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ETHYLENE SYNTHESIS INHIBITOR AFFECTS POSTHARVEST KIWIFRUIT QUALITY

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Applied Science
at
Massey University
Palmerston North
New Zealand

Jose Roberto Marques

1998

to

Anna Luisa,

my precious little 'kiwi' girl,

and her mother, great little woman,

Cris

ABSTRACT

Firmness is a key quality criteria of kiwifruit and changes significantly during fruit ripening, with premature softening being a serious commercial problem for the industry. Ethylene is involved in regulation of fruit ripening and influences a number of processes, including ethylene production, respiration rate and changes in firmness. Kiwifruit is very sensitive to ethylene, which increases fruit softening rate and reduces storage potential. Aminoethoxyvinylglycine (AVG), an inhibitor of ACC synthase, a key enzyme in the pathway for ethylene biosynthesis, has been applied to horticultural crops, especially apples, in an attempt to regulate ethylene synthesis and its mediated processes, with a number of positive effects including reduced fruit ethylene production, reduced respiration rate, and slower softening rate.

The effects of AVG (500 and 1000 $mg.l^{-1}$ a.i., or 200 and 400 $g.acre^{-1}$ a.i.) applied to 'Hayward' kiwifruit vines (6 and 4 weeks before commercial harvest) on ethylene production, respiration rate, firmness and soluble solids content of fruit at harvest and after coolstorage were investigated. Kiwifruit treated with either 500 or 1000 $mg.l^{-1}$ AVG 4 weeks before commercial harvest and maintained at 20 °C over 15 days, had a lower respiration rate, reduced ethylene production, a slower softening rate, and lower SSC than control fruit immediately after harvest and following 14 days at 0 °C, with the differences generally becoming significant after 6 days at 20 °C. These attributes are generally stimulated by ethylene, indicating that the endogenously produced ethylene was inhibited by the applied AVG, resulting in a slower fruit ripening rate at 20 °C.

However, AVG effects were transitory. There were generally no differences in the above fruit variables between AVG-treated and control fruit at 20 °C up to 20 days following 30, 52, and 80 days at 0 °C. After 110 and 180 days at 0 °C, kiwifruit treated with either 500 or 1000 $mg.l^{-1}$ AVG 4 weeks before commercial harvest and maintained at 20 °C up to 10 days, had a higher respiration rate, increased ethylene production, and accelerated softening compared with control fruit. There were basically no differences in any of the

above fruit variables between the treatments 500-AVG-6 and control, either immediately after harvest or following storage at 0 °C up to 180 days.

The short term effect of AVG in kiwifruit during and after coolstorage and the questionable efficiency of AVG uptake in kiwifruit are issues to be further addressed before any practical application can be recommended.

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TABLE OF CONTENTS

ABSTRACT....iii

ACKNOWLEDGEMENTS....iv

TABLE OF CONTENTS....vii

LIST OF TABLES....x

LIST OF FIGURES....xii

LIST OF PLATES....xiv

1 INTRODUCTION....1

1.1 Economic Importance of Kiwifruit....1

1.2 Preharvest Factors Affecting Postharvest Fruit Quality....2

1.4.1 Crop Genetics....2

1.2.2 Climate....3

1.2.3 Nutrient and Water Management....4

1.2.4 Cultural Management....5

1.2.5 Diseases....7

1.2.6 Bioregulators....7

1.2.7 Maturity (Time of Harvest)....8

1.3 Postharvest Factors Affecting Fruit Quality....10

1.3.1 Physical Damage....10

1.3.2 Storage Conditions....10

1.3.3 Postharvest Diseases....11

1.3.4 Postharvest Losses....12

1.4 Fruit Development and Physiology of Kiwifruit 14
1.4.1 Composition and Morphology 14
1.4.2 Maturation and Ripening 15
1.4.3 Softening 16
1.5 Ethylene 22
1.5.1 Biosynthesis in Higher Plants 22
1.5.2 Role in Fruit Ripening 24
1.5.3 Ethylene and Kiwifruit 26
1.6 AVG 28
1.6.1 Action in Plant Tissues 28
1.6.2 Application in Horticultural Crops 29
1.7 Objective 32
2 MATERIAL AND METHODS 33
2.1 Field Activities 33
2.1.1 Treatment Application 33
2.1.2 Harvesting 34
2.1.3 Postharvest Operations 34
2.1.4 Cool Storage 35
2.2 Laboratory Activities 35
2.2.1 Carbon Dioxide Production 36
2.2.2 Fruit Ethylene Production 37
2.2.3 Fruit Firmness 38
2.2.4 Fruit Total Soluble Solids Content 39
2.3 Statistical Analysis 39

3 RESULTS 43

3.1 Fruit Respiration (CO_2 Production) 43

3.2 Fruit Ethylene Production 48

3.3 Fruit Firmness 53

3.1 Destructive Firmness Measurement ('Texture Analyser') 53

3.2 Non-destructive Firmness Measurement ('Kiwifirm') 58

3.4 Fruit Total Soluble Solids Content 61

4 DISCUSSION 65

4.1 General Results 66

4.2 Low Temperature Effects in Fruit Physiology 69

4.3 Fruit Ethylene Production and Softening 71

4.4 Use of AVG in Kiwifruit and Suggested Future Research 72

5 REFERENCES 75

APPENDIX I 94

APPENDIX II 95

APPENDIX III 96

LIST OF TABLES

Table 1.1 - Flesh firmness and SSC of 'Hayward' kiwifruit grown in 2 locations (A and B) at harvest and after 6 months at 0 °C (Mitchell *et al.* 1992). 9

Table 1.2 - Postharvest losses of kiwifruit in New Zealand (Source: Anonymous 1997b). 13

Table 1.3 - Inhibition of ethylene synthesis (%) in apple plugs by AVG (0.1 mM) in relation to temperature (°C) (Mattoo *et al.* 1977). 29

Table 1.4 – Pre and postharvest effects of AVG on apple cultivars. 31

Table 2.1 – Measurement time (days) of fruit variables at 20 °C, after successive removals from 0 °C. 36

Table 3.1 – CO_2 production ($nmol.kg^{-1}.s^{-1}$) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time. 44

Table 3.2 – CO_2 production ($nmol.kg^{-1}.s^{-1}$) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data). 46

Table 3.3 – Ethylene production ($pmol.kg^{-1}.s^{-1}$) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time. 50

Table 3.4 - Ethylene production ($pmol.kg^{-1}.s^{-1}$) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data). 51

Table 3.5 – Loss of firmness (%) of kiwifruit on removal to 20 °C following storage for up to 180 at 0 °C. Means of 60 fruit per removal time from all treatments. Different letters indicate significant difference between removal times. 55

Table 3.6 – Firmness (N) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data). 56

Table 3.7 – Firmness ('Kiwifirm' unit) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time. 59

Table 3.8 – Firmness ('Kiwifirm' unit) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data). 60

Table 3.9 – Soluble solids content (%) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time. 62

Table 3.10 – Soluble solids content (%) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data). 64

LIST OF FIGURES

Figure 1.1 - Schematic representation of postharvest softening of kiwifruit in relation to the timing of key events in the process (MacRae & Redgwell 1992)......................18

Figure 1.2 - The major pathway of ethylene biosynthesis in higher plants and the enzymes involved (Fluhr & Mattoo 1996)..23

Figure 3.1 – CO_2 production ($nmol.kg^{-1}.s^{-1}$) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference)...43

Figure 3.2 – CO_2 production ($nmol.kg^{-1}.s^{-1}$) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means from all treatments of 60 fruit per removal time. Bar represents overall LSD (least significant difference). Means with the same letter are not significantly different...45

Figure 3.3 - CO_2 production ($nmol.kg^{-1}.s^{-1}$) of kiwifruit at 20 °C for up to 15 days, after 14 and 180 days at 0 °C. Means of 15 fruit per treatment per sampling time. Bars represent overall LSD (least significant difference). 47

Figure 3.4 – Ethylene production ($pmol.kg^{-1}.s^{-1}$) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Values plotted on logarithmic scale. Bar represents overall LSD (least significant difference)..48

Figure 3.5 – Number of fruit that exceeded 0.1 $\mu l.l^{-1}$ ethylene production per sampling time during 13 days at 20 °C immediately after harvest. Total of 15 fruit per treatment per sampling time. 49

Figure 3.6 – Percent of fruit from each time of removal from 0 °C that exceeded 4.5 $pmol.kg^{-1}.s^{-1}$ (about 0.1 $\mu l.l^{-1}$) ethylene production of kiwifruit at 20 °C for up to 20 days (4 to 5 measurements), following storage for up to 180 at 0 °C. Total of 60 to 75 fruit per treatment per removal time. 52

Figure 3.7 – Firmness (N) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference). 53

Figure 3.8 – Firmness (N) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time. Bars represent LSD (least significant difference) at each removal time....54

Figure 3.9 – Firmness (N) of kiwifruit at 20 °C for up to 20 days at harvest (A) or following storage at 0 °C for 14 (B), 30 (C), and 52 (D) days. Bar indicates overall LSD (least significant difference). 57

Figure 3.10 – Firmness ('Kiwifirm' unit) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference). 58

Figure 3.11 – Firmness ('Kiwifirm' unit) of kiwifuit during 15 days at 20 °C after 14 days storage at 0 °C. Means of 15 fruit per treatment per sampling time. 60

Figure 3.12 - Soluble solids content (%) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference). 61

Figure 3.13 – SSC (%) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means from all treatments of 60 fruit per removal time. Bar represents overall LSD (least significant difference). Means with the same letter are not significantly different. 63

LIST OF PLATES

Plate 2.1 – The 'Texture Analyser' fitted with a 7.9 mm diameter probe, material testing machine used for measuring fruit firmness destructively. 41

Plate 2.2 – The 'Kiwifirm', device used for measuring fruit firmness non-destructively. 42

1 INTRODUCTION

1.1 Economic Importance of Kiwifruit

Kiwifruit is one of the more recent internationally successful fruit crops. In the last 10 to 15 years, it has emerged as one of the fastest developing horticultural industries in the world, with trade increasing from 155,000 tonnes in 1985 to more than 800,000 tonnes in 1995 (Anonymous 1996a). The crop was first developed commercially in New Zealand, which is a major supplier of kiwifruit (about 25% of world production). New Zealand has led the development of the global industry, and exports kiwifruit to more than 50 countries. Other major producers include Italy, Chile, Japan, the United States of America, Greece and France (Anonymous 1997a).

Kiwifruit is one of New Zealand's most important horticultural earners. In the 1995/96 season, it represented 41% of New Zealand fresh fruit exports and 26% of total New Zealand horticultural exports (Anonymous 1997c). In the 1996/97 season, New Zealand exported 56.2 million trays (202,000 tonnes) of kiwifruit, with a sales value of NZ$ 565.4 million. The main markets were Europe (52%), Japan (30%), East Asia (8%) and emerging markets (10%), which include North and South America, Southeast Asia and Australia (Anonymous 1997b). Although real prices have reduced due to the rapid increase in global production and increasing competition from other fruits, New Zealand has consistently earned price premiums over its competitors (Anonymous 1997c).

1.2 Preharvest Factors Affecting Postharvest Fruit Quality

Preharvest factors play an important role in postharvest kiwifruit quality by influencing fruit size, firmness, and composition. Interactions between factors are many and complex (Hopkirk & Clark 1992b), affecting the postharvest fruit quality in several respects, including shelf life and storage ability, sensory characteristics, resistance to handling and diseases, fruit losses, and market preferences. This section outlines the most important preharvest factors that influence postharvest quality of kiwifruit, particularly firmness.

1.4.1 Crop Genetics

Cultivar

Cultivar is considered the most important single preharvest factor affecting the storage quality of kiwifruit (Beever & Hopkirk 1990). Domesticated varieties of kiwifruit are large-fruited selections of *Actinidia deliciosa*. The kiwifruit of commerce [*Actinidia deliciosa* (A. Chev) C. F. Liang et A. R. Ferguson var. *deliciosa*] is a warm-temperate vine subjected to little genetic selection and is similar to the wild plant in its natural habitat in China (Ferguson 1984).

'Hayward' (which was selected in New Zealand 50 years ago) has been the most important commercial variety of kiwifruit due to its excellent storage performance and sensory attributes compared with other cultivars. During 16 to 17 weeks storage at 0 °C, fruit from 'Hayward' showed a slower softening rate, lower respiration rate and ethylene production, and higher SSC than 'Allison', 'Bruno', and 'Monty' (Antunes & Sfakiotakis 1997; Cotter *et al.* 1991), and had better sensory attributes (sweetness, bitterness, stringiness in texture, and presence of 'off-flavours' and aromas) than 'Abbott', 'Allison', 'Bruno', 'Monty', 'Gracie', and 'Constricted' (Cotter *et al.* 1991; Papadopoulou & Manolopoulou 1997). Fruit from 'Hayward' were also larger and less variable between different geographical and climatic conditions than fruit from 'Abbott'

and 'Bruno' (Scienza *et al.* 1990). At present, it is almost the sole variety of commerce both in New Zealand (about 98%) and worldwide (Ferguson & Bollard 1990).

'Hayward' is a vigorous, deciduous vine with simple, alternate large obovate-shaped leaves (up to 20 cm diameter). Shoots of the current season develop from axillary buds on canes of the previous season's growth (Ferguson 1990). Large cream-white pistillate and staminate flowers are borne on separate plants. The fruit shape is oval, with a brown skin covered with short stiff hairs (Beever & Hopkirk 1990). Inside, the soft, bright green juicy flesh contains hundreds of small dark seeds, radially arranged around a creamy-white central core of woodier tissue. This brightly coloured flesh, along with the unique tangy taste of the fruit, are largely responsible for the success of the kiwifruit industry (McMath 1992).

Rootstock

Within a commercial orchard, vines of the same cultivar have been identified that consistently produce larger fruit than adjacent vines with similar fruit load and cultural management, suggesting an effect of rootstock in vine cropping and fruit quality (Lawes *et al.* 1990). Fruit from 'Hayward' vines on their own roots showed lower SSC at harvest and after cool storage, and were softer after harvest than fruit from eight different rootstocks (Castillo *et al.* 1997). Fruit from 'Hayward C' vines on clonal rootstocks of two genotypes were firmer after 15 weeks cold storage than fruit on five other rootstocks (Castillo *et al.* 1997). Fruit from plants on 'D1' were firmer in storage and had higher SSC than fruit from plants on 'Bruno' (Monastra & Testoni 1991).

1.2.2 Climate

The factors controlling photosynthesis (light, temperature, carbon dioxide, water, and photoperiod) are the major environmental constraints on berry fruit quality (Prange & DeEll 1997). Kiwifruit harvested from different orchards vary in the rate at which they soften in storage, and even fruit from a single orchard differ between years in their softening rate, suggesting that climatic factors influence fruit storage life (Beever &

Hopkirk 1990). Kiwifruit grown under increased temperature in controlled environments have lower SSC at harvest than control fruit, as well as remaining firmer than control fruit during long-term cool storage, indicating that warmer preharvest conditions tend to decrease fruit maturation rates compared with cooler conditions (Hopkirk *et al.* 1989; Seager *et al.* 1996).

1.2.3 Nutrient and Water Management

Development of balanced fertiliser and irrigation programme, are necessary to develop a strong and health root system, being important management strategies for producing high quality fruit (Spiers 1996).

Nutrient management

Excessive nitrogen (N) application, especially if associated with low calcium (Ca) concentrations in the plant (resulting in fruit with a high N:Ca ratio at harvest), tends to result in a rapid fruit softening rate and high incidence of rots during coolstorage of kiwifruit (Mitchell & Costa 1997; Prasad & Spiers 1992; Tagliavine *et al.* 1995). Excessive application of potassium chloride, and boron, can also cause premature softening of kiwifruit in cool storage (Prasad & Spiers 1992). Fruit Ca level is associated with variation in storage behaviour; fruit developing localised soft areas (or 'soft patches') have less Ca than healthy fruit (Davie *et al.* 1996). Fruit sprayed preharvest with $CaCl_2$ had higher Ca content, TA and lower SSC at harvest, as well as lower softening rate in storage at 0 and 20 °C, than unsprayed fruit (Gerasopoulos *et al.* 1996; Hopkirk *et al.* 1990). Similarly, postharvest $CaCl_2$ dips enhanced fruit Ca content and slowed fruit softening rate in storage (Hopkirk *et al.* 1990).

Water management

Although irrigation of kiwifruit vines was previously reported as having no effect on storage life of the fruit (Beever & Hopkirk 1990), a more recent study (Reid *et al.* 1996) showed that mild water stress (caused by withholding early-season irrigation) increased

by 30 days the time taken for firmness of cool stored kiwifruit to decrease to 1 kgf (the critical value for market acceptability).

1.2.4 Cultural Management

While on the macroclimate scale the factors controlling photosynthesis are virtually unmanageable, the microclimate within each planting can be influenced by plant spacing, pruning and training, and flower and fruit thinning. The goal is to increase both leaf and fruit exposure to light and to shift more photosynthate into the fruit, as well as to remove excessive vegetative growth, which can shift more water and calcium flow to the fruit, and improve fruit quality (Prange & DeEll 1997).

Light and fruit position on the canopy

The radiation regime within the canopy and the amount of global radiation intercepted by the canopy during the growing season influence the energy balance of the vines. Low light intensity on kiwifruit vines during fruit development reduces growth, size (fruit count), SSC and chlorophyll content of the fruit at harvest, as well as delaying harvest maturity and slightly increasing rate fruit softening rate during storage at 0 °C (Snelgar *et al.* 1991; Tombesi *et al.* 1993). Shading of vines during fruit development does not affect fruit firmness at harvest (Biasi *et al.* 1995), but shading of individual fruit for most of the growing season reduces fruit firmness at harvest and during storage and increases incidence of fruit rots (Tombesi *et al.* 1993). Thus, good light exposure of the leaves rather than fruit is essential for normal fruit development, but both fruits and leaves need exposure for high fruit quality (Biasi *et al.* 1995).

Possibly correlated with these light effects, the position of the fruit on the vines has some influence in fruit quality, particularly SSC, which is usually higher in fruit: from the ends of the leader than those from nearer the centre of the vine; from the proximal ends of canes (near the leader) than those from the distal ends of the cane or from spurs (Hopkirk *et al.* 1986; Pyke *et al.* 1996). Although fruit harvested from exposed positions on the vine, or from young vines with little leaf shading were suggested as

being firmer than fruit taken from shaded position on mature vines (Beever & Hopkirk 1990), more recent studies show that the relationships between fruit position on the vine and fruit firmness (at harvest and in storage) are not consistent (Pyke *et al.* 1996; Smith *et al.* 1994). Likewise, the relationships between fruit firmness and height above the ground, and between fruit firmness and different training systems are not consistent across different orchards and regions (Pyke *et al.* 1996).

Crop load & leaf to fruit ratio

There is a negative correlation between fruit number per unit leaf area and fruit size. As fruit density on kiwifruit vines increases, total yield increases, but mean fruit weight decreases (Antognozzi *et al.* 1992; Cooper & Marshall 1990). For the same fruit number per vine, fruit is larger when distributed over a greater cane length, which allows a larger spacing of fruit, ensuring a greater number of leaves available to size fruit. This suggests that during crop thinning, it is desirable to remove buds, flowers or fruit, rather than whole canes or shoots (Cooper & Marshall 1990).

Pollination

Good pollination (achieved by appropriate male:female planting ratio and by bringing honey-bees onto the orchard) is required to produce export-sized fruit, since larger fruit is correlated with increasing seed dry weight of the fruit (Lawes *et al.* 1990; Sale & Lyford 1990).

Fruit size

Cultural practices that affect fruit size also have another indirect effect in fruit quality, since large size fruit has a slower softening rate than medium and small size kiwifruit under both air and controlled atmosphere storage conditions (Crisosto *et al.* 1997).

1.2.5 Diseases

Botrytis cinerea is a facultative parasite widely distributed in kiwifruit orchards, growing and sporulating on dead flower parts and on dead leaf and cane tissues. The spores, conidia, are readily liberated to be a component of the air spora in orchards, available to contaminate fruit at harvest (Brook 1992), making *Botrytis cinerea* the most important postharvest disease of kiwifruit. Infected fruit are a source of ethylene production and can accelerate the softening of healthy fruit in coolstore (refer to section 1.3.3).

1.2.6 Bioregulators

CPPU, a synthetic cytokinin, has shown to affect fruit quality of kiwifruit. CPPU increases the amount of carbon allocated to fruit growth and stimulates both cell division and cell expansion, increasing fruit size (Woolley *et al.* 1992). Applied as preharvest sprays (to individual fruit or the whole vine) or by dipping of the fruitlet and peduncle base, CPPU (2.5 to 20 ppm a.i.) increased mean fruit size by 20-70 g, resulting in a higher proportion of the crop being in larger size grades (Costa *et al.* 1997; Lawes *et al.* 1992). However, CPPU use resulted in fruit with lower flesh firmness and higher SSC values at harvest (suggesting that it may induce earlier fruit maturation), darkening of flesh colour, and higher proportion of distorted fruit shape compared with untreated fruit (Antognozzi *et al.* 1996; Lawes *et al.* 1992). During cool storage, the differences in SSC and flesh firmness disappeared, and treated fruit performed as well as control fruit, maintaining good quality for up to 6 months (Antognozzi *et al.* 1996; Costa *et al.* 1997).

Plant growth regulators that affect fruit by inhibiting ethylene biosynthesis are considered in section 1.6.

1.2.7 Maturity (Time of Harvest)

In New Zealand, the minimum acceptable maturity for kiwifruit at harvest is indicated when SSC of the fruit reaches 6.2% (Harman 1981). This can be measured by a hand-held refractometer and is attained from a random sample of ten fruit (minimum) from five vines (Sale & Lyford 1990). Fruit harvested at lower soluble SSC fail to give a satisfactory flavour when ripe and have a slower softening rate in cool storage than fruit harvested at higher SSC. They also show signs of internal breakdown after storage, with the inner pericarp becoming translucent and water-soaked in appearance while the outer pericarp often remains tough and rubbery. Besides, the SSC will not reach a final concentration as high as that attained in fruit harvested later; such fruit does not develop a good flavour, and may even develop unpleasant off-flavours (Beever & Hopkirk 1990).

The maturity index of 6.2 is a minimum, not the optimum. Although fruit harvested with higher maturity index (SSC between 7-10%) is usually softer at harvest, they have lower incidence of soft patches after long term cool storage (Davie *et al.* 1996), a slower softening rate during cool storage (Table 1.1), and overall better eating quality (Abdala *et al.* 1996; MacRae *et al.* 1989b; Mitchell *et al.* 1992). The manner in which kiwifruit soften at 20 °C also varies according to maturity at harvest. When fruit is less mature at harvest, there is a lag in the onset of the first phase of softening, which does not occur in more mature fruit. Fruit held at 20 °C following storage at 0 °C for 6 weeks after harvest soften more rapidly if less mature at harvest, and show greater response to ethylene treatment than more mature fruit (MacRae *et al.* 1989b). It is suggested that this may be associated with internal ethylene concentration in the fruit. Fruit harvested later in the season presumably accumulated sufficient ethylene from their own metabolism in the closed system of a commercially packed tray to supersede a threshold level required for promotion of softening (MacRae *et al.* 1989b).

Table 1.1 - Flesh firmness and SSC of 'Hayward' kiwifruit grown in 2 locations (A and B) at harvest and after 6 months at 0 °C (Mitchell *et al.* 1992).

Harvest Date	At Harvest				After Storage	
	SSC (%)		Firmness (N)		Firmness (N)	
	Loc. A	Loc. B	Loc. A	Loc. B	Loc. A	Loc. B
26/Sep.	6.5	6.0	75	78	12	5
05/Oct.	7.4	6.1	73	75	14	7
17/Oct.	7.5	6.7	66	72	15	13
26/Oct.	8.7	6.7	58	62	16	11
06/Nov.	9.7	10.7	54	45	19	14

1.3 Postharvest Factors Affecting Fruit Quality

1.3.1 Physical Damage

Both impact bruising (injury caused by dropping of the fruit), and compression bruising at and after harvest can result in symptoms such as external flattening, flesh soaking or water soaking, and light coloured fracture lines in the flesh (Mitchell 1990). Bruising accelerates ripening by increasing ethylene production and SSC of the fruit, and by decreasing flesh firmness. This ripening response induced by tissue damage is minimised by cooling the fruit soon after harvest (Banks *et al.* 1992; Mencarelli *et al.* 1996). Impact bruises and compression can also lead to the development of localised softening ('soft patches') in fruit (refer to section 1.4.3).

Abrasion or vibration bruising (injury caused by fruit movement during handling or transport), usually causes only minor signs of surface injury, but often develops severe internal flesh injury. Such injury occurs when fruit has softened to about 22 N, with flesh translucency as the main symptom, associated with an increase in ethylene production (Mitchell 1990). Abrasion caused by brushing of kiwifruit (to remove dirt, adhering particles, and trichomes) accelerates the ripening process due to very small injuries on the epicarp, which induces ethylene production (Massantini *et al.* 1995b).

1.3.2 Storage Conditions

In contrast to many other subtropical crops, kiwifruit can be cool stored for long periods. Current requirements for satisfactory long-term conventional storage include fruit temperature of 0 ± 0.5 °C (which is the most important single factor in maintaining fruit quality during storage and transport), relative humidity in the air surrounding the fruit of at least 95% (important to minimise fruit water loss), and ethylene concentration (which is a promoter of premature softening) in the air around the fruit below 0.03 parts per million. If ethylene exceed such concentration, the New Zealand Kiwifruit Marketing

Board recommends that the coolstore room be vented. In these optimal storage conditions fruit quality can be maintained for up to 6-8 months (Anonymous 1996b; Lallu *et al.* 1992; McDonald 1990).

1.3.3 Postharvest Diseases

Fungal rots

Botrytis cinerea stem-end rot is the most important postharvest disease of kiwifruit (Brook 1992) and an important factor associated with postharvest losses (Beever & Hopkirk 1994). The fungus invades fruit through the picking wound and continues to grow even in fruit stored at 0 °C. The infection begins at the stem of the fruit and progresses to the distal end, causing extreme softness and affecting the whole fruit. Diseased flesh become glassy and watersoaked (Brook 1992).

In addition to tissue spoilage, fruit infected by *B. cinerea* contribute to further losses because they are significant sources of ethylene (Niklis *et al.* 1997; Qadir *et al.* 1997). They elevate ethylene levels in cool stores or trays, potentially accelerating the softening of healthy fruit (Henzell *et al.* 1994; Mitchell 1990), as well as increasing the incidence of ripe rot caused by other fungi when the fruit is taken from cool storage (Brook 1992).

Dicarboximide fungicides applied as preharvest sprays can reduce incidence of stem-end rot by decreasing inoculum level. However, control is not complete as the potential infection site is not directly accessible at the time of spraying, and because dicarboximides are contact fungicides with limited eradicant activity (Pyke *et al.* 1994). Also, decarboximide-resistant strains of *B. cinerea* have emerged in kiwifruit orchards in New Zealand (Poole & McLeod 1994). Experimentally, the application of chemical fungicides to picking wounds within 24 hours of harvested may provide more effective and reliable control than spray programmes, but this control method is disallowed by importing countries (Brook 1992; Pyke *et al.* 1994).

The only other industry-approved control method in current use is 'curing'. Keeping fruit at ambient temperatures for up to 48 hours between picking and packing is an effective and cheap method of reducing *Botrytis* stem-end rot (Lallu *et al.* 1997; Long & Banos 1994). A condition of about 10 °C with relative humidity higher than 92% for a period of no more than three days provides an adequate disease control, without excessive fruit weight loss and with no significant effects on ethylene production and softening rate at harvest and during cool storage (Banos 1995; Retamales *et al.* 1997). It also has no major effects on fruit SSC and chemical composition (Banos *et al.* 1997).

After fruit is taken from cool store, fungal ripe rots can develop, caused by species of *Botryosphaeria, Colletotrichum, Cryptosporiopsis, Diaporthe, Fusarium, Fusicoccum, Glomerella, and Phoma* (Brook 1992).

Physiological disorders

A recent study (Lallu 1997) suggests that kiwifruit is chilling sensitive, and suggests the term low temperature breakdown (LTB) to describe the symptoms of chilling injury. These include development of a ring or zone of granular, water soaked tissue in the outer pericarp at the stylar end of the fruit. Chilling injury may also involve the development of diffuse pitting related to a dark scald-like appearance of the skin. These symptoms are also associated with reduced firmness of kiwifruit in coolstore (Lallu *et al.* 1992). The incidence and severity of symptoms is reduced by delays at ambient conditions prior to coolstorage (Lallu 1997). Storage of fruit harvested immature is a contributory factor to premature breakdown (Beever & Hopkirk 1990).

1.3.4 Postharvest Losses

In the last four seasons, about 11-17% of packed trays in New Zealand did not reach the consumer because of postharvest losses (Table 1.2).

Table 1.2 - Postharvest losses of kiwifruit in New Zealand (Source: Anonymous 1997b).

Season	1993	1994	1995	1996
Fruit loss (millions of trays)	4.28	4.81	6.22	5.53
Fruit loss (tonnes)	15,400	17,300	22,400	19,900
Fruit loss/Total (%)	11%	11%	17%	11%
Income loss (NZ$ millions)	18.4	23.6	26.2	24.0

Soft fruit (whole fruit firmness below 10 N or approximately 1 kgf) and those with soft patches (localised areas below 10 N) are the major causes of kiwifruit losses after harvest (Banks *et al.* 1992; Hopkirk & Clark 1992a). Premature softening of kiwifruit is typically responsible for between 40 and 70% of fruit losses after harvest (Davie *et al.* 1996). The softening process is considered in detail in section 1.4.3.

Postharvest diseases can also be significant contributors to fruits losses (refer to section 1.3.3). Other causes of fruit rejections after harvest can be wind, sun scald, and damage from frost, hail and hormone sprays.

1.4 Fruit Development and Physiology of Kiwifruit

1.4.1 Composition and Morphology

The major kiwifruit components are water (80-88%) and carbohydrates (12-17.5%), mainly starch, which is converted to soluble sugars (especially fructose, sucrose, glucose, and inositol) as fruit mature (Reid *et al.* 1982). Other components include fibre, organic acids (mainly citrate, quinate, and malate), pectic compounds, volatile components, vitamins (particularly C or ascorbic acid; also, A or retinol, B_1 or thiamin, B_2 or riboflavin, B_6 or pyridoxine, and niacin or nicotinic acid), and minerals (mainly potassium, nitrogen, calcium, magnesium and phosphorus). Cell plastids containing chlorophyll give the internal tissue its characteristic green colour (Beever & Hopkirk 1990).

Distinct from many other fruits, the edible part of the kiwifruit is made of three heterogeneous portions: the core, the seed area (or inner cortex or inner pericarp) and the flesh under the skin (or outer cortex or outer pericarp). Each of these tissues differs in mineral concentration (Ferguson 1984), cell wall composition (Redgwell *et al.* 1990), and cell characteristics (Harker & Hallet 1994), each with different patterns of change during maturation, which may affect both final eating quality of the fruit and the manner in which the fruit ripen after harvest (MacRae *et al.* 1989a).

Structurally, the core is made up of a few small regularly shaped cells packed closely together with very few air spaces between them. Cells from the seed area are of two types. The divisions (locule walls) between the seed compartments are made up of cells similar to the core cells, and cells of the seed compartments are large, oblong, and fitted closely together. Cells of the outer cortex are irregular in shape and size, and are loosely arranged, with relatively large air spaces between them (MacRae 1988).

Chemically, there are major differences in carbohydrate and acid concentrations between the different tissue zones. The core has higher levels of starch and lower levels

of acids (particularly quinic), and a higher sugar/acid ratio at harvest than other tissues. The inner cortex has higher levels of acids (especially citric) and lower levels of starch than the outer cortex (MacRae *et al.* 1989a). Since starch is converted to sugar during fruit ripening, these differences affect fruit flavour when they ripen (for instance, the core is usually described as sweeter, and the seed area as more sour) (MacRae 1988).

1.4.2 Maturation and Ripening

Kiwifruit is a berry which develops from a superior multicarpellate ovary and increases in size over about 160 days from a pollinated flower to a fruit weighing approximately 120 g. This process involves both cell division and cell expansion, and does not occur at a constant rate. Physiologically mature fruit will normally continue to ripen after harvest to a stage where they are optimum for eating, and there is no visible changes in the colour of the skin or the flesh during development (Beever & Hopkirk 1990).

Carbohydrate composition and firmness are the two major attributes that change as kiwifruit mature. In the early stages of development, photosynthate is converted to starch, which accumulates as grains in the cells of the flesh from the outer cortex to the core (Okuse & Ryugo 1981). From about 20 weeks after anthesis, the starch is hydrolysed, resulting in the rapid accumulation of soluble sugars (Reid *et al.* 1982). This process continues in fruit harvested at normal maturity, and stored at either 0 or 20 °C (MacRae *et al.* 1989b). SSC, which is positively correlated with taster acceptance of kiwifruit (Mitchell *et al.* 1992), is closely related to sugar content in kiwifruit juice (Beever & Hopkirk 1990). Because a hand-held refractometer can easily measure SSC in the field, this attribute is used in preference to sugar content as a measure of maturity (Harman 1981). In addition, total organic acid content (particularly citrate-measured by titratable acidity, as anhydrous citric acid equivalents) decreases during kiwifruit ripening at either 0 or 20 °C (MacRae *et al.* 1989b). The outer cortex of kiwifruit softens before the core of the fruit, and cell wall swelling reaches a maximum in the outer cortex earlier than in the core. At later stages of fruit maturation, the core continue to synthesise starch from photosynthate after such synthesis had ceased in the outer cortex (Redgwell *et al.* 1990).

In terms of respiratory pattern, kiwifruit displays a climacteric curve, with increased ethylene production either before or concurrent with the climacteric rise, although individual fruit vary greatly in the time they enter the climacteric rise (Hyodo & Fukasawa 1985; Pratt & Reid 1974). Respiration rate of mature fruit at 20 °C decreases slowly as the fruit soften. When flesh firmness values are about 10 N, respiration increases about two fold from the basal rate for a short time, before decreasing again. Respiration may increase again before senescence (Beever & Hopkirk 1990). When respiration rate begins to rise, ethylene production increases from trace amounts to very high peak values, before falling again as senescence approaches (Pratt & Reid 1974).

1.4.3 Softening

Softening is one of the most significant quality alterations consistently associated with the ripening of fleshy fruits. Kiwifruit texture changes dramatically during fruit ripening, and flesh firmness can decline by as much as 94%, from about 60-90 N at harvest to 5-8 N when ripe (Beever & Hopkirk 1990).

Importance in kiwifruit

Firmness is a key criterion in the assessment of quality of kiwifruit for export in New Zealand, where premature softening is the main quality attribute responsible for fruit losses after harvest (refer to section 1.3.4). A crucial aspect in the commercial success of kiwifruit is the ability of fruit to maintain firmness in cool store for a prolonged period of time after harvest. Since marked differences in kiwifruit softening rate can occur between orchards, districts and seasons, it is also the main factor limiting both the length of the packing season and the overall storage ability of the fruit (McDonald & Harman 1982). The industry in New Zealand recommends that fruit for export have a flesh firmness (measured by a 7.9 mm diameter penetrometer probe) of more than 1.0 kgf (or about 10 N) at the time of shipping, since the lower the fruit firmness, the higher the susceptibility to physical damages and diseases (Mitchell 1990). At a firmness of 5-8 N, fruit are ripe for eating (McDonald 1990). Sensory evaluation has demonstrated a

significant correlation between kiwifruit flesh firmness and overall fruit acceptability (Stec *et al.* 1989)

Fruit softening, as determined by fruit firmness, occurs in a characteristic manner in kiwifruit (Figure 1.3). Depending on harvest maturity, kiwifruit will maintain harvest firmness for up to 4-5 days at 20 °C, followed by a more extensive phase, in which fruit soften to about 20% of the harvest value (Lallu *et al.* 1989). This phase can be accelerated by exposure to ethylene, which plays an important role in the process (refer to section 1.5) or delayed by storage at low temperatures with or without controlled atmosphere (Arpaia *et al.* 1986; McDonald & Harman 1982; McRae *et al.* 1989b). At a storage temperature of 0 °C, flesh firmness usually decreases from about 80 N to 30 N in 4-6 weeks, followed by a decrease to about 15 N in 12 weeks. From this stage, fruit softening rate is very slow (Beever & Hopkirk 1990). The respiratory burst is usually associated with the last phase of softening during which fruit soften through to eating ripeness (MacRae & Redgwell 1992).

<u>Cell wall and other physiological aspects of kiwifruit softening</u>

The plant cell wall is a key determinant of texture in fruit. Physiological events such as ripening can reduce the strength of cell adhesion through the biochemical dissolution of the middle-lamellar pectic polysaccharides. Several enzymes that are able to degrade wall polysaccharides have been implicated in the ripening process, including polygalacturonases, pectin methyl esterases and β–galactosidases (Waldron *et al.* 1997). None of the cell wall enzymes studied so far in kiwifruit have shown major increases in activity during fruit softening comparable to those observed in the case of polygalacturonase in tomato or cellulase in avocado (Bonghi *et al.* 1996; MacRae & Redgwell 1992).

Physiological events which accompany the softening process in kiwifruit involve starch degradation and changes in cell wall composition and structure. Major aspects include solubilisation of the pectic polysaccharides of the primary cell walls (MacRae *et al.* 1992), loss of galactose from the side chains of the polymers (Redgwell *et al.* 1997a), reduction in molecular weight of the xyloglucan (MacRae & Redgwell 1992),

degradation of hemicellulose (Soda *et al.* 1987), increase in membrane permeability (Abdala *et al.* 1996), and decrease in water and osmotic potential (Harker & Hallet 1994). Cell wall deterioration and swelling is pronounced and the thickness of walls in the outer pericarp is three to four times greater in ripe fruit than in fruit at harvest (Redgwell *et al.* 1997b). The major changes in cell wall chemistry of the outer cortex, which occur during the later stages of maturation and in response to postharvest ethylene treatments, are summarised in Figure 1.1.

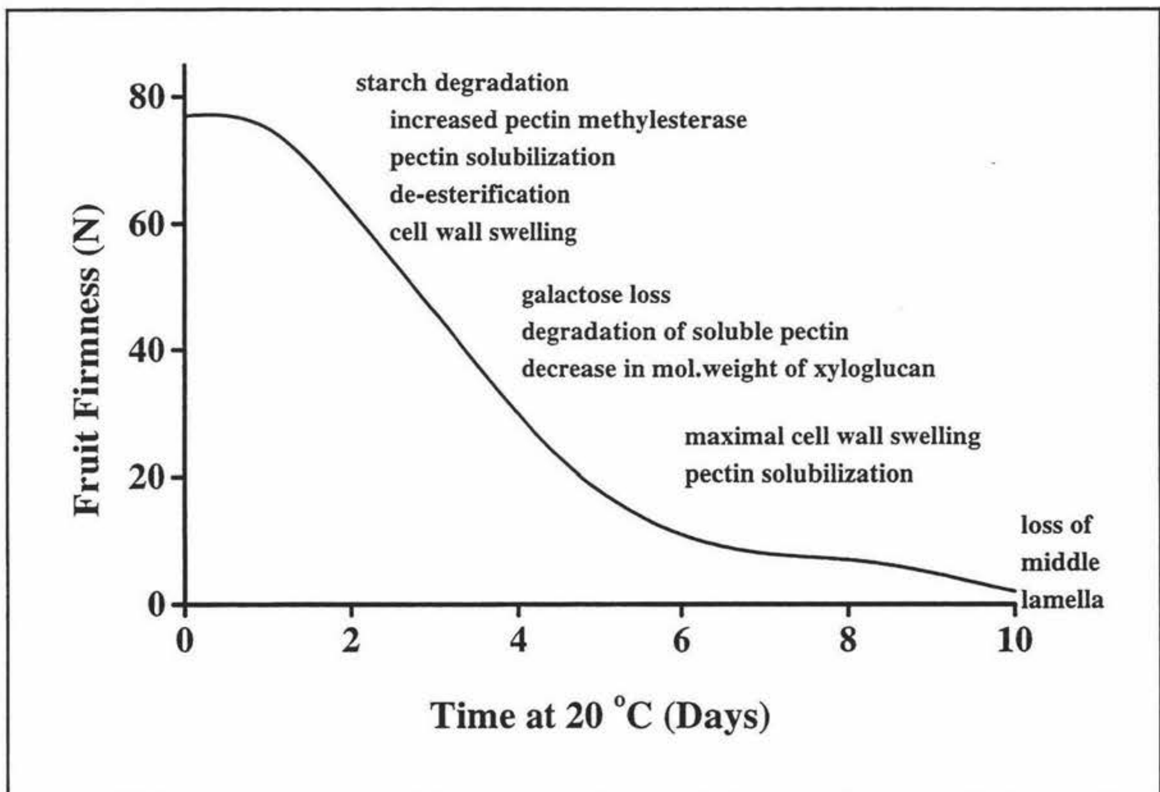

Figure 1.1 - Schematic representation of postharvest softening of kiwifruit in relation to the timing of key events in the process (MacRae & Redgwell 1992).

In kiwifruit, galactose loss and pectin solubilistion seem to affect different polysaccharides, and there is a lack of synchrony between the two process, suggesting that they are separate and unconnected events (Redgwell *et al.* 1997a). Kiwifruit discs treated with aminooxyacetic acid, an inhibitor of ethylene biosynthesis, did not soften or show any sign of pectin solubilisation after 72 hours, but did show a loss of galactose

from the cell wall materials, suggesting that galactose loss, in part, may be independent of ethylene (Redgwell & Harker 1995).

Although ethylene has an inductive effect on kiwifruit softening (Arpaia *et al.* 1986; McDonald & Harman 1982), the relationship between ethylene and the softening process is complex, and is not well understood. Distinctively from other climacteric fruit, the rise in ethylene and respiration in kiwifruit occur late in the ripening process, after the flesh has softened to the 'edible ripe' stage. Usually, the climacteric rise occurs at the same time as eating ripeness (such as in avocado and banana) or considerably earlier (such as in apple and tomato) (Beever & Hopkirk 1990).

<u>Cellular and biochemical aspects of kiwifruit softening</u>

Since kiwifruit texture is typically assessed by measuring mechanical properties of the outer pericarp tissue, the relative number of large and small cells (large cells are less likely to rupture than small cells) and the chemical composition of cells walls affect kiwifruit texture. Rapid loss of fruit firmness during the early ripening stages of kiwifruit stored at 0 °C is accompanied by reduced adhesion between cells and results in cells separating at the middle lamella rather than rupturing. Tissue softening during later stages is associated with further reductions in cell-to-cell adhesion and increases in the elasticity or plasticity of the cell walls (Harker & Hallet 1994). Also, starch degradation could play a role in the early events of fruit softening by altering cell turgor and osmotic pressure, which may affect cell walls and their relative flexibility (Arpaia *et al.* 1987; MacRae *et al.* 1989b). As fruit soften on the vine from 98 to 77 N, starch content decreases by 20%, without any detectable change in cell wall composition (MacRae & Redgwell 1992). Thus, not only cell walls, but the cells as whole play a role in the softening process.

In addition, tensile strength of kiwifruit skin is considerably higher than that of the outer pericarp tissue, and proportional differences in tensile strength of skin and pericarp tissue increase from 2 (at harvest) to 42, after 29 weeks of storage, indicating that the role of the skin in holding the fruit together becomes more important as kiwifruit soften

(Harker & Hallet 1984). This may have implications in the assessment of firmness without removing the skin (for instance, using a non-destructive mechanical device).

Firmness assessment in kiwifruit

Kiwifruit firmness after a patch of skin has been removed is usually measured using a hand-held Effegi-type penetrometer that records the maximum force required to push a probe into the flesh. Generally, force applied to the probe increases as the probe slowly penetrates further into the tissue until the force become so great that the tissue starts to disintegrate. Once tissue failure has started, the applied force does not usually increase above this maximum, no matter how deep the probe is pushed into the outer pericarp (Harker & Hallett 1994). Although it is a simple and fast test, it is dependent on the operator, as different operators can generate different values for the same fruit (Harker *et al.* 1996), since factors such as the speed of punch and depth of penetration are not constant (Blanpied *et al.* 1978). This inadequacy can be overcome using materials testing machines (such as 'Instron'), which can apply penetrometer tests to fruits at a constant speed and depth of penetration (thus providing a more consistent measurement). These are widely used in assessing texture in foods (Harker *et al.* 1996).

Another limitation with the use of penetrometer is that it is a destructive test (the probe punctures the fruit), and thus only a sub-sample, selected to represent all fruit, rather than every individual fruit, can be tested. In recent years, considerable effort has been expended towards developing alternative methods, specially non-destructive tests, that could better measure the firmness of several fruit crops. These include use of impact response, sonic vibration, and force deformation (Hopkirk *et al.* 1996). Such devices allow the same individual fruit to be monitored on successive occasions over a period of time, being especially useful where there is large variability between individual fruit (demanding a large sample), or where there is only a limited number of fruit available (Hopkirk *et al.* 1996).

'Soft patches' in kiwifruit

Apart from individual fruit softening entirely as they ripen, there is a condition in kiwifruit in which localised soft areas (named 'soft patches') develop on the fruit surface, areas that are substantially softer than the rest of the fruit. These areas, located anywhere on the fruit surface, may be only 1 cm^2 or cover large portions of the fruit surface. In same cases, soft patches are visible on the fruit surface as darkened, water soaked areas. In addition to water soaking, cutting open a fruit through a soft patch can sometimes reveal cracking and whitening of the tissue, signs of impact at harvest (Davie *et al.* 1996).

1.5 Ethylene

1.5.1 Biosynthesis in Higher Plants

The plant hormone ethylene (C_2H_4), a volatile hydrocarbon gas, is biologically active in trace amounts and its effects are commercially important in agriculture. Ethylene influences many aspects of plant growth, development and senescence, such as seed germination, diageotropism, flowering, abscission, flower and leaf senescence, sex determination in cucurbits, fruit ripening, and pathogenesis responses (Abeles *et al.* 1992; Fluhr & Mattoo 1996). It also coordinates plant responses to mechanical and environmental stresses such as wounding and chilling (Abeles *et al.* 1992; Smalle & Van Der Straeten 1997). Although sometimes the effects of ethylene on horticultural crops can be beneficial (improving the postharvest quality of the product by promoting faster and more uniform ripening before consumption), they are usually deleterious, speeding senescence and reducing shelf life, causing large losses of produce annually.

Ethylene production by plant tissues is normally low. During certain stages of growth and development, an increase in ethylene production is induced, causing important physiological consequences such as the ripening of fruit. Increased knowledge on ethylene biosynthesis (Adams & Yang 1979; Lurssen *et al.* 1979) has provided the opportunity for the biochemical and molecular genetic analysis of the pathway to be undertaken (Figure 1.2). The amino acid methionine (Met) is the biological precursor of ethylene. It is converted to S-adenosyl-L-methionine (AdoMet or SAM) by the enzyme methionine adenosyl transferase or AdoMet synthetase. The rate-limiting step in ethylene biosynthesis is the conversion of SAM to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5-methylthioadenosine (MTA), which is catalysed by ACC synthase (ACS). ACC is then converted to either: (a) ethylene, carbon dioxide (CO_2), and hydrogen cyanide (HCN), which is an oxygen (O_2) dependent reaction catalised by ACC oxidase (ACO), formerly called ethylene-forming enzyme or EFE; or (b) N-malonyl-ACC (MACC), a biologically inactive end-product, catalysed by malonyl ACC transferase, a reaction which may contribute to the regulation of ACC concentration and

ethylene production. Met is recycled through the pathway by the conversion of MTA to Met, which allows the maintenance of high ethylene biosynthesis even when the free Met pool is small. This pathway has been confirmed in all higher plants studied (Fluhr & Mattoo 1996; Kende 1993; Yang & Hoffman 1984; Zarembinski & Theologis 1994).

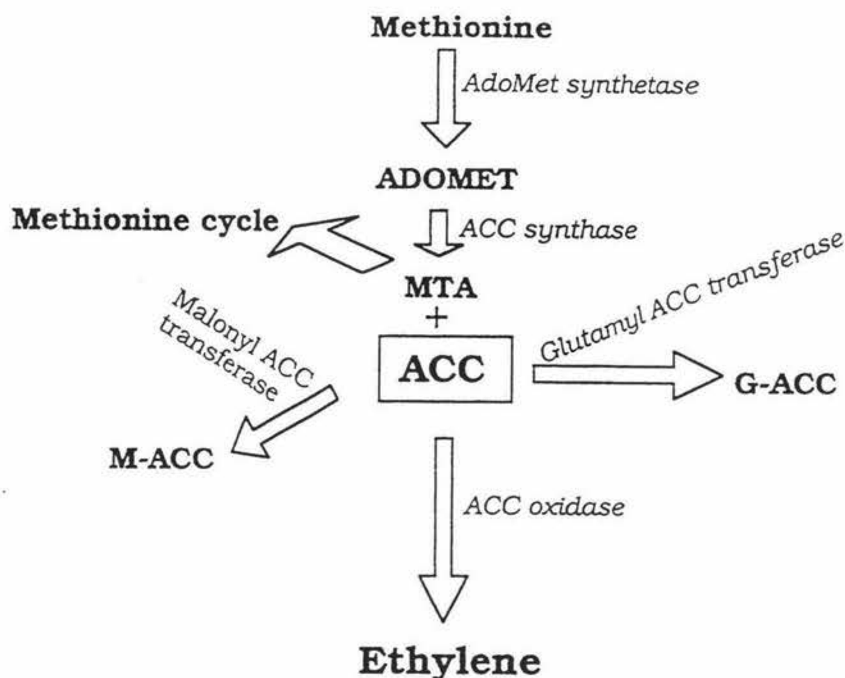

Figure 1.2 - The major pathway of ethylene biosynthesis in higher plants and the enzymes involved (Fluhr & Mattoo 1996).

While ACO has been purified to homogeneity, ACS has only partially been purified because of its low abundance and lability. ACS is a cytosolic enzyme which represents the key regulatory enzyme in the pathway (Boller *et al.* 1979; Kim & Yang 1992). Several internal cues and external inducers affect the synthesis of ACS, such as ethylene, auxin, cytokinin+auxin, calcium ions +cytokinin, wounding, fruit ripening, elicitors/pathogens, anaerobiosis, and stress (heat, cold, cadmium ions, lithium ions, UV light) (Fluhr & Mattoo 1996; Zarembinski & Theologis 1994). ACS is also strongly inhibited by some synthetic bioregulators (refer to section 1.6). ACO activity is not as highly regulated as that of ACS and requires CO_2, iron, and ascorbate for activity (Ververidis & John 1991; Zarembinski & Theologis 1994). It is constitutive in most

1.5.2 Role in Fruit Ripening

Senescence is a universal phenomenon in living organisms and is viewed as the final phase of development and differentiation. In plants, ripening of a fruit is the prelude to senescence and involves co-ordinated changes in various biochemical pathways (Theologis 1994). Experiments using ethylene inhibitors, pulse ethylene treatments, and antisense transgenic fruits have demonstrated that ethylene synthesis plays a key role in regulating fruit maturation and ripening in many important fruit crops. It coordinates expression of genes responsible for a variety of biochemical processes, which include increase in respiration rate and ethylene synthesis, texture change (cell wall softening resulting from solubilisation of pectins and cellulose), synthesis of aroma compounds, conversion of starch to sugars, colour change (chlorophyll breakdown), and abscission (Fluhr & Mattoo 1996; Oetiker & Yang 1995).

Respiratory patterns of fruits

Fruits are divided into two groups on the basis of their respiratory behaviour during maturation and ripening. Climacteric fruits (such as apple, apricot, avocado, banana, guava, fig, kiwifruit, mango, papaya, passion fruit, peach, pear, persimmon, plum, and tomato) undergo an autocatalytic production of ethylene, with a sharp increase in ethylene production and respiration, accompanied by marked changes in composition and texture; non-climacteric fruits (such as grape, lemon, lychee, orange, pineapple, mandarin, and strawberry) show no autocatalytic ethylene production or other biochemical changes associated with fruit ripening, that can be associated with distinct changes in composition (Biale & Young 1981; Theologis 1994).

System 1 and *System 2* ethylene production in fruit ripening

The ripening of climacteric fruit is divided into a preclimacteric and a climacteric stage. The different physiological roles of ethylene production during fruit ripening have led to the classification of two systems. *System 1* is the low level of ethylene present in early climacteric fruit (or preclimacteric) before the onset of ripening (most ethylene production by vegetative plant tissues and the ethylene produced by nonclimacteric fruit

can be classified as *system 1*). *System 2* is the high rate of ethylene production observed during the climacteric and its main characteristic is that this ethylene production is ethylene induced, i.e. is autocatalytic (McMurchie *et al.* 1972).

The enzymes of *system 1* and *system 2* ethylene production

As the biosynthetic pathway for ethylene has been established, ACS and ACO have been characterised and their genes cloned. Various genes of the ACS and ACO families may be involved in a complex temporal and spatial pattern of regulation, which ultimately controls the onset of fruit ripening. Both ACS and ACO are causally involved in fruit ripening (Oetiker & Yang 1995). During the preclimacteric stage ACS activity is low, but then increases several hundred fold and becomes abundant during the climacteric, indicating that ACS is tightly regulated. This is achieved through the short half life of the ACS protein (58 min), which may be an important factor contributing to an efficient control of ethylene biosynthesis and hence of fruit ripening at the transcriptional level (Kim & Yang 1992). Low levels of *system 1* ethylene are present in the preclimacteric stage and exert a positive feedback on ACO activity. Treatment with exogenous ethylene does not induce the synthesis of ACS, but of ACO in preclimacteric tomato (Liu *et al.* 1985) and apple fruit (Bufler 1986).

Response mechanisms in fruit ripening

Two types of responses are involved in ethylene action. The first is a response to a change in concentration of cellular ethylene (concentration response). The second is a change in the sensitivity of tissue to ethylene which is already present (Abeles *et al.* 1992). The ripening sensitivity of fruits in response to ethylene increases (the response threshold decreases) during fruit development, and fruits attached to the plant are less responsive to ethylene (ACS and ACO are suppressed) than detached fruits (Bradford & Trewavas 1994; Yang *et al.* 1986). Yang (1987) speculates that high levels of a 'ripening inhibitor' could desensitise the fruit towards ethylene action and, as a consequence, ripening is not initiated. As with other hormones, it is assumed that ethylene perception by plant cells involves receptors, and that binding of ethylene to these sites initiates a sequence of biochemical events leading to ripening. Thus, a

variation in sensitivity may depend on the levels and characteristics of these receptors. At least two types of ethylene-receptor complexes can be found in fruit tissues: one responsible for stimulating respiratory rate is not affected by the 'ripening inhibitor', and is, therefore, active in both mature and immature fruits; the other, responsible for autocatalytic ethylene production and other ripening processes, is regulated by the 'ripening inhibitor' and is active only in mature fruits (Yang *et al.* 1986). Therefore, fruit development can be regarded as a transition from a non-climacteric tissue (immature fruit) to a climacteric tissue (mature fruit).

The low level of *system 1* ethylene produced by the preclimacteric fruit, in conjunction with the preexisting *system 1* receptor, could play an essential role by gradually destroying the 'ripening inhibitor', which could either suppress the development of *system 2* receptor or interfere with the ethylene binding to the *system 2* receptor. As the 'ripening inhibitor' is inactivated , the fruit tissue could begin to develop a functional *system 2* receptor, resulting in a surge in ACS, ACC, autocatalytic ethylene production and climacterium (Oetiker & Yang 1995). The molecular mechanisms by which environmental and endogenous factors regulate ethylene formation, and the nature of the biochemical sensor that detects the level of the functional receptor that enhances or depresses ACS activity are unknown (Kende 1993; Theologis 1994).

1.5.3 Ethylene and Kiwifruit

Healthy kiwifruit produce very small amounts of ethylene at harvest and throughout cool storage at 0 °C, but are highly sensitive to exogenous ethylene (Pratt & Reid 1974). Concentrations as low as 0.01 $\mu l.l^{-1}$ increase softening rate in kiwifruit at 21 °C (Hyodo *et al.* 1987) and at 0 °C (Arpaia *et al.* 1987; Retamales & Campos 1997), and reduces coolstorage potential by 46% (Jeffery & Banks 1996). Kiwifruit infected with pathogens are significant sources of ethylene and can elevate ethylene levels within individual trays and within a coolstore as a whole, potentially inducing rapid fruit softening (refer to section 1.3.3).

Although internal ethylene concentration (IEC) of kiwifruit attached to the vine was shown to increase from 0.007 $\mu l.l^{-1}$ at 4.4% SSC to 0.016 $\mu l.l^{-1}$ at 6.4% SSC as fruit

matured (MacRae *et al.* 1989b), concentrations remained low during maturation. Even in fruit left on the vine for almost 170 days after bloom (with a SSC of 10.5% and a firmness value of 41 N), the IEC did not increase above 0.086 $\mu l.l^{-1}$ (Sfakiotakis *et al.* 1997). This suggests that a 'ripening inhibitor' is produced by the vine and translocated to attached fruit, and that there is no induction of autocatalytic ethylene production while fruit is on the tree.

In kiwifruit, a linear relationship is observed between ethylene production and IEC, with IEC ($\mu l.l^{-1}$) values being about 10 times higher than ethylene production ($\mu l.kg^{-1}.h^{-1}$) values (Abeles *et al.* 1992; Hyodo & Fukusawa 1985). Ethylene production is also paralleled by ACC content, and ACC oxidase in the fruit tissue (Hyodo & Fukasawa 1985; Hyodo *et al.* 1987). In any kiwifruit which is producing ethylene as part of its ripening process, the outward movement of ethylene is restricted by its skin. Skin resistance of kiwifruit to gas diffusion (22.3 $cm.h^{-1}.atm^{-1}$) is much higher than those from other crops such as apple (5.6 $cm.h^{-1}.atm^{-1}$), and banana (1.9 $cm.h^{-1}.atm^{-1}$) (Banks *et al.* 1991). In addition, this high resistance shows very little change throughout cool storage and ripening (Peter Jeffery, Massey University; personal communication, 1997).

Temperature plays a vital role in ethylene biosynthesis and ripening of kiwifruit, which behaves as a climacteric fruit (in reference to respiration and ethylene production) at 20 °C, while behaving as a non-climacteric fruit (concerning ethylene production) at 0 °C. Kiwifruit lacks the ability for autocatalysis of ethylene production below the temperature range of 11-14.5 °C, a condition in which the conversion from *System 1* to *System 2* ethylene seems to be inhibited (Sfakiotakis *et al.* 1997). Optimum ethylene production occur in the temperature range of 20-34 °C (Antunes & Sfakiotakis 1997). High ethylene production at these optimum temperatures coincide with increased IEC, ACC content, ACS activity, and ACO activity in the fruit tissue (Antunes & Sfakiotakis 1997; Hyodo & Fukasawa 1985; Hyodo *et al.* 1987). In addition, chilling induces production of ethylene on returning fruit to room temperature. Fruit stored at 0 °C for 12 days have higher ethylene production (concurrent with higher activities of ACS and ACO) during shelf life at 20 °C, than fruit previously stored at 5, 10, and 15 °C (Sfakiotakis *et al.* 1997).

1.6 AVG

1.6.1 Action in Plant Tissues

The low concentration of preclimacteric ethylene production can be reduced physiologically by holding the fruits under non-inductive conditions such as low temperature, low oxygen, low pressure (since the conversion of ACC to ethylene is an oxygen-dependent reaction), or chemically by application of an ethylene biosynthesis inhibitor such as aminoethoxyvinylglycine (AVG) or aminooxyacetic acid (AOA) (Oetiker & Yang 1995).

AVG [NH_2-CH_2-CH_2-O-CH:CH-CH(NH_2)-COOH], or (S)-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid, is one of the most effective inhibitors of ethylene biosynthesis in many plant tissues (Lieberman 1979; Yang & Hoffman 1984). It competitively inhibits activity of ACS, inhibiting conversion of SAM to ACC *in vivo* (Adams & Yang 1979) as well as *in vitro* (Boller *et al.* 1979). ACS requires pyridoxal phosphate for activity, and AVG interferes with pyridoxal phosphate-mediated enzyme reactions (Rando 1974). The K_i value for the AVG inhibition of ACS from tomato tissue was estimated to be 0.2 μM (Boller *et al.* 1979). As an analogue of rhizobitoxine, AVG is apparently a synthetic plant growth regulator, but it has also been indicated as a fungal fermentation metabolite (Curry 1992; Shafer *et al.* 1995).

Although AVG blocks the synthesis of ethylene in plant tissues by inhibiting the conversion of SAM to ACC (which is a key reaction in the biosynthetic pathway for ethylene as seen in section 1.5.1), it does not inhibit conversion of methionine to SAM or ACC to ethylene (Boller *et al.* 1979; Lieberman 1979). AVG was found to be an irreversible inhibitor of ethylene production in apple tissue, but the inhibition of AVG on ACS *in vitro* is reversible as the inhibited enzyme activity is fully recovered when AVG is removed from the incubation medium (Yang & Hoffman 1984).

In addition, according to Mattoo *et al.* (1977), inhibition of ethylene biosynthesis by AVG in apple plugs is temperature-dependent (Table 1.3).

Table 1.3 - Inhibition of ethylene synthesis (%) in apple plugs by AVG (0.1 mM) in relation to temperature (°C) (Mattoo *et al.* 1977).

Temperature (°C)	Inhibition of AVG (%)
2	20.8
5	24.6
10	38.3
15	47.6
20	54.4
25	59.4
30	68.0

1.6.2 Application in Horticultural Crops

Over the last 20 years, AVG has been applied to several horticultural crops including apples, pears, peaches, nectarines, and cut flowers, in an attempt to regulate ethylene synthesis and its mediated processes, resulting in several effects on plant physiology before and after harvest.

Apple

Preharvest sprays with AVG (mainly at a concentration of 500 $mg.l^{-1}$ applied about two to four weeks before harvest) resulted in various physiological effects on both pre and postharvest fruit quality of apples (Table 1.5). Most attributes, particularly fruit ethylene production, respiration rate, and firmness, are stimulated by ethylene, indicating that endogenously produced ethylene was inhibited by the applied AVG. Use of AVG on apple trees for prevention of preharvest fruit abscission is an area of plant research that

has generated considerable interest. 'ReTain™', a commercial formulation of AVG, has been cleared for commercial application in the United States of America at the maximum concentration of 125 $mg.l^{-1}$ active ingredient (or about 123.5 $g.ha^{-1}$ or 50 $g.acre^{-1}$ a.i.), 4 weeks before harvest.

Other horticultural crops

Preharvest sprays of AVG (400 ppm) delayed ripening of pear (Romani *et al.* 1982; 1983). Dipping or vacuum infiltration with AVG (1,000 ppm) after two months storage at –1.1 °C, inhibited ethylene production in pear fruit ripened at 20 °C (Wang & Mellenthin 1977). Postharvest dips with AVG (250, 500, and 1,000 ppm) delayed softening of peach and nectarine (Byers 1997).

AVG sprayed onto flowering potted plants (especially carnations and other flowers sensitive to ethylene) immediately before shipping and handling, extended longevity and yielded plants with significantly more viable flowers (Shafer *et al.* 1995; Woltering 1987).

Kiwifruit

Postharvest dips (0.5 mM) with another ACS inhibitor ('LAB 181 508'- BASF, an experimental compound) reduced ethylene production of kiwifruit during and after cool storage, and resulted in firmer fruit after cool storage (Retamales *et al.* 1995). AVG inhibited ethylene production by flesh kiwifruit discs exposed to 0.5 mM AVG (Hyodo & Fukasawa 1985), or to 5 mM AVG (Massantini *et al.* 1995a). However, the effects of preharvest AVG applications on kiwifruit quality (including firmness) are not known.

Table 1.4 – Pre and postharvest effects of AVG on apple cultivars.

Period	Effect	Cultivar	Reference
Pre-harvest	Reduced preharvest fruit abscission (drop), with increased fruit set.	Cox's Orange Pippin, Delicious, Golden Delicious, McIntosh, Spartan, Spencer.	Bangerth 1978; Child *et al.* 1986; Child & Williams 1983; Edgerton 1981; Greene 1980, 1983; Rahemi & Dennis 1981; Shafer *et al.* 1995; Williams 1980.
	Increased lateral branching.	Delicious.	Curry & Williams 1986.
Post-harvest	Reduced fruit ethylene production.	Cox's Orange, Delicious, Golden Delicious, Granny Smith.	Child *et al.* 1984; Curry & Patterson 1992; Bufler 1984; Treccani & Cocucci 1986.
	Reduced fruit internal ethylene concentration.	Cortland, Golden Delicious, McIntosh, Spencer, Red Delicious.	Autio & Bramlage 1982; Bangerth 1978, 1986; Bramlage *et al.* 1980; Bramlage & Autio 1982; Halder-Doll & Bangerth 1987.
	Reduced fruit respiration rate.	Golden Delicious.	Bangerth 1978, 1986; Bufler 1984; Curry & Patterson 1992; Halder-Doll & Bangerth 1987.
	Slower fruit softening rate.	Cortland, Cox's Orange, Delicious, Golden Delicious, McIntosh, Spencer, Red Delicious.	Autio & Bramlage 1982; Bangerth 1978; Bramlage *et al.* 1980; Bramlage & Autio 1982; Bufler 1984; Child *et al.* 1984; Halder-Doll & Bangerth 1987; Olsen 1982; Shafer *et al.* 1995.
	Reduced aroma volatile.	Golden Delicious	Bangerth 1986; Bangerth & Streif 1987; Halder-Doll & Bangerth 1987.

1.7 Objective

To characterise the effect of preharvest applications of different concentrations and times of application of AVG to kiwifruit vines on ethylene biosynthesis, respiration, firmness, and soluble solids content of fruit at harvest and after coolstorage.

2 MATERIAL AND METHODS

2.1 Field Activities

2.1.1 Treatment Application

40 uniform vines (six years old, trained on a winged T-bar system) of kiwifruit [*Actinidia deliciosa* (A. Chev) C. F. Liang et A. R. Ferguson], cv. 'Hayward' on their own roots, growing at the Fruit Crops Unit, Massey University, Palmerston North (Lat S 40 21, Long E 175 37), New Zealand, were selected and divided into four treatments each with five replicates, with two vines per replicate, in a Completely Randomised Block Design (CRBD), according to the layout in Appendix I. The vines (leaves and fruits) were sprayed (until runoff) with water (rate of 1,000 $l.ha^{-1}$) + surfactant ('Freeway' - adjuvant 0.1%) + AVG (aminoethoxyvinylglycine, in the commercial formulation 'ReTain™', a soluble powder with 15% (w/w) active ingredient, supplied by 'Nufarm Limited', New Zealand), at two application dates (6 and 4 weeks before commercial harvest, on 12th May, 1997), and two AVG concentrations (500 $mg.l^{-1}$ a.i. or about 494 $g.ha^{-1}$ a.i. or 200 $g.acre^{-1}$ a.i.; and 1,000 $mg.l^{-1}$ a.i. or about 988 $g.ha^{-1}$ a.i. or 400 $g.acre^{-1}$ a.i.), using a knapsack sprayer.

The treatments and respective abbreviations used in this thesis were:

1 - Control (unsprayed) ⇒ **'Control'**

2 - AVG-500 $mg.l^{-1}$ a.i. - 4 weeks before harvest ⇒ **'500-AVG-4'**

3 - AVG-500 $mg.l^{-1}$ a.i. - 6 weeks before harvest ⇒ **'500-AVG-6'**

4 - AVG-1,000 $mg.l^{-1}$ a.i. - 4 weeks before harvest ⇒ **'1000-AVG-4'**

2.1.2 Harvesting

Fruit were harvested at a commercial stage of maturity with soluble solids content (SSC) value of 7.5%, attained from a random sample of ten fruit from five vines (two fruit per vine). SSC was determined using a hand-held Atago N-20 refractometer (Brix range from 0-20%). Fruit were harvested in the morning (between 9-11 am) by snapping fruit off the stem, placed in a picking bag, and then carefully emptied into small plastic bins before transport to the laboratory.

2.1.3 Postharvest Operations

After removal of damaged, blemished and undersized fruit, the stem scar of each fruit was treated with fungicide iprodione ('Rovral' 0.075%, one droplet of approximately 30 μl of solution per fruit). After the droplet had dried (about 30 minutes), 2,400 fruit (600 fruit per treatment) were placed in 160 commercial kiwifruit cardboard trays (40 trays per treatment, with 15 fruit per tray) containing a single layer of plastic pockets (plix) count 20, surrounded by a polyethylene liner ('polyliner'), made of 10 μ high density polyethylene (HDPE) to prevent fruit water loss (McDonald 1990; Anonymous 1996b). Average individual fruit weight was 100 g, and the mean fruit weight variation between treatments was less than 5%. A sachet containing 20 g of potassium permanganate held on aluminium oxide ('Purafil'), an ethylene oxidiser (Scott *et al.* 1984) was placed in each tray. Fruit were then cured for 3 days at 10 °C, RH 98% to reduce potential for *Botrytis cinerea* infection (Banos 1995).

After curing, 20 trays were placed at 20 °C for 24 hours to stabilise fruit temperature. Open buckets with potassium permanganate ('Purafil') were placed in the room to avoid ethylene contamination between trays. In addition, the room was periodically checked for ethylene by taking three air samples, which were measured for ethylene production (refer to section 2.2.2), and averaged. After 1, 3, 6, 9, and 13 days at 20 °C, 15 fruit per treatment were taken from the trays (3 fruit per tray) for measurements of respiration rate, ethylene production, firmness, and soluble solids content. The relative humidity of the room was about 65% and fruit were left inside 'polyliners' in trays until analysed.

All measurements after 1 day at 20 °C in this thesis are referred as 'immediately after harvest'.

2.1.4 Cool Storage

The remaining 140 trays were placed at 0 °C in a coolstore which had ethylene scrubbing (by catalytic oxidation) equipment ('Swingtherm ME 40', Tubainet, Switzerland) (Knee *et al.* 1985). The coolstore was periodically checked for ethylene concentrations by taking 5 air samples from inside the chamber and injecting them into a gas chromatograph for ethylene determination (refer to section 2.2.2). After 14, 30, 52, 80, 110, and 180 days at 0 °C, 20 trays (5 trays per treatment) were removed for assessment of fruit physiology and quality (refer to section 2.2).

2.2 Laboratory Activities

Trays removed from cool store were placed at 20 °C for 24 hours, and fruit quality attributes mentioned above were measured five times, or four times when the fruit were over ripe (firmness below 5 N) after the fourth measurement. The intervals of measurement varied between removals (Table 2.1) depending on the physiological response of the fruit during measurements at 20 °C, particularly ethylene production. All measurements after 1 day at 20 °C in this thesis are referred as 'on removal'.

Table 2.1 – Measurement time (days) of fruit variables at 20 °C, after successive removals from 0 °C.

Time at 0 °C (Days)	Measurement time at 20 °C (Days)
0	1 – 3 – 6 – 9 – 13
14	1 – 4 – 7 – 10 – 15
30	1 – 4 – 7 – 12 – 17
52	1 – 5 – 10 – 15 – 20
80	1 – 5 – 10 – 14
110	1 – 4 – 7 – 10
180	1 – 3 – 6 – 9

2.2.1 Carbon Dioxide Production

Carbon dioxide (CO_2) production, as an estimate of respiration rate, was measured by placing individual fruit in separate 500 ml air-tight plastic jars fitted with a rubber septum for taking gas samples from inside the jar. Immediately after sealing, a 1 ml gas sample was removed from each flask to determine initial CO_2 concentration. Jars were then incubated at 20 °C for about 1 hour (time recorded) and 1 ml gas sample was taken from the headspace of each jar using an air tight plastic syringe. Samples were taken successively and immediately analysed to avoid gas leakage caused by holding the syringes for prolonged periods (Blankenship & Hammett 1987).

Mole fraction composition of CO_2 was determined using a miniature infra-red CO_2 transducer with electronic linearisation (Analytical Development Company, United Kingdom), with O_2-free N_2 as carrier gas (flow rate 580 $mm^3.s^{-1}$) (Yearsley 1996). Output signals from the CO_2 transducer were analysed using an integrator ('HP 3394A',

Hewlett Packard, United States of America) using peak area mode. Respiration rate was calculated according to the formula in Appendix II (Shusiri 1992). Kiwifruit density was assumed to be 1030 $kg.m^3$ (Harris & McDonald 1975). The conversion factor proposed by Banks *et al.* (1995) was used to convert the unit $ml.kg^{-1}.h^{-1}$ to $mol.kg^{-1}.s^{-1}$, which has been proposed as a standard unit in postharvest research on gas exchange. In the conditions of this experiment, 1 $nmol.kg^{-1}.s^{-1}$ is equivalent to approximately 0.087 $ml.kg^{-1}.h^{-1}$.

A humidified 0.5% CO_2 volumetrically prepared standard was used for calibration. This was prepared by injecting 9400 mm^3 of pure CO_2 from a cylinder of compressed food grade CO_2 (NZIG Special Gases, Wellington, New Zealand), into a 1.815×10^{-3} m^3 jar, and the jar atmosphere pressurised with addition of 0.185×10^{-3} m^3 of air. Ambient CO_2 concentration was assumed to be 0.033%. The atmosphere of the standard bottle was humidified by injecting a drop of water into the jar and stirring. A fresh standard was made when the bottle partial pressure has decreased to near atmospheric pressure (Yearsley 1996).

2.2.2 Fruit Ethylene Production

Ethylene production was calculated using the same gas samples used for CO_2 production. After incubation at 20 °C for about 1 hour (time recorded), 1 ml gas samples were injected into a gas chromatograph ('Photovac 10S50', Photovac International Incorporated, United States of America), which was fitted with a photoionization detector for low ethylene concentration (minimum detection limit 0.001 $\mu l.l^{-1}$), a 45 cm nylon tubing XE-60 column at 20 °C, and clean air as the carrier gas (flow rate 250 $mm^3.s^{-1}$). Output signals from the gas chromatograph were analysed using an integrator ('HP 3394A', Hewlett Packard, United States of America) using peak height mode. Ethylene production was calculated according to the formula in Appendix II (Shusiri 1992). Kiwifruit density was assumed to be 1030 $kg.m^3$ (Harris & McDonald 1975). The conversion factor proposed by Banks *et al.* (1995) was used to convert the unit $\mu l.kg^{-1}.h^{-1}$ to $mol.kg^{-1}.s^{-1}$. In the conditions of this experiment, 1 $pmol.kg^{-1}.s^{-1}$ is

equivalent to approximately 0.087 $\mu l.kg^{-1}.h^{-1}$. For a fruit of 100 g, incubated for 60 minutes, 1 $pmol.kg.^{-1}.s^{-1}$ ethylene production is equivalent to about 0.022 $\mu l.l^{-1}$.

A commercially prepared certified gas mixture (NZIG Special Gases, Wellington, New Zealand) was used as standard for ethylene analysis (0.101 ± 0.001 $\mu l.l^{-1}$ ethylene, balance N_2).

In graphs, rates of ethylene production were plotted on a logarithmic scale to illustrate small but physiologically significant changes at the initiation of ripening, while keeping high rates within the bounds of the graph (Pratt & Reid 1974).

2.2.3 Fruit Firmness

Destructive measurement

Fruit firmness (N; the force required to penetrate the outer pericarp) of kiwifruit equilibrated to 20 °C, was measured with a materials testing machine ('TA-XT2 'Texture Analyser', Stable Micro Systems, England; refer to Plate 2.1, page 41), fitted with a 7.9 mm diameter probe ('Effegi', Facchini, Italy). A 1 mm thick slice of skin/outer pericarp was removed from opposite sides of the equatorial surface of the kiwifruit, as sites for penetration of the probe, which was driven 9 mm radially into the fruit. Distance and speed control in the machine was achieved by a stepping motor attached to a fine lead screw that moved the probe carrier up and down. The machine was controlled by a microprocessor interfaced to an appropriate software ('Texture Expert for Windows', Version 1.0, Stable Micro Systems, England). The puncture speed was 10 mm/s. Fruit firmness was taken as the mean of the two measurements in Newtons (N), which is equivalent to 1 kgf multiplied by 9.81.

Non-destructive measurement

Fruit firmness was measured non-destructively with a 'Kiwifirm', a small hand-held device developed by 'Industrial Research Limited', Auckland, New Zealand (refer to

Plate 2.2, page 42). The device is based on a known quantum of energy applied to the fruit surface through a small, non-penetrating tip; it contains an in-built processor which converts characteristics of the resulting collision to display a digital value which ranges from 0.0 to 9.9 and decreases as fruit soften (Hopkirk *et al.* 1996). Firmness was obtained as the mean of four measurements per fruit, taken on opposite sides of each fruit, around the equator. At the beginning of each day of measurement, a rubber sample was used for checking calibration of the device.

The 'Kiwifirm' scale is unitless, but a correlation curve between 'Kiwifirm' and the values of the destructive test described below (in N) was made from the samples taken in the experiment (Appendix III). The values of 5.4, 6.0, 7.0 and 8.0 in the 'Kiwifirm' scale were approximately equivalent to 10, 25, 55, and 80 N respectively.

2.2.4 Fruit Total Soluble Solids Content

Total soluble solids content (SSC, %) of kiwifruit equilibrated to 20 °C was determined using a hand held refractometer (0-20% 'Atago N-20', Atago Co., Ltd., Japan), calibrated at 20 °C to 0% with distilled water. Measurements were made by mixing a drop of juice from 15 mm thick slices taken from the two opposite ends (proximal & distal) of kiwifruit. The prism surface and the light plate were washed and dried with a clean soft tissue paper between each reading. Two readings were taken on each fruit and averaged.

2.3 Statistical Analysis

Statistical data analyses were conducted using SAS system (Anonymous 1990). Analysis of Variance (ANOVA) was done (General Linear Model Procedure), followed by Multiple Comparison Procedures. All measurements had equal sample size (balanced data), and Fisher's Least Significant Difference (LSD) was used to find out treatment differences at the 5% level of significance, i.e. $P<0.05$ indicates a significant difference (Ott 1993).

Data 'at harvest' and 'on removal' were analysed for each fruit variable considering the means of 15 fruit per treatment for each one of the 7 removal times shown in Table 2.1 (1 time at harvest, and 6 storage times).

Data from each of the 4 to 5 measurements (or sampling time) at 20 °C, immediately after harvest were analysed for each fruit variable considering the means of 15 fruit per treatment per sampling time.

In addition, data from each one of the 4 to 5 measurements (or sampling times) at 20 °C (which represent the means of 15 fruit per treatment), for each one of the 7 removal times (shown in Table 2.1) were analysed considering the average of all 4 or 5 sampling times per variable per removal time. The total fruit number averaged was then 75 (when fruit were measured 5 times at 20 °C, immediately after harvest, and after 14, 30, and 52 days at 0 °C), or 60 (when fruit were measured 4 times at 20 °C, after 80, 100, and 180 days at 0 °C). All data shown in this thesis in which this procedure was used is referred as 'pooled data'.

Treatment differences are indicated by letters following mean values on tables, and by a vertical LSD line of the overall data on graphs. Residual analysis was also made, and when required, data were appropriately transformed to validate the basic assumptions for analysis of variance (Fernandez 1992).

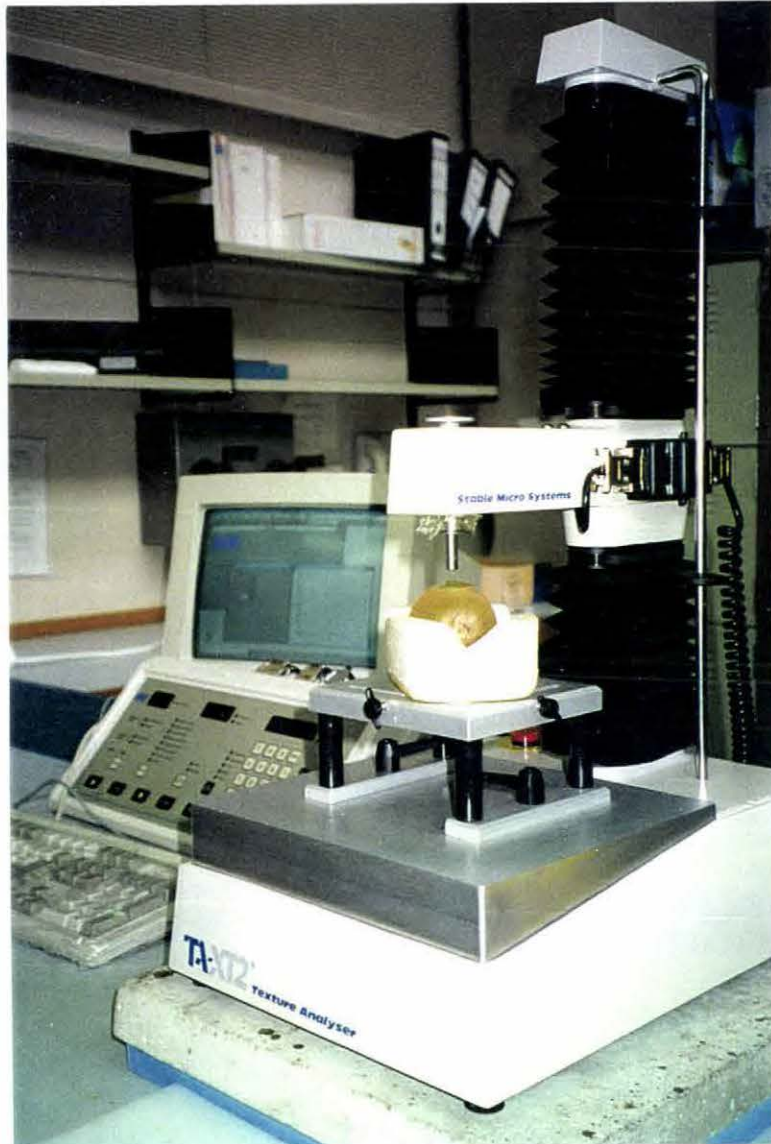

Plate 2.1 – The 'Texture Analyser' fitted with a 7.9 mm diameter probe, material testing machine used for measuring fruit firmness destructively.

Plate 2.2 – The 'Kiwifirm', device used for measuring fruit firmness non-destructively.

3 RESULTS

3.1 Fruit Respiration (CO_2 Production)

Immediately after harvest, carbon dioxide (CO_2) production at 20 °C of fruit from treatment 500-AVG-4 decreased steadily for 9 days, then remained steady through 13 days (Figure 3.1). Fruit from other treatments had a shorter period of decrease, followed by a rise (similar to a climacteric). Treatment 1000-AVG-4 had a rise after 6 days, with a peak after 9 days and then a decrease after 13 days. Treatments 500-AVG-6 and control had a rise after 3 days, with a peak after 9 days and a decrease after 13 days.

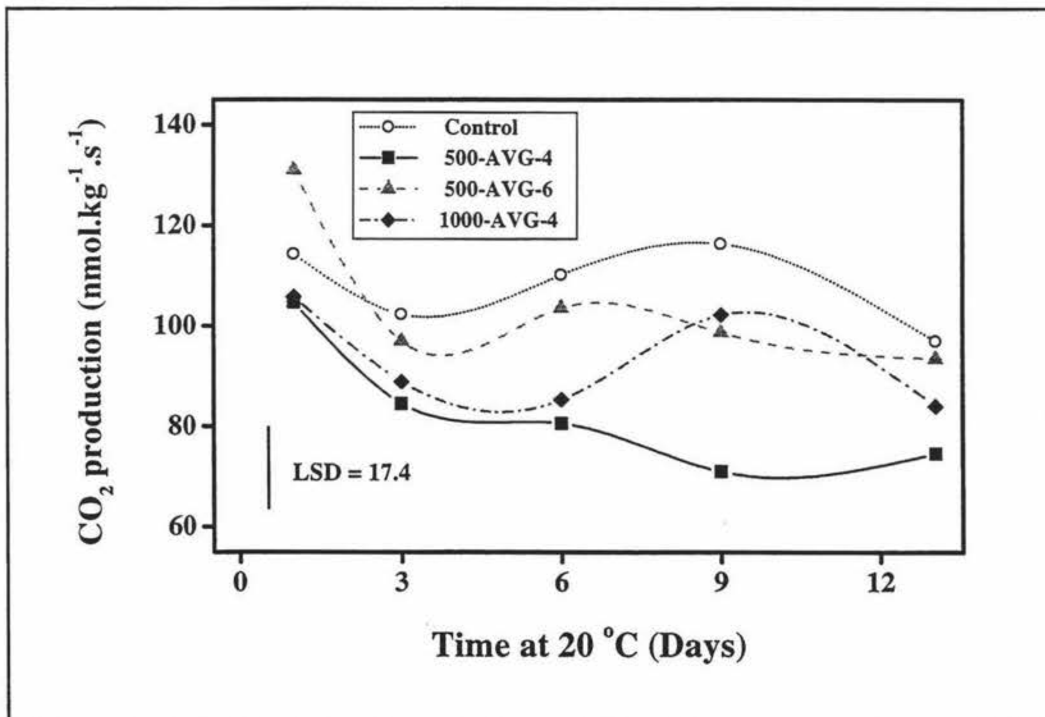

Figure 3.1 – CO_2 production (nmol.kg^{-1}.s^{-1}) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference).

There was no difference in CO_2 production between treatments after 1 or 3 days at 20 °C, but after 6, 9, or 13 days, CO_2 production of fruit from treatment 500-AVG-4 was lower than control fruit (Figure 3.1). Fruit from treatment 1000-AVG-4 had lower CO_2 production than control fruit only after 6 days, while fruit from treatment 500-AVG-6 had the same CO_2 production as control fruit (Figure 3.1).

There was no difference in CO_2 production between treatments following removal to 20 °C after 14, 30, 52, 80, or 110 days at 0 °C (Table 3.1), indicating that AVG did not affect fruit respiration at 0 °C. After 180 days at 0 °C, CO_2 production of fruit from treatment 500-AVG-4 was higher than control fruit (Table 3.1).

Table 3.1 – CO_2 production ($nmol.kg^{-1}.s^{-1}$) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time.

Time at 0 °C	CO_2 production ($nmol.kg^{-1}.s^{-1}$)				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	114 a	105 a	131 a	106 a	29.7
14	200 a	199 a	211 a	207 a	19.7
30	170 a	168 a	162 a	176 a	37.5
52	163 a	173 a	160 a	157 a	21.0
80	155 a	143 a	149 a	157 a	15.8
110	217 a	245 a	229 a	220 a	35.6
180	262 b	342 a	285 ab	290 ab	65.9

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

CO_2 production on removal to 20 °C following storage at 0 °C was consistently higher than immediately after harvest for all treatments (Table 3.1). Mean CO_2 production from all treatments was significantly affected by time at 0 °C (Figure 3.2). Following storage at 0 °C, mean CO_2 production was between 32 to 158% higher than immediately after harvest.

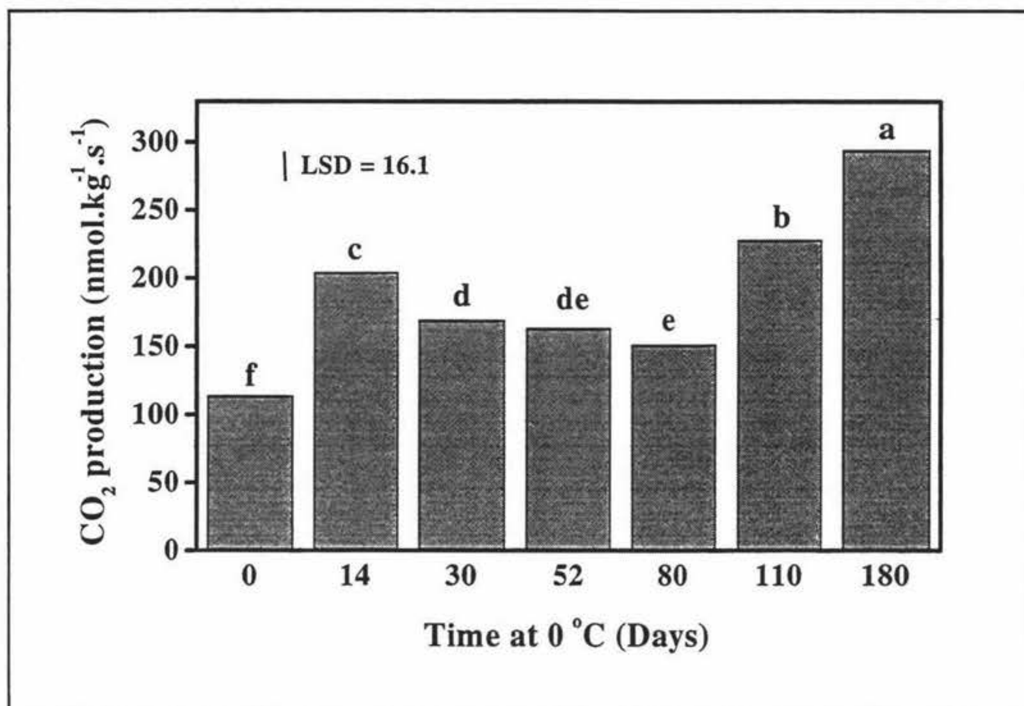

Figure 3.2 – CO_2 production (nmol.kg^{-1}.s^{-1}) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means from all treatments of 60 fruit per removal time. Bar represents overall LSD (least significant difference). Means with the same letter are not significantly different.

Average CO_2 production up to 20 days at 20 °C (pooled data, refer to section 2.3) of fruit from treatment 500-AVG-4 was lower than control fruit immediately after harvest, and after 14 days at 0 °C (Table 3.2). Fruit from treatments 500-AVG-6 and 1000-AVG-4 had the same CO_2 production as control fruit at harvest, but had lower CO_2 production than control fruit after 14 days at 0 °C. There was no difference in CO_2 production between treatments after 30, 52, or 80 days at 0 °C (Table 3.2). After 110 or 180 days at 0 °C, fruit from treatment 500-AVG-4 had higher CO_2 production than control fruit, as did fruit from treatment 1000-AVG-4 after 110 days at 0 °C, which was the opposite effect from that found at harvest or after 14 days at 0 °C (Table 3.2).

Table 3.2 – CO_2 production ($nmol.kg^{-1}.s^{-1}$) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data).

Time at 0 °C	CO2 production ($nmol.kg^{-1}.s^{-1}$)				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	108 a	83 b	105 a	93 ab	17.4
14	138 a	119 b	128 b	124 b	9.5
30	115 a	115 a	111 a	115 a	8.7
52	150 a	151 a	152 a	141 a	13.7
80	160 a	168 a	151 a	165 a	19.7
110	209 b	243 a	226 ab	236 a	17.6
180	247 c	277 a	254 bc	271 ab	21.9

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

The opposed results of AVG-treated fruit observed between the beginning and the end of the storage were illustrated by plot of CO_2 production over time at 20 °C, comparing data from treatments 500-AVG-4 and control after 14 days at 0 °C with those after 180 days at 0 °C (Figure 3.3). After 14 days at 0 °C, CO_2 production of fruit from both treatments decreased after 4 days at 20 °C, increased to a maximum after 10 days, and decreased after 15 days. CO_2 production of AVG-treated fruit was 20-28% lower than control fruit after 7 and 10 days at 20 °C. However, after 180 days, CO_2 production of AVG-treated fruit was 31% higher than control fruit after 1 day at 20 °C, but decreased to control levels after 3 days, and then remained relatively constant for both treatments after 6 and 9 days (Figure 3.3). CO_2 production after 180 days at 0 °C was consistently higher than after 14 days at 0 °C for both treatments.

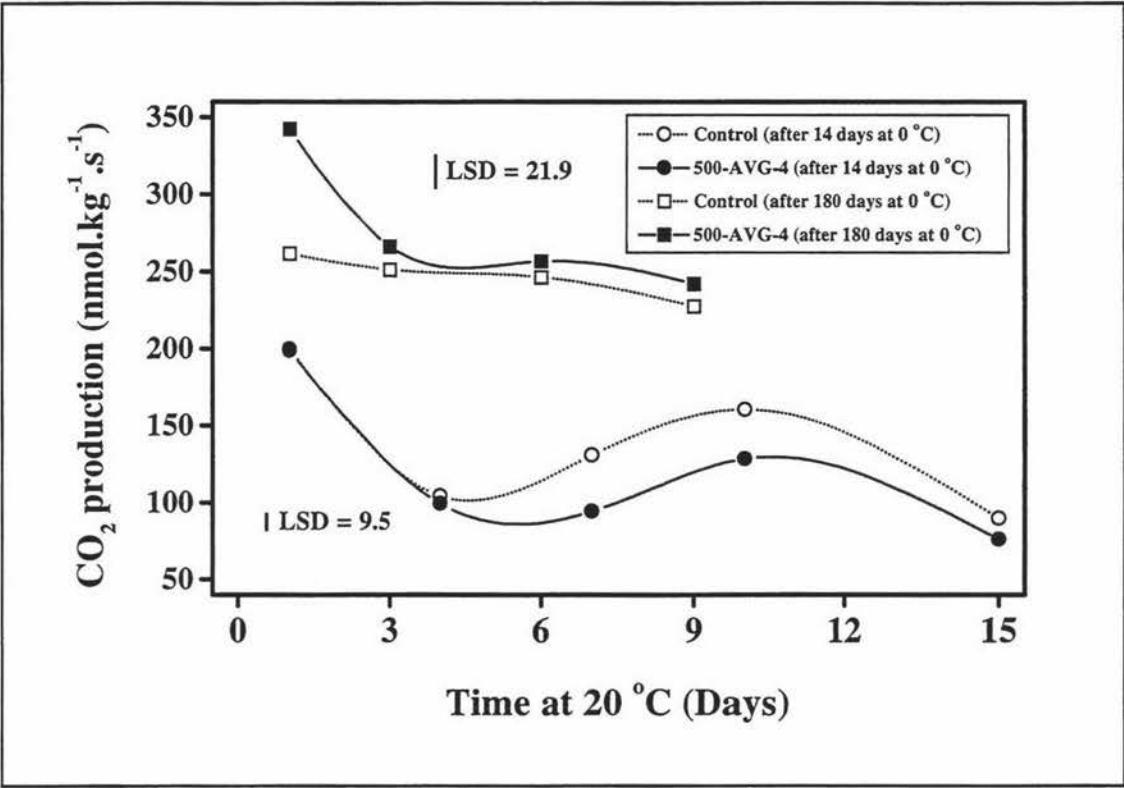

Figure 3.3 - CO_2 production (nmol.kg^{-1}.s^{-1}) of kiwifruit at 20 °C for up to 15 days, after 14 and 180 days at 0 °C. Means of 15 fruit per treatment per sampling time. Bars represent overall LSD (least significant difference).

3.2 Fruit Ethylene Production

Immediately after harvest, ethylene production at 20 °C of fruit from treatments 500-AVG-4 and 1000-AVG-4 remained below 1 pmol.kg^{-1}.s^{-1} throughout 13 days (Figure 3.4). Ethylene production of fruit from treatments 500-AVG-6 and control increased after 3 days at 20 °C, reaching peak values above 100 pmol.kg^{-1}.s^{-1} after 9 days at 20 °C (Figure 3.4). This rise in ethylene production for these 2 latter treatments basically coincided with the rise in CO_2 production (refer to Figure 3.1).

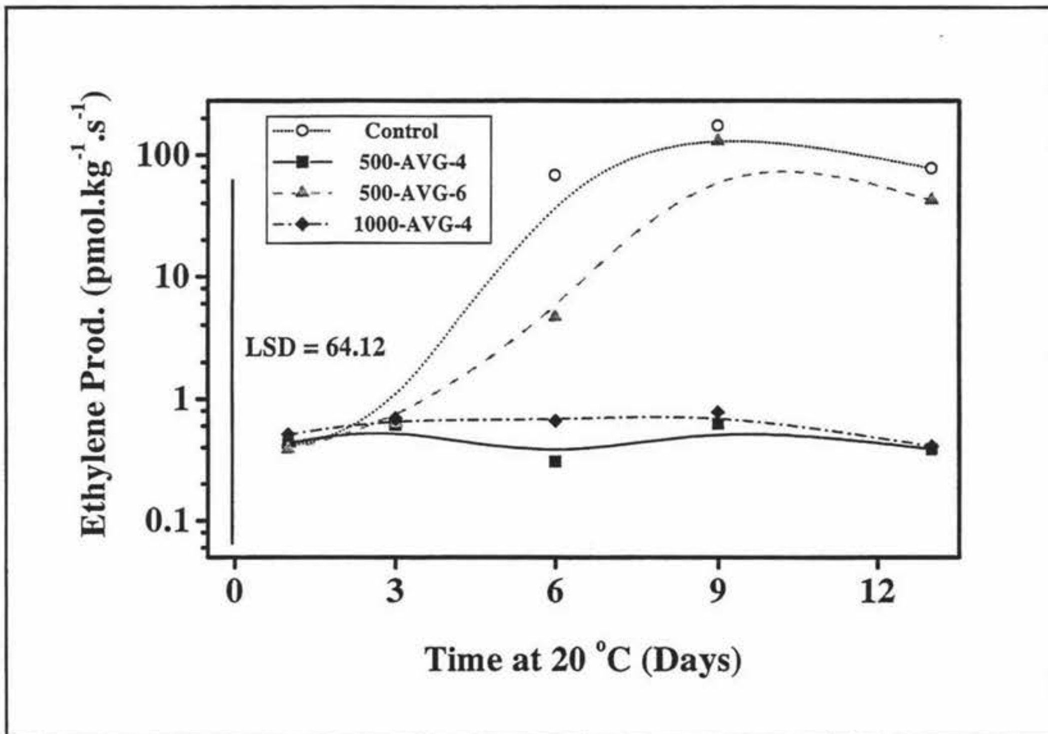

Figure 3.4 – Ethylene production (pmol.kg^{-1}.s^{-1}) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Values plotted on logarithmic scale. Bar represents overall LSD (least significant difference).

However, the large fruit to fruit variation in ethylene production resulted in very high LSD (least significant difference) values, and the differences between treatments were not significant (Figure 3.4). This occurs because normally the time when fruit start

producing autocatalytic ethylene, which results in very high amounts of ethylene production, varies according to factors such as fruit maturity (refer to sections 1.5.2 and 1.5.3). As an example, considering control fruit used in this experiment after 9 days at 20 °C, 11 out of 15 fruit produced rates of ethylene from 0.35 to 0.64 $pmol.kg^{-1}.s^{-1}$, while the other 4 fruit produced from 705.97 to 1099.75 $pmol.kg^{-1}.s^{-1}$.

Because of this large fruit to fruit variation, the number of fruit in each sampling time after harvest (out of a total of 15 fruit per treatment) that exceeded a threshold level of 4.5 $pmol.kg^{-1}.s^{-1}$ (or about 0.1 $\mu l.l^{-1}$) ethylene production were plotted over time at 20 °C (Figure 3.5). No fruit from treatment 500-AVG-4, exceeded the threshold throughout 13 days at 20 °C, and only 1 fruit from treatment 1000-AVG-4 did so after 6 days at 0 °C. Two, 4, and 3 control fruit exceeded the threshold after 6, 9, and 13 days at 20 °C, respectively, as did 1, 3, and 2 fruit from treatment 500-AVG-6 (Figure 3.5). These results indicate some AVG inhibition on ethylene production at 20 °C for up to 13 days immediately after harvest.

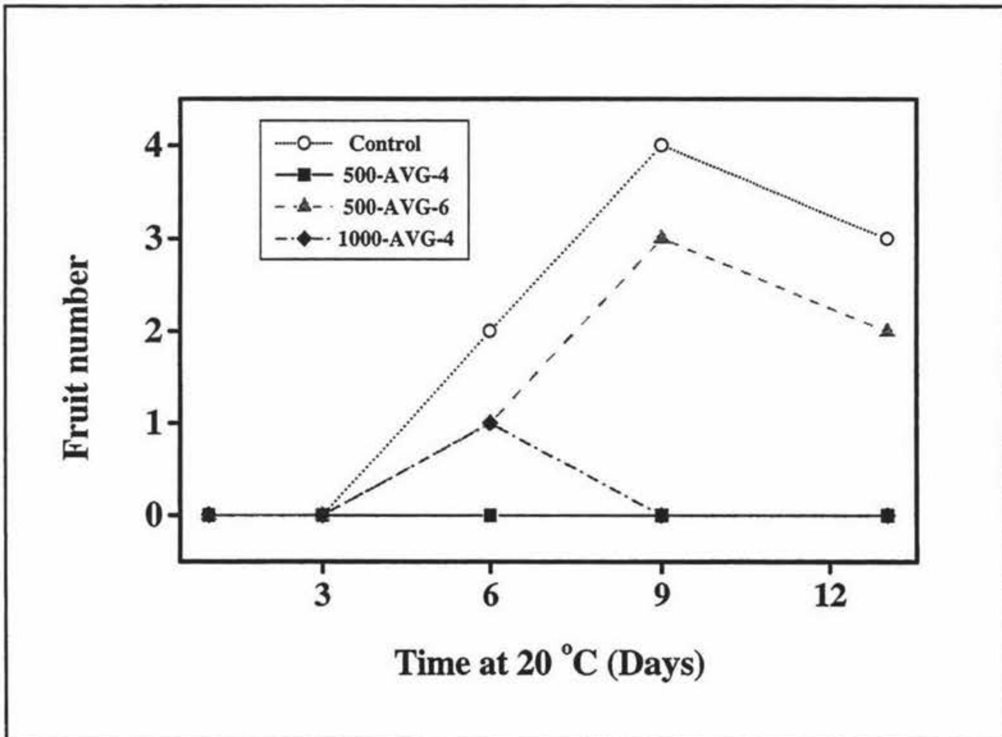

Figure 3.5 – Number of fruit that exceeded 0.1 $\mu l.l^{-1}$ ethylene production per sampling time during 13 days at 20 °C immediately after harvest. Total of 15 fruit per treatment per sampling time.

Ethylene production following removal to 20 °C after up to 180 days at 0 °C, remained below 1 $pmol.kg^{-1}.s^{-1}$ for all treatments except 500-AVG-6 after 30 days (Table 3.3). Ethylene production of fruit from treatment 500-AVG-4 was slightly higher than control fruit after 14, 80, and 180 days at 0 °C. Ethylene production of fruit from treatment 1000-AVG-4 was also slightly higher than control fruit after 80 and 180 days at 0 °C, as was ethylene production of fruit from treatment 500-AVG-6 after 180 days at 0 °C (Table 3.3). There was no difference in ethylene production between treatments on removal to 20 °C immediately after harvest, or after 30, 52, and 110 days at 0 °C (Table 3.3).

Table 3.3 – Ethylene production ($pmol.kg^{-1}.s^{-1}$) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time.

Time at 0 °C (Days)	Ethylene production ($pmol.kg^{-1}.s^{-1}$)				LSD
	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	0.41 a	0.43 a	0.39 a	0.51 a	0.17
14	0.42 b	0.54 a	0.54 a	0.52 ba	0.11
30	0.47 a	0.54 a	1.18 a	0.63 a	0.87
52	0.60 a	0.55 a	0.48 a	0.55 a	0.20
80	0.55 c	0.65 ab	0.59 cb	0.68 a	0.09
110	0.73 a	0.94 a	0.70 a	0.77 a	0.31
180	0.53 b	0.70 a	0.68 a	0.76 a	0.11

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

There was no difference in ethylene production at 20 °C for up to 20 days (pooled data, refer to section 2.3) between treatments immediately after harvest, or after 14, 30, 52, 80, and 180 days at 0 °C (Table 3.4). After 110 days at 0 °C, ethylene production of fruit

from treatments 500-AVG-4 and 1000-AVG-4 was higher than control fruit. Basically, ethylene production at 20 °C increased with increased storage time at 0 °C for all treatments (Table 3.4).

Table 3.4 - Ethylene production (pmol.kg^{-1}.s^{-1}) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data).

Time at 0 °C	Ethylene production (pmol.kg^{-1}.s^{-1})				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	63.71 a	0.47 a	35.63 a	0.61 a	64.12
14	0.65 a	0.50 a	0.51 a	0.54 a	0.16
30	0.49 a	0.52 a	0.58 a	11.13 a	14.95
52	9.53 a	22.36 a	31.05 a	26.36 a	43.59
80	90.85 a	173.19 a	78.31 a	127.98 a	99.69
110	30.74 b	117.80 a	61.28 ab	100.66 a	58.14
180	72.01 a	136.05 ab	105.80 ab	190.93 a	86.75

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

Because of the high values of LSD in the data due to fruit to fruit variation (as seen in the results immediately after harvest), residual analysis of the ethylene data was made (refer to section 2.3). Such analysis indicated that log of the data was the most appropriate transformation. The log transformation of the raw data for ethylene production (the raw data used to generate the average values in Table 3.4) confirmed the treatment differences in Table 3.4, except for the data at harvest, in which ethylene production from treatment 500-AVG-4 (log transformed = -0.81 pmol.kg^{-1}.s^{-1}) was significantly lower than control fruit (log transformed = 0.13 pmol.kg^{-1}.s^{-1}).

The percentage of individual fruit sampled at each removal time that exceeded a threshold level of 4.5 $pmol.kg^{-1}.s^{-1}$ (or about 0.1 $\mu l.l^{-1}$) ethylene production was calculated (Figure 3.6). Immediately after harvest, fruit from treatments 500-AVG-4 and 1000-AVG-4 had lower ethylene production than fruit from treatments 500-AVG-6 and control, but this effect did not persist throughout cool storage. In fact, after 110 and 180 days at 0 °C, the percentage of fruit exceeding 4.5 $pmol.kg^{-1}.s^{-1}$ ethylene production increased markedly in all treatments, and ethylene production of fruit from treatments 500-AVG-4 and 1000-AVG-4 was consistently higher than treatments 500-AVG-6 and control.

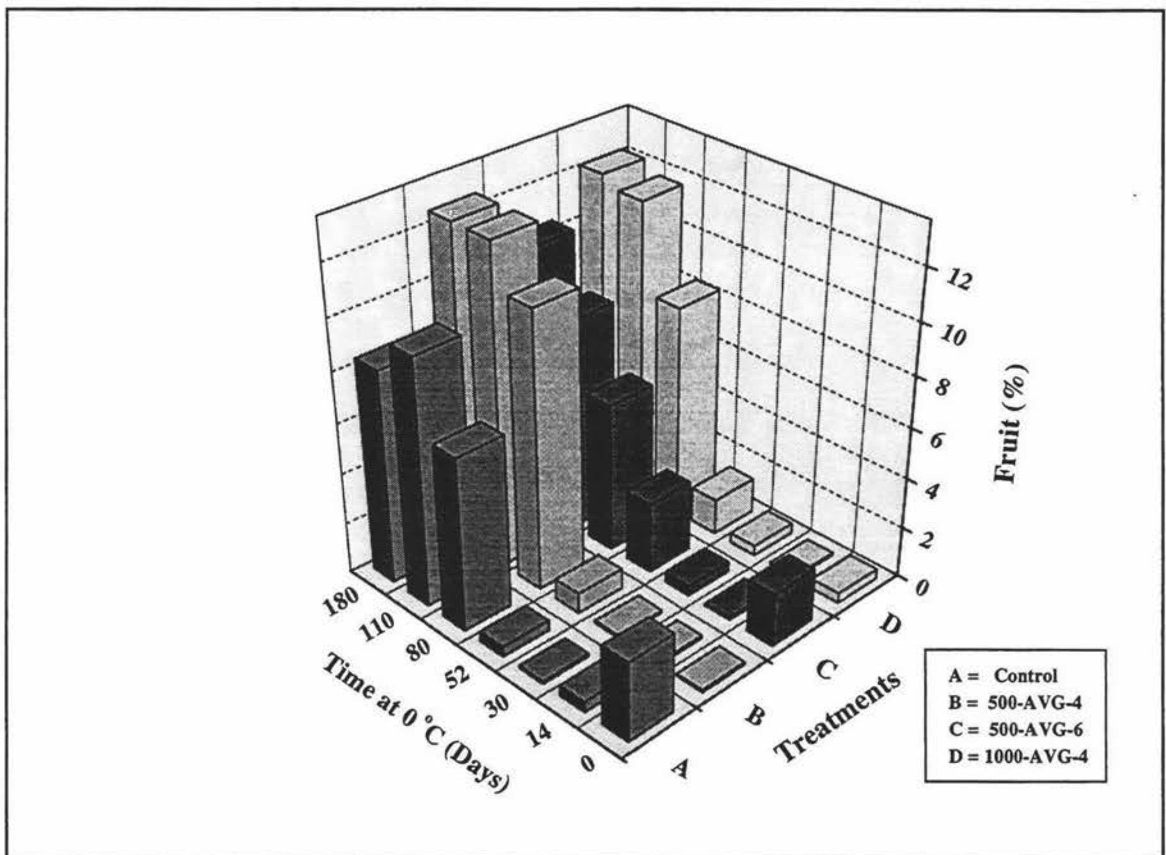

Figure 3.6 – Percent of fruit from each time of removal from 0 °C that exceeded 4.5 $pmol.kg^{-1}.s^{-1}$ (about 0.1 $\mu l.l^{-1}$) ethylene production of kiwifruit at 20 °C for up to 20 days (4 to 5 measurements), following storage for up to 180 at 0 °C. Total of 60 to 75 fruit per treatment per removal time.

3.3 Fruit Firmness

3.1 Destructive Firmness Measurement ('Texture Analyser')

Immediately after harvest, softening rates at 20 °C of fruit from treatments 500-AVG-4 and 1000-AVG-4 were slower than those of fruit from treatments 500-AVG-6 and control (Figure 3.7). Firmness of control fruit reduced steadily by 24 N through 9 days. Firmness of fruit from treatment 500-AVG-4 remained constant through 6 days and reduced by just 6 N after 9 days (Figure 3.7). There was no difference in firmness between treatments after 1, 3, and 13 days at 20 °C. After 6 and 9 days, fruit from treatments 500-AVG-4 and 1000-AVG-4 were firmer than fruit from treatments 500-AVG-6 and control (Figure 3.7).

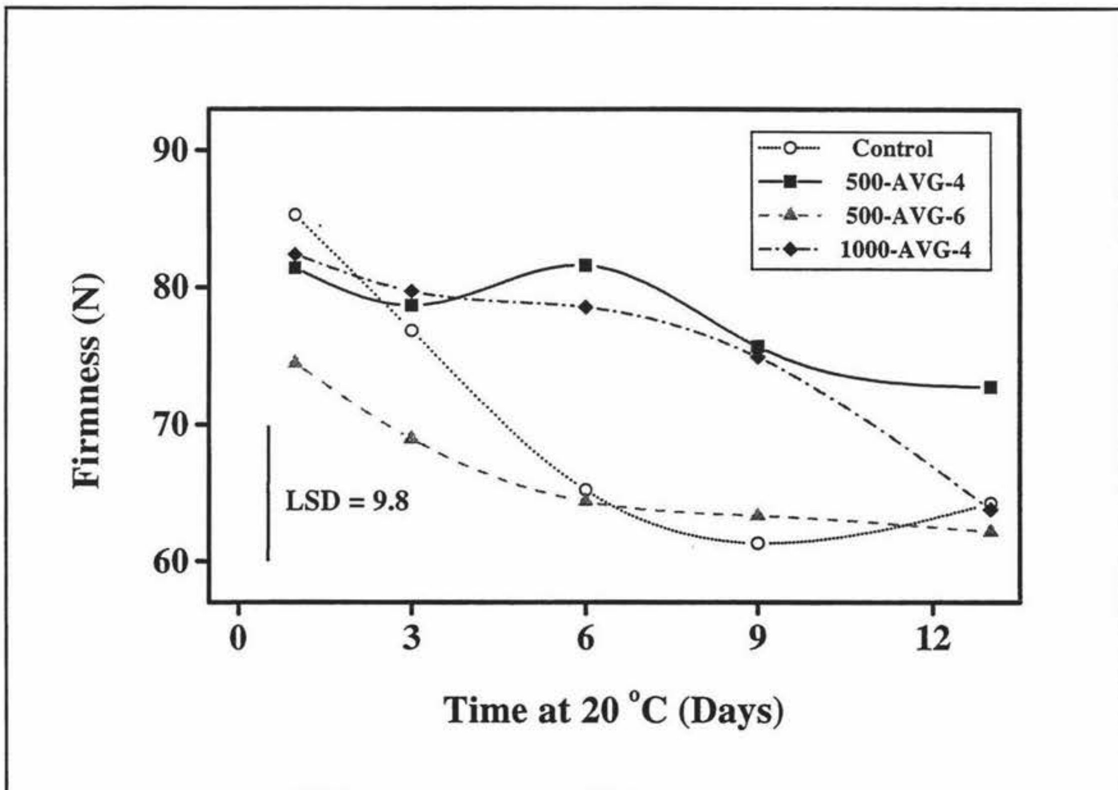

Figure 3.7 – Firmness (N) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference).

Fruit firmness at 0 °C decreased rapidly by more than 40 N throughout the first 52 days in all treatments, following removal to 20 °C after storage for up to 180 days at 0 °C (Figure 3.8). From 52 up to 110 days at 0 °C firmness decreased more slowly than the first 52 days, followed by the slowest decrease from 110 to 180 days (Figure 3.8). There was no difference in firmness between treatments following removal to 20 °C after 14, 80, 110, or 180 days at 0 °C (Figure 3.8). After 30 days at 0 °C, fruit from treatment 1000-AVG-4 were firmer than control fruit, while fruit from treatments 500-AVG-4 and 500-AVG-6 were firmer than control fruit after 52 days at 0 °C (Figure 3.8).

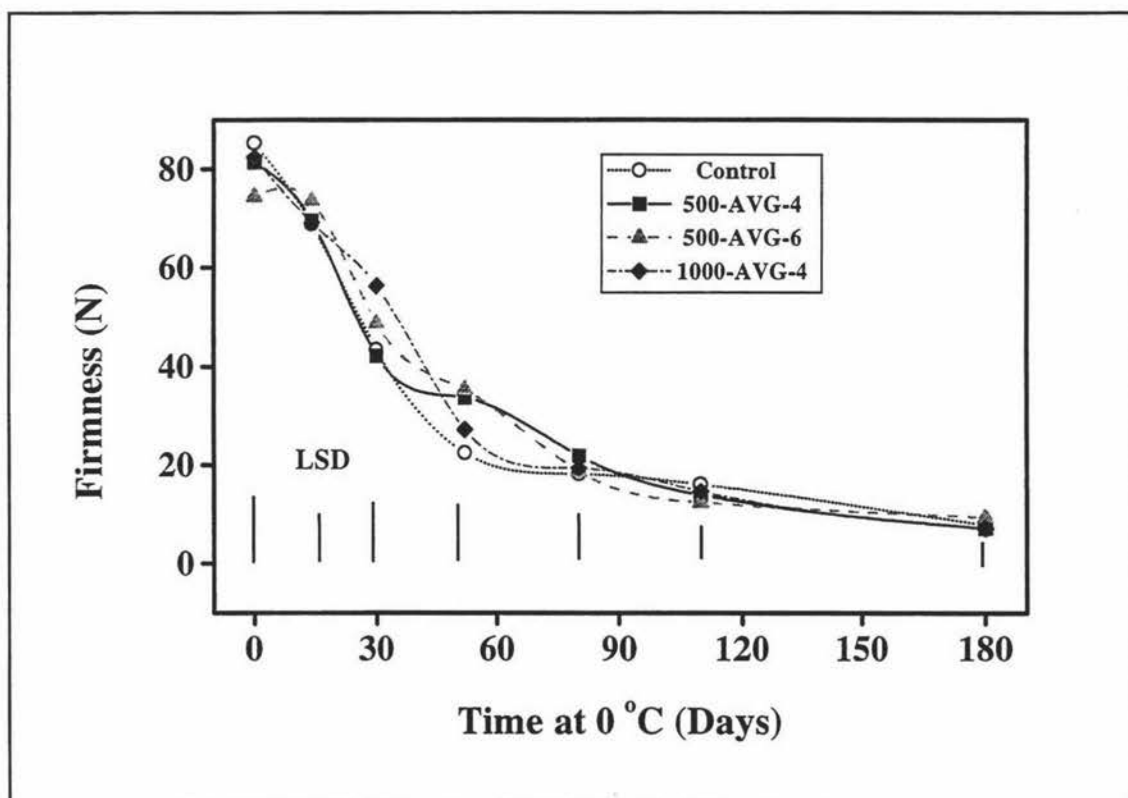

Figure 3.8 – Firmness (N) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time. Bars represent LSD (least significant difference) at each removal time.

Average firmness from all treatments on removal to 20 °C was significantly affected by time at 0 °C (Table 3.5). The average loss of fruit firmness throughout storage from all treatments increased from 0.76 N per day in the first 14 days at 0 °C, to a maximum of 1.41 N per day in the period between 14 and 30 days at 0 °C. From this period, loss of firmness decreased steadily to 0.82, 0.36, and 0.17 N per day after 52, 80, and 110 days at 0 °C respectively. From 110 to 180 days at 0 °C, loss of firmness was 0.09 N per day, the slowest rate during storage (Table 3.5).

Table 3.5 – Loss of firmness (%) of kiwifruit on removal to 20 °C following storage for up to 180 at 0 °C. Means of 60 fruit per removal time from all treatments. Different letters indicate significant difference between removal times.

Time at 0 °C (Days)	Firmness (N)	Firmness (%)	Firmness loss (N)	Firmness loss per day (N)
0	80.9 a	100	0	0
14	70.3 b	87	10.6	0.76
30	47.7 c	59	22.6	1.41
52	29.7 d	37	18	0.82
80	19.5 e	24	10.2	0.36
110	14.3 f	12	5.2	0.17
180	7.9 g	10	6.4	0.09

Firmness at 20 °C for up to 20 days (pooled data, refer to section 2.3) of fruit from all 3 AVG-treatments was higher than control fruit after 14 days at 0 °C (Table 3.6). Fruit from treatment 500-AVG-4 were firmer than control fruit after 52 days at 0 °C. After 110 days at 0 °C, fruit from all 3 AVG treatments were softer than control fruit. There were no differences in firmness between control and AVG-treated fruit immediately after harvest, or after 30, 80, and 180 days at 0 °C (Table 3.6).

Table 3.6 – Firmness (N) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data).

Time at 0 °C	Firmness (N)				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	70.6 ab	78.0 a	66.7 b	75.9 ab	9.8
14	56.9 b	63.8 a	65.7 a	65.6 a	6.8
30	37.6 a	38.7 a	41.9 a	41.7 a	5.4
52	19.0 b	22.7 a	18.9 b	19.5 ab	3.5
80	12.4 a	13.0 a	13.6 a	11.1 a	3.3
110	10.8 a	8.3 b	9.0 b	9.0 b	1.5
180	6.5 ab	5.8 b	7.3 a	6.1 b	0.9

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

The differences between treatments in firmness at 20 °C for up to 20 °C following storage after 14, 30, and 52 days at 0 °C decreased progressively as storage time increased (Figure 3.9).

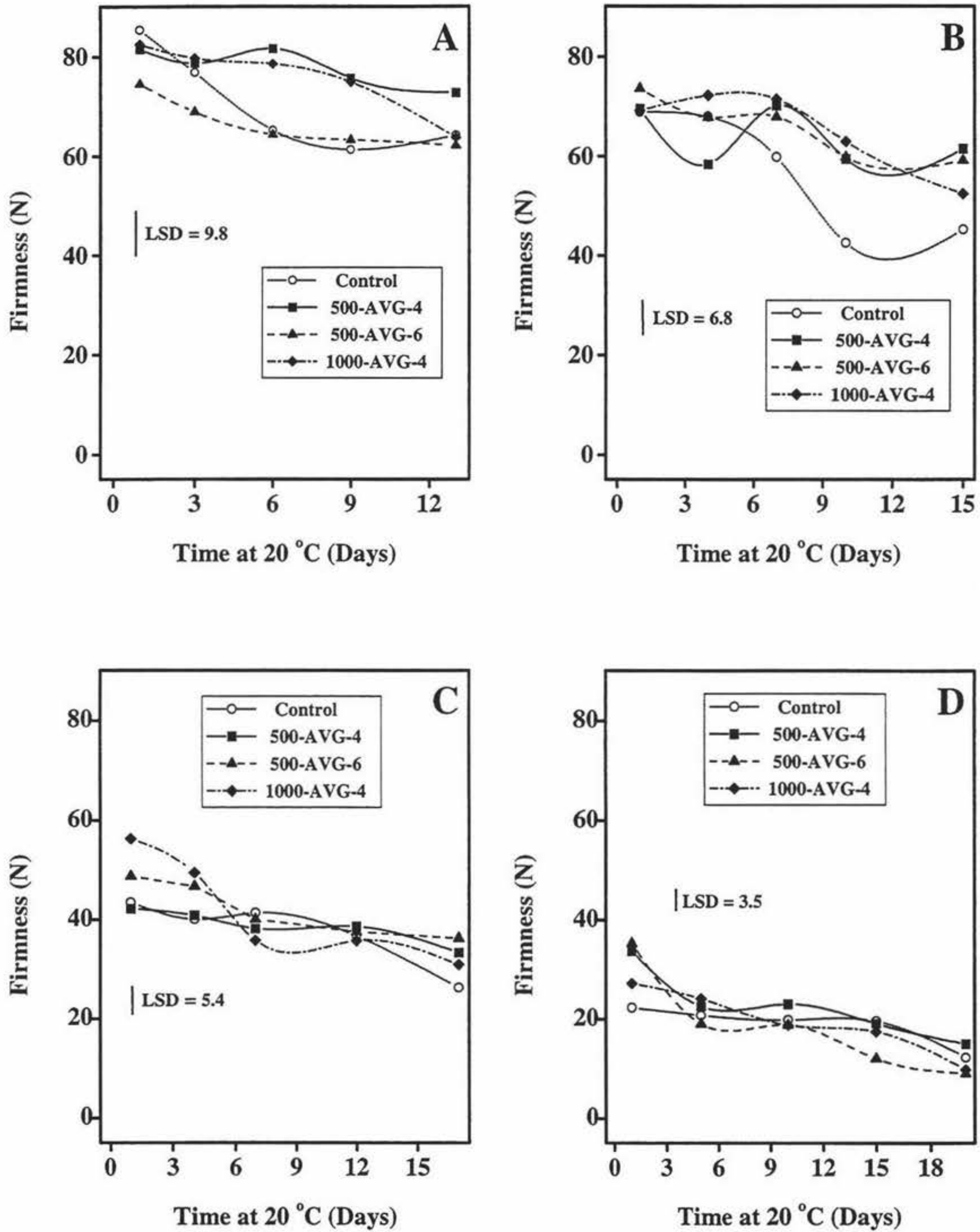

Figure 3.9 – Firmness (N) of kiwifruit at 20 °C for up to 20 days at harvest (A) or following storage at 0 °C for 14 (B), 30 (C), and 52 (D) days. Bar indicates overall LSD (least significant difference).

3.2 Non-destructive Firmness Measurement ('Kiwifirm')

Immediately after harvest, softening rates at 20 °C of fruit from treatments 500-AVG-4 and 1000-AVG-4 were slower than those of fruit from treatments 500-AVG-6 and control as measured with the 'Kiwifirm' (Figure 3.10), which is consistent with the results obtained with the 'Texture Analyser' (refer to Figure 3.7). Firmness of fruit from treatments control and 500-AVG-6 decreased steadily through 13 days at 20 °C. Firmness of fruit from treatment 500-AVG-4 remained relatively constant through 3 days, decreased through 9 days, then remained constant until 13 days (Figure 3.10). There was no difference in firmness between treatments up to 3 days at 20 °C. From 6 to 13 days, fruit from treatments 500-AVG-4 and 1000-AVG-4 were firmer than fruit from treatments 500-AVG-6 and control (Figure 3.10).

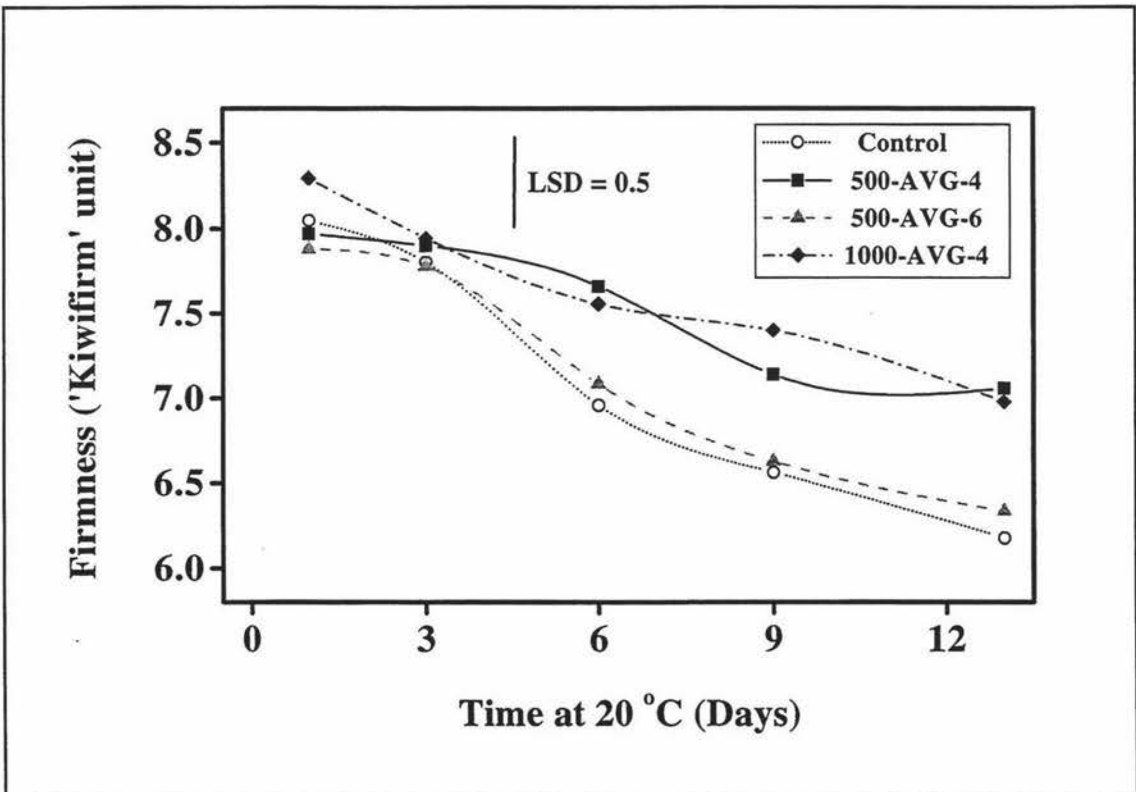

Figure 3.10 – Firmness ('Kiwifirm' unit) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference).

There was no difference in firmness between treatments following removal to 20 °C up to 180 days at 0 °C (Table 3.8), indicating that AVG did not affect fruit firmness measured with the 'Kiwifirm' throughout storage at 0 °C.

Table 3.7 – Firmness ('Kiwifirm' unit) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time.

Time at 0 °C	Firmness ('Kiwifirm' unit)				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	8.1 a	8.0 a	7.9 a	8.3 a	0.5
14	7.6 a	7.8 a	7.8 a	7.7 a	0.5
30	7.2 a	7.2 a	7.0 a	7.3 a	0.5
52	6.4 a	7.0 a	7.0 a	6.8 a	0.7
80	6.4 a	6.8 a	6.5 a	6.5 a	0.8
110	6.3 a	6.3 a	6.4 a	6.3 a	0.8
180	4.8 ab	4.5 b	5.4 a	4.8 ab	0.7

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

Firmness ('Kiwifirm' unit) at 20 °C for up to 20 days (pooled data, refer to section 2.3) of fruit from treatments 500-AVG-6 and 1000-AVG-4 was higher than control fruit after 14 days at 0 °C (Table 3.8). There were no differences in firmness measured with the 'Kiwifirm' between treatments immediately after harvest, or after 30 and 80 days at 0 °C, and inconsistent differences after 52, 110, and 180 days (Table 3.8).

After 14 days at 0 °C, AVG-treated fruit were firmer than control fruit after 10 and 15 days at 20 °C, with no differences between treatments up to 7 days (Figure 3.11). This was because control fruit softened rapidly from 7.5 to 6.5 'Kiwifirm' units between 7 and 10 days.

Table 3.8 – Firmness ('Kiwifirm' unit) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data).

Time at 0 °C	Firmness ('Kiwifirm' unit)				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	7.1 a	7.6 ab	7.1 ab	7.6 a	0.5
14	7.1 b	7.3 ab	7.4 a	7.5 a	0.24
30	6.9 a	7.0 a	6.8 a	6.9 a	0.18
52	6.2 ab	6.5 a	6.1 b	6.3 ab	0.31
80	5.4 a	5.3 a	5.7 a	5.3 a	0.6
110	5.4 a	5.0 c	5.4 ab	5.1 bc	0.34
180	4.4 ab	4.2 b	4.7 a	4.4 ab	0.35

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

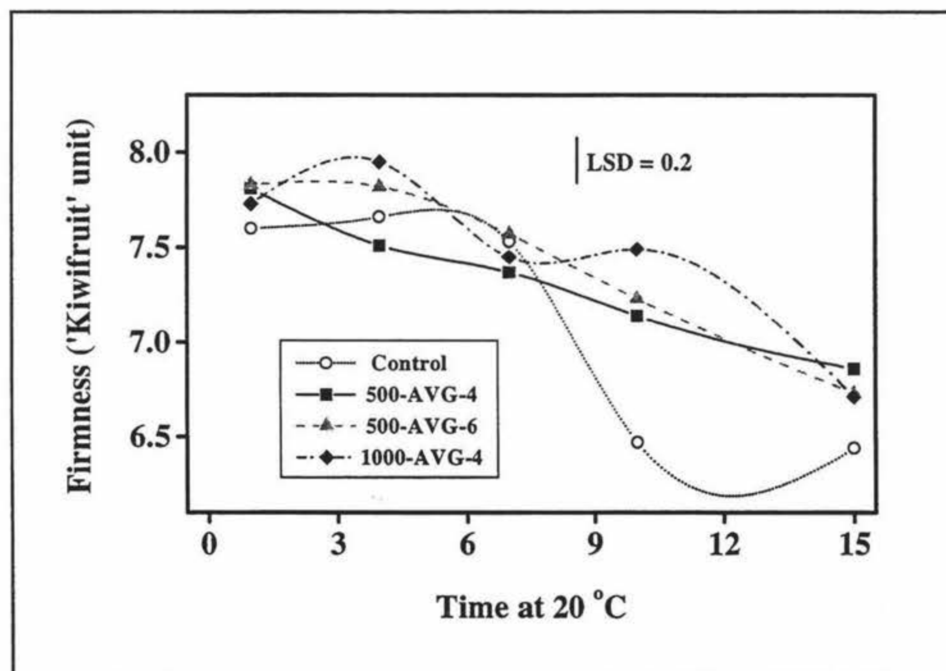

Figure 3.11 – Firmness ('Kiwifirm' unit) of kiwifuit during 15 days at 20 °C after 14 days storage at 0 °C. Means of 15 fruit per treatment per sampling time.

3.4 Fruit Total Soluble Solids Content

Immediately after harvest, soluble solids content (SSC) increased steadily to a maximum of 10.6% through 13 days at 20 °C in fruit from treatments control and 500-AVG-6 (Figure 3.12). In contrast, there was a lag of at least 6 days before SSC increased in fruit from treatments 500-AVG-4 and 1000-AVG-4, with SSC in fruit from these two treatments being lower than control and 500-AVG-6 treatments, especially after 6 and 9 days at 0 °C (Figure 3.12). SSC in fruit from treatment 500-AVG-4 was also higher than control after 13 days at 20 °C (Figure 3.12).

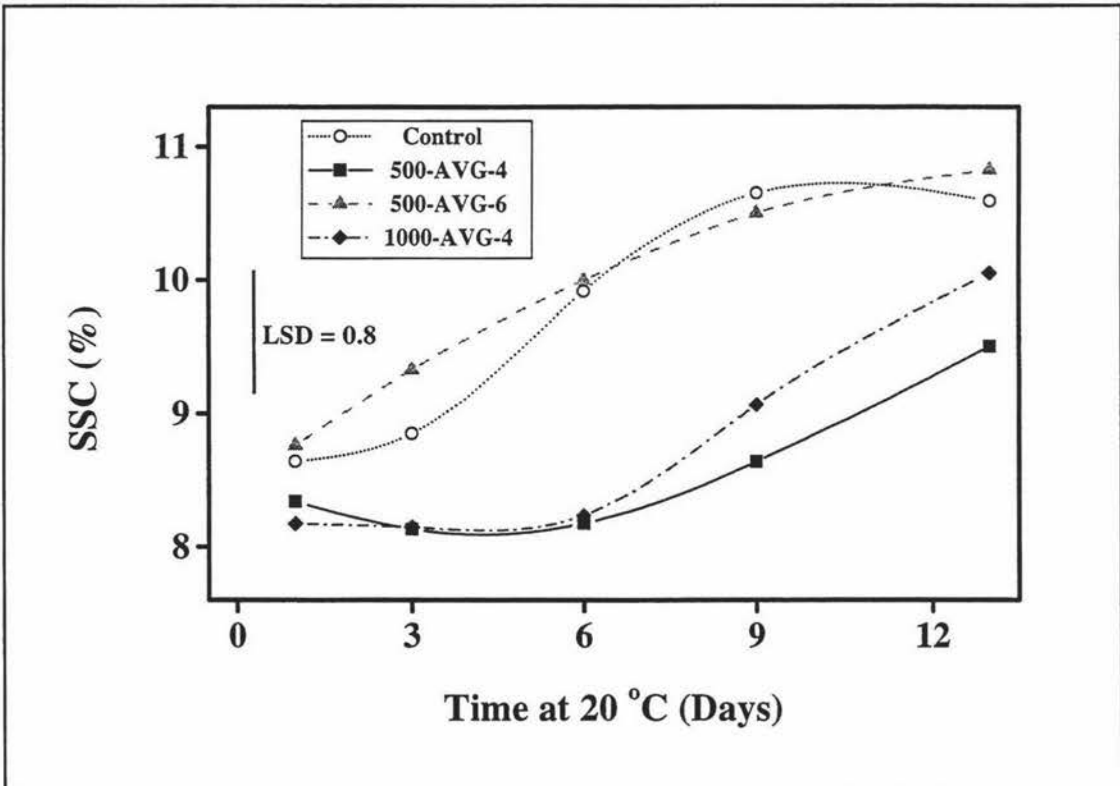

Figure 3.12 - Soluble solids content (%) of kiwifruit during 13 days at 20 °C immediately after harvest. Means of 15 fruit per treatment per sampling time. Bar represents overall LSD (least significant difference).

There was no difference in SSC between treatments following removal to 20 °C after 14, 30, 80, 110, or 180 days at 0 °C (Table 3.9), indicating that AVG did not affect SSC at 0 °C. After 52 days at 0 °C, SSC of fruit from treatment 500-AVG-4 was lower than control fruit (Table 3.9).

Table 3.9 – Soluble solids content (%) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means of 15 fruit per treatment per removal time.

Time at 0 °C	Soluble solids content (%)				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	8.6 a	8.3 a	8.8 a	8.2 a	1.5
14	11.8 a	11.3 a	11.5 a	11.3 a	0.7
30	13.2 a	12.9 a	13.0 a	12.2 a	1.1
52	14.7 a	13.8 b	13.9 ab	14.4 ab	0.8
80	14.7 a	14.3 a	14.8 a	14.0 a	0.9
110	14.7 a	14.1 a	14.7 a	13.7 a	1.1
180	14.3 a	14.3 a	14.2 a	13.9 a	1.4

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

Mean SSC from all treatments on removal to 20 °C was significantly affected by time at 0 °C (Figure 3.13). As time at 0 °C increased from 0 to 52 days, SSC increased progressively by 70 %. After 52 days at 0 °C, SSC on removal remained constant at about 14.2% (Figure 3.13).

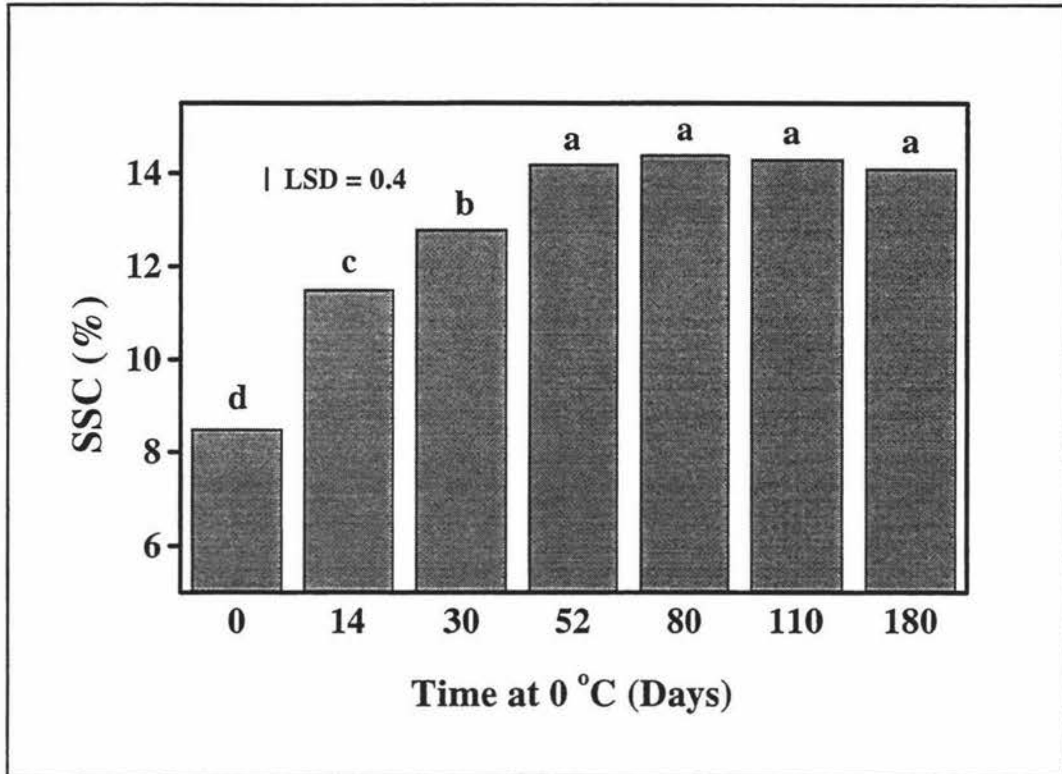

Figure 3.13 – SSC (%) of kiwifruit on removal to 20 °C following storage for up to 180 days at 0 °C. Means from all treatments of 60 fruit per removal time. Bar represents overall LSD (least significant difference). Means with the same letter are not significantly different.

SSC at 20 °C for up to 20 days (pooled data, refer to section 2.3) of fruit from treatments 500-AVG-4 and 1000-AVG-4 was lower than control fruit immediately after harvest, and after 14 and 52 days at 0 °C (Table 3.10). SSC of fruit from treatment 1000-AVG-4 was also lower after 80 and 110 days at 0 °C. There was usually no difference in SSC of fruit from treatment 500-AVG-6, except after 14 and 80 days at 0 °C, in which results were opposed. There were no differences in SSC between treatments after 30 and 180 days at 0 °C (Table 3.10).

Table 3.10 – Soluble solids content (%) of kiwifruit. Average values of 4 to 5 measurements at 20 °C following storage for up to 180 days at 0 °C. Means of 60 to 75 fruit per treatment per removal time (pooled data).

Time at 0 °C	Soluble solids content (%)				LSD
(Days)	Control	500-AVG-4	500-AVG-6	1000-AVG-4	
0	9.7 a	8.6 b	9.9 a	8.7 b	0.9
14	12.7 a	11.8 b	11.9 b	11.8 b	0.5
30	13.6 a	13.3 ab	13.4 ab	13.2 ab	0.4
52	14.6 a	14.0 b	14.3 ab	14.0 b	0.4
80	14.7 b	14.4 bc	14.9 a	14.2 c	0.5
110	14.9 a	14.5 ab	14.9 a	14.2 b	0.6
180	14.6 a	14.3 a	14.5 a	14.1 a	0.5

(LSD = least significant difference; mean values within rows followed by the same letter are not significantly different at 5 % level).

4 DISCUSSION

Kiwifruit texture changes significantly during fruit ripening, with firmness being a key quality criteria (McDonald 1990). Premature softening is a serious commercial problem for the kiwifruit industry in New Zealand and may cost many millions of dollars annually (refer to section 1.3.4). Ethylene is involved in regulation of fruit ripening, including ethylene production, respiration and changes in texture, colour, aroma and flavour (Oetiker & Yang 1995). Kiwifruit is very sensitive to ethylene; concentrations as low as 0.01 $\mu l.l^{-1}$ have been shown to increase the rate of softening and to reduce storage potential (Jeffery & Banks 1996).

In ethylene biosynthesis, formation of the key ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) from S-adenosyl-L-methionine (SAM) is catalysed by the rate limiting enzyme ACC synthase (ACS), while ACC oxidase (ACO) catalyses the conversion of ACC to ethylene (Fluhr & Mattoo 1996). Preclimacteric ethylene production can be reduced by holding the fruits under non-inductive conditions (such as low temperature, low oxygen, and low pressure), or chemically by application of an ethylene biosynthesis inhibitor such as aminoethoxyvinylglycine (AVG) or aminooxyacetic acid (AOA) (Oetiker & Yang 1995). AVG inhibits the activity of ACS, thus blocking ethylene biosynthesis in plant tissues (Boller *et al.* 1979). AVG has been applied to several horticultural crops including apples, pears, and cut flowers, in an attempt to regulate ethylene synthesis and its mediated processes, with a number of positive effects including reduced fruit ethylene production, reduced respiration rate, and slower softening rate. Another ACS inhibitor (coded 'Lab 181 508') applied to kiwifruit showed similar results (Retamales *et al.* 1995). This project characterised the effects of AVG on ripening of kiwifruit at harvest and after coolstorage.

4.1 General Results

Immediately after harvest there were no differences between treatments in fruit respiration rate (carbon dioxide production), ethylene production, firmness, and soluble solids content (SSC) at 20 °C (refer to Figures 3.1, 3.4, 3.7, 3.11, and 3.13). Since these variables usually change during fruit maturation, it appeared that AVG had little or no effect on the rate at which kiwifruit matured on the vine during the period of 4 to 6 weeks before commercial harvest.

The present results confirm previous trials with kiwifruit (Kim *et al.* 1997), in which 250 or 500 $mg.l^{-1}$ AVG, sprayed either 2 or 4 weeks before harvest, had no effect on fruit ethylene production and firmness immediately after harvest. These results are also consistent with those found in apples for firmness and SSC at harvest, in which there were no differences between fruit from trees sprayed with AVG in similar conditions (500 $mg.l^{-1}$ AVG, about 4 weeks before harvest) and control fruit immediately after harvest (Autio & Bramlage 1982; Bangerth 1978; Bramlage *et al.* 1980; Child e*t al.* 1984; Williams 1980). However, internal ethylene concentration (IEC) was reduced immediately after harvest following AVG treatment (Autio & Bramlage 1982; Bangerth 1978). Likewise, ethylene production was also reduced (Child *et al.* 1984). Some of the possible reasons for these differences in ethylene production between apple and kiwifruit immediately after harvest are considered later.

Kiwifruit treated with either 500 or 1000 $mg.l^{-1}$ AVG 4 weeks before commercial harvest and maintained during 13 days at 20 °C after harvest, had reduced carbon dioxide (CO_2) and ethylene production, a slower softening rate, and lower SSC compared with control fruit (refer to Figures 3.1, 3.4, 3.7, 3.11, and 3.13). Differences were generally significant after 6 days at 20 °C. These results suggest that, although AVG apparently did not affect fruit maturation on the vine, it did affect ripening of harvested fruit at 20 °C, thus confirming previous results with kiwifruit (Kim *et al.* 1997) in which fruit from vines sprayed with 500 $mg.l^{-1}$ AVG 4 weeks before harvest had reduced fruit ethylene production and had a slower softening rate than control fruit during 21 days at 20 °C immediately after harvest. These results are also consistent with

those found in apples for respiration rate, ethylene production, and firmness (Autio & Bramlage 1982; Bangerth 1978; Bramlage *et al.* 1980; Child *et al.* 1984; Williams 1980), but not for SSC where there were no differences between AVG-treated and control fruit (Autio & Bramlage 1982; Bramlage *et al.* 1980).

Overall, there were no consistent differences between treatments in fruit respiration rate, ethylene production, firmness and SSC following removal to 20 °C after storage for up to 180 days at 0 °C (refer to Tables 3.1, 3.3, 3.7, and 3.9, and Figure 3.8). This suggests that AVG did not affect the above fruit variables throughout storage at 0 °C.

In 'Golden Delicious' apples there were differences, especially in IEC and in firmness between AVG-treated and control fruit over 2 to 8 weeks at 3 °C (Bangerth 1978; Halder-Doll & Bangerth 1987). The fact that kiwifruit behave as a non-climacteric fruit, with low amounts of ethylene production when fruit are maintained below 10 °C (Sfakiotakis *et al.* 1997) might be the reason for the lack of response to AVG between treatments especially during coolstorage. The apparent lack of AVG response in kiwifruit in the first weeks of coolstorage could be related to differences in AVG uptake between kiwifruit and apple, which is considered later.

After 14 days at 0 °C, kiwifruit from treatments 500-AVG-4 and 1000-AVG-4 had lower CO_2 and ethylene production, slower softening rate, and lower SSC than control fruit over 15 days at 20 °C (refer to tables 3.2, 3.4, 3.6, 3.8, and 3.10). These results were similar to those at harvest with delayed ripening of AVG-treated fruit. However, there were basically no differences between treatments for the above fruit variables after 30, 52, or 80 days at 0 °C (refer to tables 3.2, 3.4, 3.6, 3.8, and 3.10); treated fruit appeared to have ripened almost as much as control fruit, suggesting that an AVG effect on delaying ripening was lost after 14 to 30 days at 0 °C.

These results were similar to those from some apple cultivars such as 'McIntosh' and 'Spencer' (Bramlage *et al.* 1980), but differ from others such as 'Delicious' (Williams 1980) and 'Golden Delicious' (Bangerth 1978; Bufler 1984; Halder-Doll & Bangerth 1987). The present results also differ from those found when an experimental compound named 'Lab 181 508', another ethylene biosynthesis inhibitor, was applied as a

postharvest dip in kiwifruit, resulting in reduced fruit ethylene production and slower softening rate at room temperature after 7 and 15 days, following up to 120 days at 0 °C (Retamales *et al.* 1995). Differences in the method used to apply AVG to fruit and the application time, resulting in probable differences in AVG uptake, does not allow a direct comparison between both experiments.

Since AVG is less effective in inhibiting ethylene synthesis at lower than at higher temperatures (Matoo *et al.* 1977), endogenous triggering of ripening at 0 °C is a possible reason for this lack of response to AVG following that time at 0 °C, which is discussed later. The possibility that exogenous ethylene concentration in the room triggered ripening of the AVG-treated fruit is negligible. The cool store room had ethylene scrubbing equipment. Ethylene concentrations in the room were periodically checked and kept in the very low range of 0.005 to 0.014 $\mu l.l^{-1}$ (which is close to the minimum detection limit of the equipment used to measure ethylene production). In addition, each tray had a sachet containing ethylene oxidiser on top of fruit enclosed by a 'polyliner', assuring that ethylene in each tray was minimal.

Following 110 and 180 days at 0 °C, fruit from treatments 500-AVG-4 and 1000-AVG-4 had higher CO_2 and ethylene production, and were softer than control fruit over 10 days at 20 °C. Such results where preharvest AVG treatments enhance fruit respiration and ethylene production have not been reported in the literature for other crops, and the effects of AVG on kiwifruit following long term storage condition have not been tested before. The reason for such effects are not known.

Overall, there were no differences in any of the fruit variables between treatment 500-AVG-6 and the control, either immediately after harvest or following storage at 0 °C up to 180 days. These results suggest that application time is an important factor influencing the effectiveness of AVG on kiwifruit.

Individual fruit variability throughout this experiment was considerable, especially in attributes such as ethylene production and SSC. One of the major reasons for this might be individual fruit maturity. The residuals from the raw data were plotted and checked, and no consistent patterns among blocks or treatments were found, confirming that the

variability was mainly due to mixed fruit maturity. Immediately after harvest, average SSC varied from 8.17 to 8.76% between treatments, but from 5.7% to 13.7% between individual fruit. A contributing factor for this large variation was possibly the fact that fruit were harvested from different position on the vine, which is known to affect fruit SSC (Hopkirk *et al.* 1986; Pyke *et al.* 1996). The limited number of vines available for the experiment restricted the possibility of harvesting from selected positions on the vines, which could have reduced such variation.

In this experiment, it was observed that the SSC means of AVG-treated kiwifruit had consistently lower standard deviation values than control fruit after 1, 3, 6, 9, and 13 days at 20 °C immediately after harvest. This could suggest less fruit to fruit variation in maturity of AVG-treated than in control fruit. It was also observed that the means of respiration rate, ethylene production, and firmness of AVG-treated fruit had lower standard deviation values than control fruit after 6, 9, and 13 days at 20 °C, suggesting that AVG could perhaps promote a more uniform ripening at 20 °C immediately after harvest. However, further investigation is required to access these observations, including statistical significance of any possible effect.

4.2 Low Temperature Effects in Fruit Physiology

CO_2 production on removal to 20 °C following storage at 0 °C for up to 180 days was consistently higher than immediately after harvest for all treatments (refer to Table 3.1). Ethylene production at 20 °C up to 20 days basically increased with increased time at 0 °C for all treatments (refer to Table 3.4). These results suggest an inductive effect of low temperatures on respiration rate and ethylene production in kiwifruit following transfer to higher temperatures, an effect (particularly with ethylene production) known to occur in apples and pears (Jobling *et al.* 1991; Knee *et al.* 1983). In these crops, cold acts by stimulating both ACC synthase (ACS) and ACC oxidase (ACO) (Gerasopoulos & Richardson 1997; Lelievre *et al.* 1995). In 'Passe-Crassane' pear, ACS gene expression is thought to be regulated by ethylene only during, or after chilling treatment, while ACO gene expression can be induced separately by either chilling or ethylene (Lelievre *et al.* 1997). In kiwifruit at 20 °C, ACS and ACO increased from 2.3 units.mg^{-1} and 0.2

$nl.g^{-1}.h^{-1}$ (respectively), immediately after harvest, to 45.7 $units.mg^{-1}$ and 51.9 $nl.g^{-1}.h^{-1}$ (respectively), following storage for 12 days at 0 °C (Sfakiotakis *et al.* 1997), which seems to confirm the same trend observed in apples and pears.

In kiwifruit, possible mechanisms for a similar response can be envisaged. Although autocatalytic ethylene production is inhibited in fruit at 0 °C (Sfakiotakis *et al.* 1989), with very low ethylene production, there is an accumulation of ACS and ACO (Sfakiotakis *et al.* 1997), possibly associated with a progressive increase in the sensitivity of fruit tissue to ethylene action. This could help to explain the burst of ethylene production upon removal to higher temperatures, with a trend for higher ethylene production rates the longer the fruit remained stored at low temperatures.

According to Mattoo *et al.* (1977), inhibition of ethylene biosynthesis by AVG in apple plugs is temperature-dependent. It seems reasonable to expect that AVG inhibition in kiwifruit would be similarly limited at low temperatures. Considering that ACS activity is stimulated when kiwifruit is stored at 0 °C (Sfakiotakis *et al.* 1997), it is possible to that the effects of AVG might be reduced in a low temperature environment. These could be strong reasons for the lack of response to AVG when fruit is stored at 0 °C for more than 30 days.

In some apple cultivars, there was an increase in brown core (which is a chilling related problem) in AVG-treated compared to control fruit, suggesting that AVG could possibly increase chilling sensitivity of these cultivars (Autio & Bramlage 1982; Bramlage *et al.* 1980). The fact that AVG-treated fruit showed higher CO_2 and ethylene production, and were softer than control fruit at 20 °C following 110 and 180 days at 0 °C, suggests that these effects could be related to an increased kiwifruit chilling sensitivity caused by AVG. It has been suggested that kiwifruit sustains chilling injury, which is manifested especially after long term cool storage (Lallu 1997). During the present research, it was observed that the flesh of many fruit measured after 180 days at 0 °C (when fruit skin was removed to measure firmness, or fruit were cut to measure SSC), tended to present a grainy texture, markedly different from the normal fruit texture. However, no attempt was made to access possible differences between AVG-treated and control fruit.

The reason for this higher sensitivity could be related to fruit maturation. Less mature apples are more susceptible to brown core than more mature ones (Bramlage *et al.* 1980). AVG-treated kiwifruit in the present experiment seemed to be less mature (as indicated by their lower SSC during 13 days at 20 °C after harvest) than control fruit (refer to Figure 3.12). It is possible that AVG kept fruit less mature and hence more sensitive to chilling than more mature fruit after long term coolstorage. It is known that more mature kiwifruit is usually softer at harvest, but firmer after a long term coolstorage than less mature fruit (MacRae *et al.* 1989b; Mitchell *et al.* 1992). In this experiment, control fruit were softer than AVG-treated fruit during 13 days at 20 °C after harvest (refer to Figure 3.7), but firmer during 10 days at 20 °C after 110 and 180 days at 0 °C (refer to Table 3.6), thus confirming the effect of AVG on delaying kiwifruit maturity after harvest.

4.3 Fruit Ethylene Production and Softening

It has been reported that ethylene induces kiwifruit softening at 21 °C (Hyodo et al. 1987) and at 0 °C (Arpaia et al. 1987; Retamales & Campos 1997). Pectin solubilisation and degradation is a major feature of cell wall changes occurring during ripening at both 0 °C and higher temperatures when kiwifruit is treated with ethylene immediately after harvest, suggesting a relationship between ethylene and kiwifruit softening (McRae & Redgwell 1992).

In this experiment, fruit firmness following removal from 0 °C to 20 °C, decreased substantially from about 85 N to 7 N immediately after harvest following 180 days at 0 °C in all treatments (refer to figure 3.8). In contrast, ethylene production of fruit in the same conditions remained at very low concentrations, generally below 1 $pmol.kg^{-1}.s^{-1}$, or about 0.087 $\mu l.kg^{-1}.h^{-1}$ (refer to Table 3.3). There was not a consistent relationship between ethylene production and softening rate at 20 °C up to 20 days of AVG-treated and control fruit (refer to Tables 3.4 and 3.6).

The reasons for this apparent lack of consistent relationship between ethylene production and softening are not known. As seen in section 1.4.3, such a relationship is

complex and is not well understood. Temperatures below 11 °C are shown to inhibit initiation of autocatalytic ethylene production in kiwifruit (induced by propylene, an ethylene analogue), but not ripening as measured by decrease in firmness and increase in SSC (Sfakiotakis *et al.* 1989), suggesting that ethylene biosynthesis and ripening in kiwifruit may be regulated by two independent mechanisms. Accordingly, the inductive effect of ethylene in fruit softening may not necessarily mean that the reduction of ethylene will result in inhibition of the softening process.

It is possible that even those small concentrations of ethylene produced by the kiwifruit in this experiment throughout storage were enough to trigger fruit ripening, including softening, as measured upon removal to 20 °C. It appears also that the inhibition of ethylene production caused by AVG in the conditions of this experiment was not enough to appreciably affect kiwifruit softening, especially during and immediately after storage at 0 °C for more than 14 days.

4.4 Use of AVG in Kiwifruit and Suggested Future Research

This research indicates that kiwifruit treated with either 500 or 1000 $mg.l^{-1}$ AVG 4 weeks before commercial harvest and maintained at 20 °C up to 15 days, had a lower respiration rate, reduced ethylene production, a slower softening rate, and lower SSC than control fruit after harvest and after 14 days at 0 °C. These attributes are generally stimulated by ethylene, indicating that the endogenously produced ethylene was inhibited by the applied AVG, resulting in a slower rate of fruit ripening at 20 °C. However, this effect was transitory; when fruit were stored at 0 °C for more than 14 days and up to 80 days, there were generally no differences in those fruit variables between AVG-treated and control fruit. AVG-treated fruit also tended to have less fruit to fruit variation in respiration, ethylene production, firmness, and SSC than control fruit over 15 days at 20 °C immediately after harvest and following 14 days at 0 °C.

Overall, results with kiwifruit appear less impressive than those found in apples. One reason for this might be that there are differences in the skin of both crops, including the presence of stiff hairs in kiwifruit. Skin resistance of kiwifruit to gas diffusion (22.3

$cm.h^{-1}.atm^{-1}$) is about 4 times higher than apple (5.6 $cm.h^{-1}.atm^{-1}$, for 'Granny Smith') (Banks *et al.* 1991), suggesting that the uptake of AVG in kiwifruit may not be as efficient as in apples. Differences in the trees between both crops could also result in variation in AVG uptake; for instance, spray may not reach fruit in certain positions on kiwifruit vine, particularly where there are large number of leaves next to the fruit. Such fruit would have a lower AVG uptake than fruit in open positions on the vine, assuming little or no AVG translocation from leaves to fruit.

Before drawing a firm conclusion about the potential use of AVG in kiwifruit, it will be necessary to carry out further investigation. Topics of future research should include a better evaluation of AVG uptake by using different methods to apply AVG (such as dips immediately after harvest) or use of different surfactants. Internal concentrations of AVG in fruit tissue should be measured and possible metabolic inactivation of AVG studied. Because of vine and fruit skin characteristics, spraying the chemical on fruit may not be the best way of applying AVG to kiwifruit. It is not known what impact factors such as different harvest date, fruit from different seasons, orchards, and locations have on AVG uptake and response, so these are issues to be considered as well. Furthermore, the possibility that AVG could affect the uniformity of fruit maturity and of fruit ripening at 20 °C immediately after harvest deserves further investigation, due to the beneficial implications in reducing fruit to fruit variability.

So far, the magnitude of AVG effects in terms of delaying kiwifruit ripening immediately after harvest and after a short term coolstorage, does not seem to offer strong appeal to possible commercial application of AVG. A more extended effect throughout coolstorage, which could contribute to the reduction of premature fruit softening, would be required. In addition, there is an increasing trend to reduce inputs and improve sustainability of food production (reflected in programs such as the 'KiwiGreen' in New Zealand). This would certainly not warrant any new input into the kiwifruit industry unless it brings very clear benefits in terms of crop production or quality, as well as being entirely safe for human consumption.

Kiwifruit is a crop very sensitive to exogenous ethylene (Arpaia et al. 1987; Pratt & Reid 1974), and is usually stored for long periods. As indicated in this experiment, AVG does seem to have a short term effect on kiwifruit quality. Accordingly, another area for future research is the use of bioregulators that could provide a more extended response, such as inhibitors of ethylene action (Sisler & Serek 1997). These appear to bind to the ethylene receptor and prevent the physiological action of ethylene for more extended periods.

5 REFERENCES

Abdala, A., Gerasopoulos, D., Stavroulakis, G. 1996: Effects of harvest maturity and storage on ripening and membrane permeability of Hayward kiwifruit. Advances in Horticultural Science 10: 3-7.

Abeles, F. B., Morgan, P. W., Saltveit, M. E. 1992: Ethylene in plant biology. 2nd Ed. San Diego, Academic Press. 414 p.

Adams, D. O., Yang, S. F. 1979: Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proceedings of the National Academy of Sciences of the United States of America 76: 170-174.

Anonymous 1990: SAS/STAT User's Guide, Version 6. 4th Ed., Vol. 1 and 2. Cary, SAS Institute Inc.

Anonymous 1996a: Industry focus 1996. Wellington, New Zealand Kiwifruit Marketing Board. 8 p.

Anonymous 1996b: NZKMB Field Manual 1996. Auckland, New Zealand Kiwifruit Marketing Board.

Anonymous 1997a: Agriculture. Pp 429-454 *in*: New Zealand Official Yearbook 1997. Wellington, GP Publications.

Anonymous 1997b: Annual report 1997. Auckland, New Zealand Kiwifruit Marketing Board. 44p.

Anonymous 1997c: Fruit research in New Zealand 1996. Wellington, Fruit Research Council of New Zealand. 48 p.

Antognozzi, E., Battistelli, A., Famiani, F., Moscatello, S., Stanica, F., Tombesi, A. 1996: Influence of CPPU on carbohydrate accumulation and metabolism in fruits of *Actinidia deliciosa* (A. Chev.). Scientia Horticulturae 65: 37-47.

Antognozzi, E., Tombesi, A., Palliotti, A. 1992: Relationship between leaf area, leaf area index and fruiting in kiwifruit (*Actinidia deliciosa*). Acta Horticulturae 297: 435-442.

Antunes, M. D. C., Sfakiotakis, E. M. 1997: Biochemical basis of thermoregulation of ethylene production and ripening of 'Hayward' kiwifruit. Acta Horticulturae 444: 541-552.

Arpaia, M. L., Labavitch, J. M., Greve, C., Kader, A. A. 1987: Changes in the cell wall components of kiwifruit during storage in air or controlled atmosphere. Journal of the American Society for Horticultural Science 112: 474-481.

Arpaia, M. L., Mitchell, F. G., Kader, A. A., Mayer, G. 1986: Ethylene and temperature effects on softening and white core inclusions of kiwifruit stored in air or controlled atmospheres. Journal of the American Society for Horticultural Science 111: 149-153.

Autio, W. R., Bramlage, W. J. 1982: Effects of AVG on maturation, ripening, and storage of apples. Journal of the American Society for Horticultural Science 107: 1074-1077.

Bangerth, F. 1978: The effect of a substituted amino acid on ethylene biosynthesis, respiration, ripening and preharvest drop of apple fruits. Journal of the American Society for Horticultural Science 103: 401-404.

Bangerth, F. 1986: Effect of ethylene biosynthesis and/or action on the production of volatile flavour substances of stored apple fruits. Acta Horticulturae 179: 823-824.

Bangerth, F., Streif, J. 1987: Effect of aminoethoxyvinylglycine and low-pressure storage on the post-storage production of aroma volatiles by Golden Delicious apples. Journal of the Science of Food and Agriculture 41: 351-360.

Banks, N. H., Cleland, D. J., Cameron, A. C., Beaudry, R. M., Kader, A. A. 1995: Proposal for a rationalized system of units for postharvest research in gas exchange. HortScience 30: 1129-1131.

Banks, N. H., Dingle, M. A., Davie, I. J., Jeffery, P. B., Mowatt, C., Banos, S. B. 1992: Effects of handling damage on storage behaviour of kiwifruit. Pp 12-14 *in*: National Research Conference, Rotorua. New Zealand Kiwifruit Marketing Board.

Banks, N. H., Jefferey, P., Hewett, E. W,, Cameron, A. C., Cleland, D. J. 1991: Ethylene – the enemy within. New Zealand Kiwifruit Journal 75: 20-21.

Banos, S. B. 1995: Postharvest environmental factors affecting infection of kiwifruit by *Botrytis cinerea*. PhD Thesis. Massey University, Palmerston North, New Zealand.

Banos, S. B., Long, P. G., Ganesh, S. 1997: Curing of kiwifruit for control of postharvest infection by *Botrytis cinerea*. Postharvest Biology and Technology 12: 137-145.

Beever, D. J., Hopkirk, G. 1990: Fruit development and fruit physiology. Pp 97-126 *in*: Kiwifruit Science and Management. Warrington, I. J., Weston, G. C. *eds*. Auckland, New Zealand Society for Horticultural Science.

Beever, D. J., Hopkirk, G. 1994: Postpacking losses in kiwifruit. Pp 87-90 *in*: Proceedings of the Australasian Postharvest Conference. Lawes, The University of Queensland Gatton College.

Biale, J. B., Young, R. E. 1981: Respiration and ripening in fruits - retrospect and prospect. Pp 1-39 *in*: Recent Advances in the Biochemistry of Fruits and Vegetables. Friend, J., Rhodes, M. J. C. *eds*. London, Academic Press.

Biasi, R., Costa, G., Manson, P. J. 1995: Light influence on kiwifruit (*Actinidia deliciosa*) quality. Acta Horticulturae 379: 245-251.

Blankenship, S. M., Hammett, L. K. 1987: Changes in oxygen, carbon dioxide, and ethylene mixtures stored in plastic or glass syringes. HortScience 22: 278-280.

Blanpied, G. D., Bramlage, W. J., Dewey, D. H. 1978: A standardized method for collecting apple pressure test data. New York's Food and Life Sciences Bulletin 74: 1-8.

Boller, T., Herner, R. C., Kende, H. 1979: Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Planta 145: 293-303.

Bonghi, C., Pagni, S., Vidrih, R., Ramina, A., Tonutti, P. 1996: Cell wall hydrolases and amylase in kiwifruit softening. Postharvest Biology and Technology 9: 19-26.

Bradford, K. J., Trewavas, A. J. 1994: Sensitivity thresholds and variable time scales in plant hormone action. Plant Physiology 105: 1029-1036.

Bramlage, W. J., Autio, W. R. 1982: Use of AVG to control ripening of apples. Pp 349-358 *in*: Richardson, D. G., Meheriuk, M. *eds*. Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities. Oregon State University, Symposium Series no. 1.

Bramlage, W. J., Greene, D. W., Autio, W. R., McLaughlin, J. M. 1980: Effects of aminoethoxyvinylglycine on internal ethylene concentrations and storage of apples. Journal of the American Society for Horticultural Science 105: 847-851.

Brook, P. J. 1992: *Botrytis* stem-end rot and other storage diseases of kiwifruit - a review. Acta Horticulturae 297: 545-550.

Bufler, G. 1984: Ethylene-enhanced 1-aminocyclopropane-1-carboxylic acid synthase activity in ripening apples. Plant Physiology 75: 192-195.

Bufler, G. 1986: Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene on peel of apple at various developmental stages. Plant Physiology 80: 539-543.

Byers, R. E. 1997: Peach and nectarine fruit softening following aminoethoxyvinylglycine sprays and dips. HortScience 32: 86-88.

Castillo, J. G. C., Lawes, G. S., Woolley, D. J. 1997: The influence of rootstock and 'Hayward' scion selection on fruit firmness and soluble solids. Acta Horticulturae 444: 513-516.

Child, R. D., Atkins, H. A., Rodriguez, A. F. R. 1986: Effects of suppression of ethylene biosynthesis with aminoethoxyvinylglycine (AVG) on fruit set in Cox's Orange Pippin apple. Acta Horticulturae 179: 375-376.

Child, R. D., Williams, R. R. 1983: The effects of the interaction of aminoethoxyvinylglycine (AVG) with mixtures of growth regulators on fruit set and shape in Cox's Orange Pippin apple. Journal of Horticultural Science 58: 365-369.

Child, R. D., Williams, A. A., Hoad, G. V., Baines, C. R. 1984: The effects of aminoethoxyvinylglycine on maturity and post-harvest changes in Cox's Orange Pippin apples. Journal of the Science of Food and Agriculture 35: 773-781.

Cooper, K., Marshall, R. 1990: Improving fruit size through crop-loading and canopy management. Pp 17-19 *in*: National Research Conference, Rotorua. New Zealand Kiwifruit Marketing Board.

Costa, G., Biasi, R., Succi, F., Quadretti, R., Morigi, M., Miserocchi, O. 1997: Effect of CPPU on fruiting performance, fruit quality and storage life of kiwifruit (cv Hayward). Acta Horticulturae 444: 467-472.

Cotter, R. L., MacRae, E. A., Ferguson, A. R., McMath, K. L., Brennan, C. J. 1991: A comparison of the ripening, storage and sensory qualities of seven cultivars of kiwifruit. Journal of Horticultural Science 66: 291-300.

Crisosto, C. H., Garner, D. T., Saenz, K. 1997: Relationship between kiwifruit size and the rate of softening under controlled atmosphere conditions. Pp 101 *in*: Proceedings of the 7th International Controlled Atmosphere Research Conference. Davis, The University of California (Abstract).

Curry, E. A., Patterson, M. E. 1992: Controlling ethylene biosynthesis with natural compounds. Pp 312-313 *in*: Proceedings of the Eighty-eighth Annual Meeting of the Washington State Horticultural Association, Pullman. Washington State University.

Curry, E. A., Williams, M. W. 1986: Physiological changes associated with increased lateral branching of apple trees treated with aminoethoxyvinylglycine. Journal of the American Society for Horticultural Science 111: 237-240.

Davie, I. J., Banks, N. H., Studman, C. J. 1996: Soft patches in kiwifruit. New Zealand Kiwifruit Journal 114: 16-17.

Edgerton, L. J. 1981: Some effects of aminoethoxyvinylglycine, 6-benzylamino purine and gibberellins on fruit set and vegetative growth of apple. Acta Horticulturae 120: 125-132.

Ferguson, A. R. 1984: Kiwifruit: a botanical review. Horticultural Reviews 6: 1-64.

Ferguson, A. R. 1990: The genus *Actinidia*. Pp 15-35 *in*: Kiwifruit Science and Management. Warrington, I. J., Weston, G. C. *eds*. Auckland, New Zealand Society for Horticultural Science.

Ferguson, A. R., Bollard, E. G. 1990: Domestication of the kiwifruit. Pp 165-246 *in*: Kiwifruit Science and Management. Warrington, I. J., Weston, G. C. *eds*. Auckland, New Zealand Society for Horticultural Science.

Fernandez, G. C. J. 1992: Residual analysis and data transformations: important tools in statistical analysis. HortScience 27: 297-300.

Fluhr, R., Mattoo, A. K. 1996: Ethylene - biosynthesis and perception. Critical Reviews in Plant Sciences 15: 479-523.

Gerasopoulos, D., Chouliaras, V., Lionakis, S. 1996: Effects of preharvest calcium chloride sprays on maturity and storability of Hayward kiwifruit. Postharvest Biology and Technology 7: 65-72.

Gerasopoulos, D., Richardson, D. G. 1997: Storage-temperature-dependent time separation of softening and chlorophyll loss from the autocatalytic ethylene pathway and other ripening events of Anjou pears. Journal of the American Society for Horticultural Science 122: 680-685.

Greene, D. W. 1980: Effect of silver nitrate, aminoethoxyvinylglycine, and gibberellins A_{4+7} plus 6-benzylamino purine on fruit set and development of 'Delicious' apples. Journal of the American Society for Horticultural Science 105: 717-720.

Greene, D. W. 1983: Some effects of AVG on fruit set, fruit characteristics, and vegetative growth of apples trees. Journal of the American Society for Horticultural Science 108: 410-415.

Halder-Doll., H., Bangerth, F. 1987: Inhibition of autocatalytic C_2H_4-biosynthesis by AVG applications and consequences on the physiological behaviour and quality of apple fruits in cool storage. Scientia Horticulturae 33: 87-96.

Harker, F. R., Hallett, I. C. 1994: Physiological and mechanical properties of kiwifruit tissue associated with texture change during cool storage. Journal of the American Society for Horticultural Science 119: 987-993.

Harker, F. R., Maindonald, J. H., Jackson, P. J. 1996: Penetrometer measurement of apple and kiwifruit firmness: operator and instrument differences. Journal of the American Society for Horticultural Science 121: 927-936.

Harman, J. E. 1981: Kiwifruit maturity. The Orchardist of New Zealand 54:126-127.

Harris, S., McDonald, B. 1975: Physical data for kiwifruit (*Actinidia chinensis*). New Zealand Journal of Science 18: 307-312.

Henzell, R. F., Allison, P., Miles, D. 1994: What factors affect the quality and sweetness of kiwifruit. Pp 26-29 *in*: National Research Conference, Rotorua. New Zealand Kiwifruit Marketing Board.

Hopkirk, G., Beever, D. J., Triggs, C. M. 1986: Variation in soluble solids concentration in kiwifruit at harvest. New Zealand Journal of Agricultural Research 29: 475-484.

Hopkirk, G., Clark, C. 1992a: Postharvest fruit losses in the New Zealand kiwifruit industry. Acta Horticulturae 297: 611-616.

Hopkirk, G., Clark, C. 1992b: Orchard factors affecting fruit quality. Pp 5-7 *in*: National Research Conference, Rotorua. New Zealand Kiwifruit Marketing Board.

Hopkirk, G., Harker, F. R., Harman, J. E. 1990: Calcium and the firmness of kiwifruit. New Zealand Journal of Crop and Horticultural Science 18: 215-219.

Hopkirk, G., Maindonald, J. H., White, A. 1996: Comparison of four new devices for measuring kiwifruit firmness. New Zealand Journal of Crop and Horticultural Science 24: 273-286.

Hopkirk, G., Snelgar, W. P., Horne, F., Manson, P. J. 1989: Effect of increased preharvest temperature on fruit quality of kiwifruit (*Actinidia deliciosa*). Journal of Horticultural Science 64: 227-237.

Hyodo, H., Aizawa, S., Ao, S. 1987: Ethylene formation during ripening of kiwifruit. Journal of the Japanese Society for Horticultural Science 56: 352-355.

Hyodo, H., Fukasawa, R. 1985: Ethylene production in kiwifruit. Journal of the Japanese Society for Horticultural Science 54: 209-215.

Jeffery, P. B., Banks, N. H. 1996: Effects of ethylene on kiwifruit softening in coolstorage. New Zealand Kiwifruit Journal 114: 9.

Jobling, J., McGlasson, W. B., Dilley, D. R. 1991: Induction of ethylene synthesising competency in Granny Smith apples by exposure to low temperature in air. Postharvest Biology and Technology 1: 111-118.

John, P. 1997: Ethylene biosynthesis: the role of 1-aminocyclopropane-1-carboxylate (ACC) oxidase, and its possible evolutionary origin. Physiologia Plantarum 100: 583-592.

Kende, H. 1993: Ethylene biosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 44: 283-307.

Kim, H. O., Marques, J. R., Hewett, E. W. 1997: AVG effects on ethylene biosynthesis and quality of kiwifruit. *In*: Proceedings of the Australasian Postharvest Horticulture Conference, University of Western Sydney Hawkesbury, Richmond, Australia (*in press*).

Kim, W. T., Yang, S. F. 1992: Turnover of 1-aminocyclopropane-1-carboxylic acid synthase in wounded tomato fruit tissue. Plant Physiology 100: 1126-1131.

Knee, M., Looney, N. E., Hatfield, S. G. S., Smith, S. M. 1983: Initiation of rapid ethylene synthesis by apple and pear fruits in relation to storage temperature. Journal of Experimental Botany 34: 1207-1212.

Knee, M., Proctor, F. J., Dover, C. J. 1985: The technology of ethylene control: use and removal in post-harvest handling of horticultural commodities. Annals of Applied Biology 107: 581-595.

Lallu, N. 1997: Low temperature breakdown in kiwifruit. Acta Horticulturae 444: 579-587.

Lallu, N., Manning, M., Pak, H. 1997: Best curing practices for the orchard and packhouse. New Zealand Kiwifruit Journal 120: 35-36.

Lallu, N., Searle, A., MacRae, E. A. 1989: An investigation of ripening and handling strategies for early season kiwifruit (Actinidia deliciosa cv Hayward). Journal of the Science of Food and Agriculture 47: 387-400.

Lallu, N., Yearsley, C. W., Elgar, H. J. 1992: Storage temperature affects the quality of kiwifruit. Pp 11-12 *in*: National Research Conference, Rotorua. New Zealand Kiwifruit Marketing Board.

Lawes, G. S., Woolley, J. G., Castillo, J. G. C. 1992: Field responses of kiwifruit to CPPU (cytokinin) application. Acta Horticulturae 297: 351-356.

Lawes, G. S., Woolley, D. J., Lai, R. 1990: Seeds and other factors affecting fruit size in kiwifruit. Acta Horticulturae 282: 257-264.

Lelievre, J. M., Tichit, L., Dao, P., Fillion, L., Larrigaudiere, C., Vendrell, M., Pech, J. C. 1995: Cold-induced accumulation of 1-aminocyclopropane 1-carboxylate

oxidase protein in Granny Smith apples. Postharvest Biology and Technology 5 : 11-17.

Lelievre, J. M., Tichit, L., Dao, P., Fillion, L., Nam, Y. W., Pech, J. C., Latche, A. 1997: Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (*Pyrus communis* L.) fruits. Plant Molecular Biology 33: 847-855.

Lieberman, M. 1979: Biosynthesis and action of ethylene. Annual Review of Plant Physiology 30: 533-591.

Liu, Y., Su, L. Y., Yang, S. F. 1985: Ethylene promotes the capability to malonylate 1-aminocyclopropane-1-carboxylic acid and d-amino acids in preclimacteric tomato fruits. Plant Physiology 77: 891-895.

Long, P. G., Banos, S. B. 1994: Curing and infection of kiwifruit by *Botrytis cinerea*. Pp 31-34 *in*: National Research Conference. New Zealand Kiwifruit Marketing Board.

Lurssen, K., Naumann, K., Schroder, R. 1979: 1-aminocyclopropane-1-carboxylic acid. An intermediate of the ethylene biosynthesis in higher plants. Z. Pflanzenphysiology 92: 285-294.

MacRae, E. A. 1988: The core of the soft fruit problem. New Zealand Kiwifruit Journal 43: 20-21.

MacRae, E. A., Bowen, J. H., Stec, M. G. H. 1989a: Maturation of kiwifruit (*Actinidia deliciosa* cv Hayward) from two orchards: differences in composition of the tissue zones. Journal of the Science of Food and Agriculture 47: 401-416.

MacRae, E. A., Lallu, N., Searle, A. N., Bowen, J. H. 1989b: Changes in the softening of kiwifruit (*Actinidia deliciosa*) affected by maturity of harvest and postharvest treatments. Journal of the Science of Food and Agriculture 49: 413-430.

MacRae, E. A., Quick, W. P., Benker, C., Stitt, M. 1992: Carbohydrate metabolism during postharvest ripening in kiwifruit. Planta 188: 314-323.

MacRae, E. A., Redgwell, R. 1992: Softening in kiwifruit. Postharvest News and Information 3: 49N-52N.

Massantini, R., Gobattoni, E., Botondi, R., Mencarelli, F. 1995a: Role of alcohol dehydrogenase on ripening of ethanol-treated tomato and kiwifruit. Acta Horticulturae 379: 297-304.

Massantini, R., Lanzarotta, R., Botondi, R., Mencarelli, F. 1995b: Ripening of kiwifruit following simulated brushing with commercial brushes. HortScience 30: 566-569.

Mattoo, A. K., Baker, J. E., Chalutz, E., Lieberman, M. 1977: Effect of temperature on the ethylene-synthesizing systems in apple, tomato, and *Penicillium digitatum*. Plant and Cell Physiology 18: 715-719.

McDonald, B. 1990: Precooling, storage, and transport of kiwifruit. Pp 429-459 *in*: Kiwifruit Science and Management. Warrington, I. J., Weston, G. C. *eds*. Auckland, New Zealand Society for Horticultural Science.

McDonald, B., Harman, J. E. 1982: Controlled-atmosphere storage of kiwifruit. I. Effect on fruit firmness and storage life. Scientia Horticulturae 17: 113-123.

McMath, K. L. 1992: Fruit taste - the world's finest. Pp 41-42 *in*: National Research Conference, Rotorua. New Zealand Kiwifruit Marketing Board.

McMurchie, E. J., McGlasson, W. B., Eaks, I. L. 1972: Treatment of fruit with propylene gas gives information about the biogenesis of ethylene. Nature 237: 235-236.

Mencarelli, F., Massantini, R., Botondi, R. 1996: Influence of impact surface and temperature on the ripening response of kiwifruit. Postharvest Biology and Technology 8: 165-177.

Mitchell, F. G. 1990: Postharvest physiology and technology of kiwifruit. Acta Horticulturae 282: 291-307.

Mitchell, F. G., Costa, G. 1997: Nitrogen influences kiwifruit storage life. Acta Horticulturae 444: 285-289.

Mitchell, F. G., Mayer, G., Biasi, W. 1992: Effect of harvest maturity on storage performance of 'Hayward' kiwifruit. Acta Horticulturae 297: 617-625.

Monastra, F., Testoni, A. 1992: Horticultural performance and quality characteristics of fruit from kiwifruit plants (cv. Hayward) obtained by in vitro-propagation, by cuttings and by grafting. Acta Horticulturae 297: 197-203.

Niklis, N., Sfakiotakis, E., Thanassoulopoulos, C. C. 1997: Ethylene production by *Botrytis cinerea*, kiwifruit and *Botrytis* rotted kiwifruit under several storage temperatures. Acta Horticulturae 444: 733-737.

Oetiker, J. H., Yang, S. F. 1995: The role of ethylene in fruit ripening. Acta Horticulturae 398: 167-178.

Okuse, I., Ryugo, K. 1981: Compositional changes in the developing 'Hayward' kiwifruit in California. Journal of the American Society for Horticultural Science 106: 73-76.

Olsen, K. L. 1982: Effect of AVG on storage behaviour of apples. Pp 345-348 *in*: Richardson, D. G., Meheriuk, M. *eds*. Controlled Atmospheres for Storage and Transport of Perishable Agricultural Commodities. Oregon State University.

Ott, R. L. 1993: An introduction to statistical methods and data analysis. 4th Ed. Belmont, Duxbury Press. 132 p.

Papadopoulou, P., Manolopoulou, H. 1997: The effect of air storage on fruit firmness and sensory quality of kiwifruit cultivars. Acta Horticulturae 444: 607-612.

Poole, P. R., McLeod, L. 1994: Development of resistance to picking wound entry *Botrytis cinerea* storage rots in kiwifruit. New Zealand Journal of Crop and Horticultural Science 22: 387-392.

Prange, R. K., DeEll, J. R. 1997: Preharvest factors affecting postharvest quality of berry crops. HortScience 32: 824-829.

Prasad, M., Spiers, M., 1992: The effect of nutrition on the storage quality of kiwifruit (a review). Acta Horticulturae 297: 579-585.

Pratt, H. K., Reid, M. S. 1974: Chinese gooseberry: seasonal patterns in fruit growth and maturation, ripening, respiration and the role of ethylene. Journal of the Science of Food and Agriculture 25: 747-757.

Pyke, N. B., Hopkirk, G., Alspach, P. A., Cooper, K. M. 1996: Variation in harvest and storage quality of fruit from different positions on kiwifruit vines. New Zealand Journal of Crop and Horticultural Science 24: 39-46.

Pyke, N. B., Manktelow, D. G., Elmer, P. A. G., Tate, K. G. 1994: Postharvest dipping of kiwifruit in iprodione to control stem-end rot caused by *Botrytis cinerea*. New Zealand Journal of Crop and Horticultural Science 22: 81-86.

Qadir, A., Hewett, E. W., Long, P. G. 1997: Ethylene production by *Botrytis cinerea*. Postharvest Biology and Technology 11: 85-91.

Rahemi, M., Dennis Jr., F. G. 1981: The role of ethylene in apple fruit set. Acta Horticulturae 120: 257.

Rando, R. R. 1974: Chemistry and enzymology of k_{cat} inhibitors. Science 185: 320-324.

Redgwell, R., Fischer, M., Kendal, E., MacRae, E. A. 1997a: Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. Planta 203: 174-181.

Redgwell, R., Harker, R. 1995: Softening of kiwifruit discs: effects of inhibition of galactose loss from cell walls. Phytochemistry 39: 1319-1323.

Redgwell, R., MacRae, E. A., Hallet, I., Fischer, M., Perry, J., Harker, R. 1997b: In vivo and in vitro swelling of cell walls during fruit ripening. Planta 203: 162-173.

Redgwell, R. J., Melton, L. D., Brasch, D. J. 1990: Cell wall changes in kiwifruit following post harvest ethylene treatment. Phytochemistry 29: 399-407.

Reid, J. B., Brash, D. W., Sorensen, I. B., Bycroft, B. 1996: Improvement in kiwifruit storage life caused by withholding early-season irrigation. New Zealand Journal of Crop and Horticultural Science 24: 21-28.

Reid, M. S., Heatherbell, D. A., Pratt, H. K. 1982: Seasonal patterns in chemical composition of the fruit of *Actinidia chinensis*. Journal of the American Society for Horticultural Science 107: 316-319.

Retamales, J., Campos, R. 1997: Extremely low ethylene levels in ambient air are still critical for kiwifruit storage. Acta Horticulturae 444: 573-578.

Retamales, J., Cooper, T., Montealegre, J. 1997: Effects of curing and cooling regime on ethylene production and storage behaviour of kiwifruit. Acta Horticulturae 444: 567-571.

Retamales, J., Perez, A., Callejas, R. 1995: Ethylene biosynthesis inhibitor improves firmness of kiwifruit. Acta Horticulturae 394: 159-164.

Romani, R., Labavitch, J., Yamashita, T., Hess, B., Rae, H. 1983: Preharvest AVG treatment of 'Bartlett' pear fruits: effects on ripening, color change, and volatiles. Journal of the American Society for Horticultural Science 108: 1046-1049.

Romani, R., Puschmann, R., Finch, J., Beutel, J. 1982: Effects of preharvest applications of AVG on ripening of 'Bartlett' pears with and without cold storage. HortScience 17: 214-215.

Sale, P. R., Lyford, P. B. 1990: Cultural, management and harvesting practices for kiwifruit in New Zealand. Pp 247-296 *in*: Kiwifruit Science and Management. Warrington, I. J., Weston, G. C. *eds*. Auckland, New Zealand Society for Horticultural Science.

Scienza, A., Failla, O., Visai, C., Nardin, K. 1990: Quality aspects of *Actinidia deliciosa* coming from different Italian districts. Acta Horticulturae 282: 317-324.

Scott, K. J., Giugni, J., Bailey, W. M. 1984: The use of polyethylene bags and ethylene absorbent to extend the life of kiwifruit (Actinidia chinensis Planch) during cool storage. Journal of Horticultural Science 59: 563-566.

Seager, N., Warrington, I. J., Hewett, E. W. 1996: Maturation of kiwifruit grown at different temperatures in controlled environments. Journal of Horticultural Science 71: 639-652.

Sfakiotakis, E., Antunes, D., Stavroulakis, G., Niklis, N., Ververidis, P., Gerasopoulos, D. 1997: Ethylene biosynthesis and its regulation in ripening 'Hayward' kiwifruit. Pp 47-56 *in*: Kanellis, A. K., Chang, C., Kende, H., Grierson, D. *eds*. Biology and Biotechnology of the Plant Hormone Ethylene. Dordrecht, Kluwer Academic Publishers.

Sfakiotakis, E., Stavroulakis, G., Ververidis, P., Gerasopoulos, D. 1989: Induction of autocatalytic ethylene production and ripening by propylene in 'Hayward' kiwifruit. Pp 173-178 *in*: Clijsers, H., Proft, M. D., Marcelle, R., Poucke, M. V.

eds. Biochemical and Physiological Aspects of Ethylene Production in Lower and Higher Plants. Dordrecht, Kluwer Academic Publishers.

Shafer, W. E., Gregory, C., Hansen, J., Woolard, D., Devisetty, B. N., Fritts Jr., R. 1995: Practical applications of aminoethoxyvinylglycine. Pp 11-15 *in*: Proceedings of the 22nd Annual Meeting of Plant Growth Regulators Society of America.

Shusiri, B. 1992: Effect of high carbon dioxide treatment before storage on the physiology and quality of apples. DipHortSci Dissertation. Massey University, Palmerston North, New Zealand.

Sisler, E. C., Serek, M. 1997: Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiologia Plantarum 100: 577-582.

Smalle, J., Van Der Straeten, D. 1997: Ethylene and vegetative development. Physiologia Plantarum 100: 593-605.

Smith, G. S., Gravett, I. M., Edwards, C. M., Curtis, J. P., Buwalda, J. G. 1994: Spatial analysis of the canopy of kiwifruit vines as it relates to the physical, chemical and postharvest attributes of the fruit. Annals of Botany 73: 99-111.

Snelgar, B., Manson, P., Hopkirk, G. 1991: Effect of overhead shading on fruit size and yield potential of kiwifruit (*Actinidia deliciosa*). Journal of Horticultural Science 66: 261-273.

Soda, I., Hasegawa, T., Suzuki, T. 1987: Changes in hemicellulose during ripening of kiwifruit. Journal of Agricultural Science 31: 261-264.

Spiers, M. 1996: Fruit firmness - does the answer lie in the soil? New Zealand Kiwifruit Journal 114: 21.

Stec, M. G. H., Hodgson, J. A., MacRae, E. A., Triggs, C. M. 1989: Role of fruit firmness in the sensory evaluation of kiwifruit (*Actinidia deliciosa* cv Hayward). Journal of the Science of Food and Agriculture 47: 417-433.

Tagliavini, M., Toselli, M., Marangoni, B., Stampi, G., Pelliconi, F. 1995: Nutritional status of kiwifruit affects yield and fruit storage. Acta Horticulturae 383: 227-237.

Theologis, A. 1994: Control of ripening. Current Opinion in Biotechnology 5: 152-157.

Tombesi, A., Antognozzi, E., Palliotti, A. 1993: Influence of light exposure on characteristics and storage life of kiwifruit. New Zealand Journal of Crop and Horticultural Science 21: 85-90.

Treccani, C. P., Visai, C., Mignani, I. L., Cocucci, M. 1986: Effects of 2,5-norbornadiene and aminoethoxyvinylglycine on ethylene levels and ripening of apple fruits. Acta Horticulturae 179: 755-759.

Ververidis, F., John, P. 1991: Complete recovery *in vitro* of ethylene-forming enzyme activity. Phytochemistry 30: 725-727.

Waldron, K. W., Smith, A. C., Parr, A. J., Ng. A., Parker, M. L. 1997: New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. Trends in Food Science and Technology 8: 213-221.

Wang, C. Y., Mellenthin, W. M. 1977: Effects of aminoethoxy analog of rhizobitoxine on ripening of pears. Plant Physiology 59: 546-549.

Williams, M. W. 1980: Retention of fruit firmness and increase in vegetative growth and fruit set of apples with aminoethoxyvinylglycine. HortScience 15: 76-77.

Woltering, E. J. 1987: Effects of ethylene on ornamental pot plants: a classification. Scientia Horticulturae 31: 283-294.

Woolley, D. J., Lawes, G. S., Castillo, J. G. C. 1992: The growth and competitive ability of *Actinidia deliciosa* 'Hayward' fruit: carbohydrate availability and response to the cytokinin-active compound CPPU. Acta Horticulturae 297: 467-474.

Yang, S. F. 1987: The role of ethylene and ethylene synthesis in fruit ripening. Pp 156-166 *in*: Plant senescence: its biochemistry and physiology. Thomson, W. W., Nothnagel, E. A., Huffaker, R. C. *eds*. American Society of Plant Physiologists.

Yang, S. F., Hoffman, N. E. 1984: Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology 35: 155-189.

Yang, S. F., Liu, Y., Lau, O. L. 1986: Regulation of ethylene biosynthesis in ripening apple fruit. Acta Horticulturae 179: 711-719.

Yearsley, C. W. 1996: Internal lower oxygen limits of apple fruit. PhD Thesis. Massey University, Palmerston North, New Zealand.

Zarembinski, T. I., Theologis, A. 1994: Ethylene biosynthesis and action: a case of conservation. Plant Molecular Biology 26: 1579-1597.

APPENDIX I

Layout of kiwifruit vines - Plant treatment number shown

(Fruit Crops Unit - Massey University)

Site Number	- ROW - A	B	C	D	E
1	x	x	**3**	x	x
2	m	m	m	m	m
3	x	x	**3**	x	x
4	x	x	**1**	x	x
5	x	x	**1**	x	x
6	m	m	m	m	m
7	x	x	**2**	x	x
8	x	x	**2**	x	x
9	x	x	**4**	x	x
10	x	x	**4**	x	x
11	m	m	m	m	m
12	x	x	**4**	x	x
13	x	x	**4**	x	x
14	x	x	**2**	x	x
15	x	x	**2**	x	x
16	m	m	m	m	m
17	x	x	**1**	x	x
18	x	x	**1**	x	x
19	x	x	x	**3**	**4**
20	x	x	**3**	**3**	**4**
21	m	m	m	m	m
22	x	x	**3**	**2**	**4**
23	x	x	x	**2**	**4**
24	x	x	x	**1**	**2**
25	x	x	x	**1**	**2**
26	m	m	m	m	m
27	x	x	x	**4**	x
28	x	x	x	**4**	x
29	x	x	x	**3**	**1**
30	x	x	x	**3**	**1**
31	m	m	m	m	m
32	x	x	x	**3**	x
33	x	x	x	**3**	x
34	x	x	x	**1**	x
35	x	x	x	**1**	x
36	m	m	m	m	m
37	x	x	x	**2**	x
38	x	x	x	**2**	x
39	x	x	x	x	x

Legend:

x = female plant

m = male plant

1-4 = AVG treatments:

1 = Control

2 = 500-AVG-4

3 = 500-AVG-6

4 = 1000-AVG-4

APPENDIX II

Formulas used for calculating fruit carbon dioxide and ethylene production rates (Shusiri 1992):

Fruit carbon dioxide production:

- ♦ FCO_2 = [(CO_2)final - (CO_2)initial] / 100 x (Vjar-Vfruit) x 1000 / wfruit x 60 / t

Fruit ethylene production:

- ♦ FC_2H_4 = (C_2H_4) / 1000 x (Vjar-Vfruit) x 1000 / wfruit x 60 / t

Where:

- FCO_2 = carbon dioxide production ($cm^3.kg^{-1}.h^{-1}$)
- FC_2H_4 = ethylene production ($\mu l.\ kg^{-1}.h^{-1}$)
- (CO_2)initial = initial carbon dioxide concentration (%)
- (CO_2)final = final carbon dioxide concentration (%)
- (C_2H_4) = ethylene concentration ($\mu l.l^{-1}$)
- Vjar = jar volume (cm^3)
- Vfruit = fruit volume (cm^3) = wfruit / dfruit
- wfruit = fruit weight (g)
- dfruit = fruit density = 1030 kg/m^3 for kiwifruit (Harris & McDonald 1975)
- t = time (min)

Conversion factors (Banks *et al.* 1995):

- $cm^3.kg^{-1}.h^{-1}$ to mol. $kg^{-1}.s^{-1} \Rightarrow 3.341 \times 10^{-11}\ p^{tot} / (T+273.15)$
- $\mu l.\ kg^{-1}.h^{-1}$ to mol. $kg^{-1}.s^{-1} \Rightarrow 3.341 \times 10^{-14}\ p^{tot} / (T+273.15)$

Where:

p^{tot} = standard atmosphere pressure (101,325 Pa)

T = temperature = 20 °C

APPENDIX III

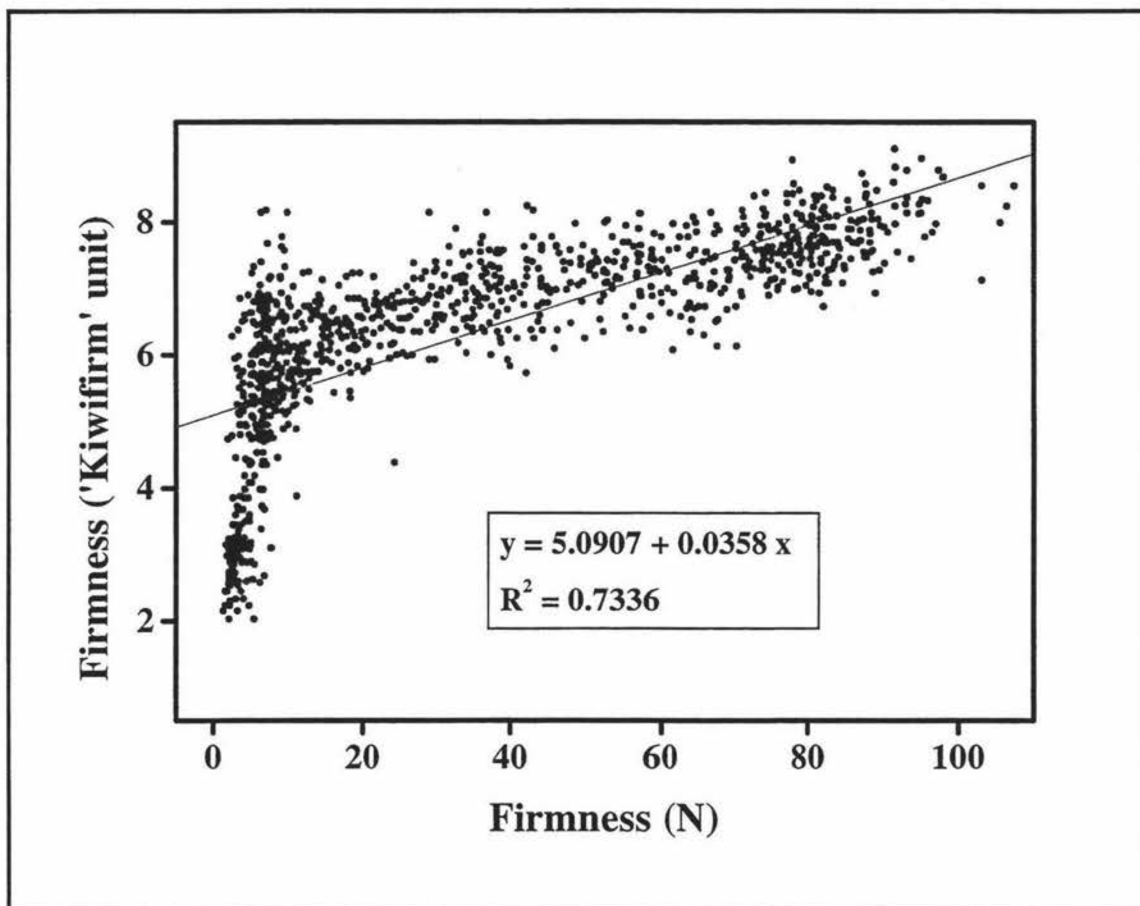

Figure 1 - Scatter plot of firmness of kiwifruit measured destructively (N) and non destructively ('Kiwifirm' unit), from individual fruit measurements at 20 °C, at harvest, and after 30, 80, and 180 days at 0 °C. Total points: 1080.