a single CPPU application could have been due to stimulation of endosperm or nucellis development in sterile ovules, which are thought to be sites of hormones in some parthenocarpic fruits (Nitsch, 1970, and references therein). However while seed produced/directed hormones presumably diffuse from near the centre of the fruit, external applications are applied to the fruit surface and therefore may not

effectively reach the normal sites of action. Thus effects on fruit growth of exogenous hormones may be unrelated to the effects normally elicited by endogenous seed produced/directed hormones. It is possible that kiwifruit seeds start to synthesise cytokinins at a later stage of development than during the cell division period which occurs immediately after fruit set. There is a large increase in fruit cytokinins which occurs between 40 days after anthesis and maturity (Lewis et al., 1996b; Woolley unpublished). Unless cytokinin import increased markedly during this time, this would suggest either that synthesis by seed or fruit tissues occurred or that there was decreased catabolism after this time. A negative effect of ABA on inner pericarp callus growth originating from kiwifruit harvested 124 DAFB was able to be overcome either by addition of cytokinin or the presence of seeds (Cruz-Castillo, 1994), which suggested that seeds of this age could produce cytokinins.

However un-pollinated kiwifruit have a low rate of post-anthesis cell division (Lewis, 1994), and abscise soon after anthesis (McKay, 1976). This suggests that pollination is required to stimulate commencement of post-anthesis cell division. A promising candidate for a seed or pollination factor which stimulates the onset of post-anthesis cell division in kiwifruit is auxin. It is well known that cytokinins stimulate cell division in cultured cells, but this only occurs if threshold levels of auxin are present (Skoog et al., 1965). It has been shown that cytokinins in kiwifruit could be derived entirely from xylem sap (Lewis, 1994). Although seed associated tissue (inner pericarp) in kiwifruit has been found to contain the highest cytokinin concentration (Lewis et al., 1996a; Chapter eight) this could be due to the high number of vascular connections which occur close to seeds (Schmid, 1978) and subsequently unloading of cytokinins from the xylem stream could occur mainly in the inner pericarp tissue. If at the time of cell division cytokinins are derived from an external source, and kiwifruit seeds are a source of auxin, only a small weight of seeds may be required to provide a threshold concentration of auxin for cell division to occur normally. The formation of seeds also appears to be essential for longer term growth of kiwifruit as is indicated by the long term effect of seed number on fruit growth rates (chapter six) and the main cellular effect of seeds which is on cell size (Lewis et al., 1996a; Chapter six). The role of auxins in promotion of cell expansion is well documented (Cleland, 1995), and this is consistent with the role of seed in stimulating cell expansion. At this time there is a clear necessity for further

investigation of auxins in fruit and seed tissue of kiwifruit. However development of viable embryos may not in fact be necessary for continued kiwifruit growth as indicated by continued growth of kiwifruit following ovule abortion (Harvey et al., 1992).

## 9.4 The Importance of Fruit Cell Number for Sink Strength

Although the potential number of cells in a fruit may be modified throughout the entire period of floral/fruit cell division, the contribution of fruit cell numbers to fruit size in kiwifruit is unclear. An organ with more cells might be expected to be larger than an organ with fewer cells, however this can only be the case if cells in each organ attain the same size. As has been pointed out by several authors, division of cells, does not in itself contribute to increased overall volume (Davies, 1995; Jones, 1973). Unless each divided cell expands at the same absolute rate ($cm^3.day^{-1}$) or at a higher relative rate ($cm^3.cm^{-3}.day^{-1}$), cell division in itself would not increase fruit size. There is some evidence that increased cell expansion can partly compensate for lower cell numbers. For example, cell division in tomato was severely inhibited following GA induced parthenocarpic fruit set, however cells grew to a significantly larger size than in fruit where cell division proceeded normally (Bunger-Kibler and Bangerth, 1982). Similarly, cucumbers with reduced cell numbers were able to recover by increased cell expansion rate (Marcelli, 1993). Thinning of apples can result in increased fruit weight due to increased cell numbers (Denne, 1960; Quinlan and Preston, 1968), however thinning can also increase fruit size solely due to increased cell size (Sharples, 1964). Although distal tomato fruit contain fewer cells than proximal fruit at anthesis, the relative sink strength of these fruits is determined by the sequence of fruit set, and this over-rides cell number differences (Bangerth and Ho, 1984).

Although the controlling step for sink activity in fruit is probably the enzymatic conversion and accumulation of reserves within cells (Ho, 1992), this does not automatically link sink activity to cell number, as large cells may contain proportionally higher amounts of the enzymes required for assimilate conversion/storage. In addition, additional carbohydrate resource would be used in respiration associated with generation of new cellular components before storage of incoming carbohydrate could occur. Thus the suggestion of Ho (1992) that sink strength is the product of cell number (as a measure of sink size) and enzymatic conversion and accumulation of carbohydrates is probably an over-simplification.

The evidence supporting a role for cell numbers in kiwifruit sink strength includes:

1. Cell numbers of the different kiwifruit selections which were tested in this thesis were correlated with fruit size differences between the selections (Chapter six).
2. Differences in cell numbers at anthesis, related to earliness of flowers, is correlated with fruit size (Cruz-Castillo, 1994).
3. Kiwifruit on lateral shoots with a high leaf:fruit ratio which are girdled after the time of cell division, have a lower rate of fruit expansion than fruit on lateral shoots girdled during the cell division phase, but accumulate a higher percentage of dry matter (Chapter three). As girdling at the earlier time stimulates cell division, this suggests that later girdled fruit reach a cellular limit, and excess carbohydrates are used for storage rather than expansion.

However there is evidence that cell numbers of kiwifruit may determine only a relatively minor proportion of kiwifruit sink strength. Although the influence of pre-anthesis cell division is probably important, the contribution of post-anthesis cell division may be quite limited. Post-anthesis factors which have been found to strongly influence kiwifruit growth appear to do so by their ability to drive cell expansion:

1. Seeds which are the main determinant of fruit sink strength in kiwifruit, mainly affect fruit growth by affects on cell expansion unless seed numbers are very low (Chapter six). Although cell division was affected at very low seed numbers, the contribution of lower cell numbers at these seed numbers to fruit growth may not be important compared with the continuing effect of seeds late in fruit growth, presumably to stimulate continued cell expansion (Chapter six).
2. The effect of carbohydrate supply on kiwifruit expansion are mainly exhibited through effects on cell size (Chapter three). Although limitation of carbohydrate supply reduced fruit cell numbers, cell diameter was most significantly affected.
3. Application of CPPU to kiwifruit ovaries 24 dafb was found to increase both radial cell number and size in the outer pericarp leading to larger ultimate fruit size

(Woolley et al., 1992). Similarly (Lewis, 1994) found that CPPU applied to un-pollinated kiwifruit ovaries increased both the number and size of cells. Although this is consistent with a role for cytokinins in fruit cell division, the contribution of cell division induced by CPPU is not certain. Patterson et al. (1993) found that CPPU applied 21 days after flowering, increased the transverse cell size (area) of outer pericarp small parenchyma cells, and this increase in cell size could account for the resulting increase in fruit size. Lewis (1994) found that CPPU application to pollinated kiwifruit did not result in significant differences in radial cell number from fruit harvested 40 DAFB, by which time pericarp cell division is expected to have mostly ceased. Although no counts were made at harvest by (Lewis, 1994), it would be expected that subsequent differences could only involve cell expansion. If the mechanism by which CPPU is able to enhance fruit size is closely linked to it's ability to stimulate cell division, it might be expected that application times closer to anthesis would produce an enhanced response, as stimulation of cell division would occur over a more extended period of the cell division phase. However kiwifruit grew to a larger size when CPPU was applied at three weeks after anthesis rather than immediately after anthesis (Iwahori et al., 1988). Application of CPPU at 38 or 21 DAFB provided the same stimulation to fruit growth, while application at 11 DAFB produced the least effect (Lawes et al., 1992). Given that the main effect of CPPU on cell division appears to be in the outer pericarp (Woolley et al., 1992), and in this tissue the majority of cell division has ceased well prior to 38 DAFB (Hopping, 1976b; Woolley and Lai, 1990; Chapter three), late application of CPPU seems unlikely to provide stimulation to cell division, unless cell division is re-activated. If cell division is not re-activated, this strongly suggests that increased fruit cell numbers as a result of CPPU application at 24 DAFB (Woolley et al., 1992), is probably not necessary for the improvement in competitive ability induced by CPPU.

An extension to the hypothesis of Ho (1988) that the number of cells in a sink organ determines the potential sink size is proposed here:

***"Fruit cell number determines potential sink size and forms the ultimate limitation to the size that fruit can attain. However unless competition from other sinks is low, apparent sink strength often bears little relation to the potential sink size".***

## 9.5 Directions for Future Research

The use of girdling in kiwifruit production is potentially worthwhile. Depending on the status of the market for large kiwifruit, girdling could be used by growers to readily produce very large fruit suitable for export in jumbo categories. However at this stage, girdling has not demonstrated itself to be profitable due to the negative effect on fruit growth on non-girdled parts of the vine. Although girdling always increased fruit size on the girdled cane, the response to girdling diminished as more canes on a vine were girdled. There is evidence that the cause of reduced fruit growth on girdled canes may be increased vegetative growth caused by an increase in cytokinin concentration in xylem sap. A number of avenues exist to further investigate this problem:

1. Experimental work could be directed at timing of girdle application. For example, later girdles may avoid the seed formation period immediately after fruit set, which appears to be particularly sensitive to inhibitory signals. Evidence from lateral shoot girdling suggests that later girdles increase percentage dry matter more than early girdles (chapter three), which may be beneficial for fruit quality.

2. Other girdling techniques may yield results worth pursuing. For example, trunk or cordon girdling may be worthwhile. However some cordon (main leader) girdling trials have suggested that negative effects on fruit growth from the non-girdled cordon occur, similar to those found in chapter four (Woolley, unpublished).

3. The results in this thesis may bring into question the use of 'hormone pruning' systems which apply NAA to pruned lateral shoots to inhibit lateral bud development (Henzell et al., 1986). If auxin signals are inhibitory for seed development, then hormone pruning during this time could have a similar effect to dominant fruits, girdling or vegetative growth. In contrast if this is not found to be the case, gel pruning could limit the amount of vegetative growth on girdled canes, and thus remove the inhibition to fruit growth that this is thought to impose.

4. Replacement of the auxin signal at the girdle could reduce the cytokinin levels in xylem sap. Application of a stable auxin to the cut surface could achieve this, particularly if the increased cytokinin arises from reserves stored in bark rather than roots. A relatively stable, mobile auxin such as NAA may be suitable.

If further experimental work on the negative effects on fruit size of increased girdling can yield solutions to the problem, then girdling has the potential to become an extremely worthwhile operation for growers. In addition to the effects on fruit size, late girdling may increase the percentage dry matter of fruit, which may be important in terms of increasing post-harvest storage potential, and improving fruit flavour, both of which are becoming increasingly relevant in export markets. In addition, girdling has been found to alter the endogenous hormone signals in xylem sap. The current consumer emphasis appears to be on natural "organic" cultivation methods which shun artificial sprays, and in particular there is a shift away from application of '*hormones*'. Natural means of altering endogenous plant growth hormones by girdling or pruning that are beneficial to production could become increasingly important (Cutting and Wolstenholme, 1993). Manipulation of plant growth hormones by girdling could thus become a useful technique, although more research is required into this area to fine tune the benefits. This could include:

1. Further characterisation of the effects of girdling on xylem sap cytokinins, with more samples during the first two to three weeks after application of the girdle, as it appears that effects are limited to this time period.
2. Characterisation of xylem sap gibberellins, as evidence in the literature (Cutting and Lyne, 1993) suggests that these may also be affected by girdling, and they may be important for vegetative growth.
3. Correlation of concentrations of xylem sap growth regulators with fruit concentrations and also fruit growth, as this would provide more conclusive evidence of the role of seed versus external growth regulators in fruit development.

The role of vegetative growth in inhibition of fruit growth may be important in designing pruning strategies. Results in this thesis have indicated that vegetative growth may have an inhibitory effect on fruit growth that is independent of the effects of competition for assimilate. A similar effect of a large number of leaves on fruiting lateral shoots of kiwifruit was found by Lai et al. (1989b), although it was possible in that work that leaf

expansion prior to anthesis may have competitively inhibited development of ovaries perhaps by retarding pre-anthesis cell division. However in this thesis, it has been suggested that vegetative growth from girdled canes which arises after fruit set was able to inhibit fruit development. Although this hypothesis requires further investigation, it does suggest that careful canopy management is especially required to limit the effects of newly arising vegetative growth on fruit development during the weeks after fruit set. On non-girdled canes, the effect of newly arising vegetative growth during this period could be even more severe, as there will be competition between fruit and expanding leaves on lateral shoots for a limited supply of assimilate. Despite the immediate effect on fruit growth rate, this could also affect fruit growth in the long term. It appears that assimilate supply may affect the amount of fruit cell division (chapter three), and seed abortion in low seeded fruit. In addition, if seed development is sensitive to inhibitory auxin signals as has been suggested, new vegetative growth may provide a dominating auxin signal, which also may increase seed abortion in fruit, with subsequent long term effects on fruit growth.

With the release of new kiwifruit cultivars by the New Zealand kiwifruit marketing board and New Zealand HortResearch, physiological information on requirements for maximum fruit growth will be important for growers. This thesis has highlighted a number of requirements that are likely for the new cultivars. Firstly, it has been demonstrated that pollination of the new varieties is likely to be crucial to fruit development similar to 'Hayward' kiwifruit. Positive relationships were found between seed number and fruit weight in seven different selections from three *Actinidia* species, which confirms that appropriate pollinating varieties will need to be present in any orchard planting of new species. For example, flowering of *A. chinensis* occurs approximately one month earlier than 'Hayward' (Ferguson, 1992), thus pollinisers with coincident flowering will be important. Additionally, honeybee pollination or artificial pollination will be required at this time. The possibility that metaxenic influences of parental pollination on seed development were raised in chapter six. As total weight of seeds has been found to correlate with fruit weight over all kiwifruit selections in this study, research aimed at optimising polliniser selections which are highly compatible and possibly produce larger seeds in both 'Hayward' and the new kiwifruit selections could

be worthwhile. Alternatively, breeding programs may be able to utilise large seed size characteristics as one of the selection criteria for parental stock.

Experimental work with the auxin transport inhibitor NPA applied to kiwifruit pedicels has raised a number of questions regarding the role of auxin transport in kiwifruit growth. Although NPA was demonstrated to inhibit transport of IAA through kiwifruit pedicels, it was considered that NPA itself was probably transported into fruit. In addition it was not certain whether inhibitory effects of NPA were due to direct phytotoxicity, or indirect due to auxin accumulation/deficiency. However it was clear that seed development was inhibited. This work has opened a number of potential routes for further research which would help clarify the role of auxin transport for fruit development, which is probably important also for identifying dominance relationships which occur. Also this research could help clarify the role of seeds in kiwifruit:

1. The rate of transport of NPA from kiwifruit pedicels into fruit and seeds needs to be measured using radio-labelled NPA. This could separate the role of auxin transport out of fruit from more direct effects of NPA within fruit and seeds.
2. Further investigation is required into the effects of NPA on seed viability, when NPA is applied at different times during the season. There appears to be no effect of NPA on fruit growth when it is applied later than four weeks after full bloom, thus it would be useful to know if seed viability is also unaffected.
3. Measurement of IAA concentrations in fruit following NPA application is potentially useful. NPA could cause an increase in concentration as IAA builds up being unable to be transported out of fruit. Alternatively feedback responses could reduce IAA concentration.
4. Investigation of the location of NPA binding proteins, which are responsible for active transport of IAA would be worthwhile. There has been no report of the location of NPA binding proteins in kiwifruit, although it seems likely from the response of $^{14}$C-IAA transport in kiwifruit pedicels to NPA that they are present at least in vascular material. However there is little evidence as to whether NPA binding proteins are present in fruit tissue not associated with phloem.

## 9.6 References

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