TEMPERATURE EFFECTS ON KIWIFRUIT MATURATION

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ABSTRACT

The effect of temperature on rate of kiwifruit maturation was studied using container-grown vines placed in controlled environments and field-grown vines from four orchards (Kerikeri, Te Puke, Palmerston North and Riwaka) at the geographical extremes of the kiwifruit growing regions. Soluble solids concentration (SSC) and partitioning of carbohydrate between starch and total sugar concentrations were studied at different stages of maturation in both the controlled environment and field work. Flesh firmness, dry matter concentration and fruit growth changes during fruit maturation were also measured. The effect of carbohydrate status on fruit maturation was determined by manipulating it using girdling of field-grown vines. A model relating changes in SSC to temperature was derived using data collected from controlled environment treatments. This model was applied to field-grown vines using meteorological data from kiwifruit growing regions.

Use of controlled environments quantified changes in kiwifruit during maturation. Increase in SSC and total sugar concentration, and decrease in starch concentration were faster at cooler than warmer mean temperatures, irrespective of minimum temperature *per se* or magnitude of the difference between maximum and minimum temperature. A temperature perturbation altered the partitioning of carbohydrate compared to treatments where a perturbation did not occur. In some years fruit were not responsive to any temperature treatments; these fruit had not reached the stage of development at which they were able to respond to temperature. Differences in rate of fruit maturation were found among orchard sites. Some of these differences, such as decrease in starch concentration and increase in total sugar and SSC could be attributed to the effect of temperature.

Girdling kiwifruit laterals altered carbohydrate concentration and affected rate of fruit maturation. Carbohydrate concentration was higher in fruit from the 5:1 than 1:1 leaf:fruit ratio treatment. Fruit in the 5:1 treatment matured similarly to fruit from ungirdled vines, compared to delayed maturation in fruit from the 1:1

treatment. Carbohydrate concentration in this treatment may be insufficient to support fruit maturation.

The model developed to predict the rate of change in SSC during kiwifruit maturation was made up of two components; a state-dependent physiological response function and a temperature-dependent rate function. The base + exponential model was chosen to represent the state-dependent physiological response function, based on SSC being separated into two components; basal SSC and maturation SSC. The temperature-dependent rate function from container-grown vines placed in controlled environments was successfully transported to fit SSC in field-grown vines at different orchard locations. The model was developed using continuous temperature records but was later modified to use daily maximum and minimum temperatures allowing greater practical application. The partial rate coefficient accounted for most of the physiological differences between years, orchards and experiments; it required fitting at each orchard location. Transportability of the partial rate coefficient was demonstrated between years for two orchard locations. The model, therefore, has great potential for prediction of harvest date of kiwifruit in different regions and seasons.

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CHAPTER 1

INTRODUCTION

1.1 Kiwifruit; origins, biology and domestication

Kiwifruit (*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson var. *deliciosa*) is one of the few new commercial fruit crops to have appeared in world trade this century. New Zealand is now accepted as the home of the cultivated kiwifruit that originated in China. A comprehensive text 'Kiwifruit Science and Management' (Warrington and Weston, 1990) covers all aspects of the history and domestication of kiwifruit, its biology, production, postharvest handling, processing and marketing. Some of this material has been used for the following short summary of the origin, domestication and biology of kiwifruit.

Cultivated kiwifruit are large-fruited selections of *A. deliciosa*, one of more than 50 species and 100 taxa in the genus *Actinidia*. Most of these are found in the temperate forests of the mountains and hills of south-west China, although some species are found in regions as diverse as Siberia and Indonesia. The species are remarkably widespread in distribution and habitat, from 50°N to the equator, and from cold-temperate or arctic forests to the tropics (Ferguson, 1990a). The geographic centre of the genus is a narrow band between 25° and 30°N in the mountains and hills of south China, between the Chang Jiang and Xi Jiang Basins. *A. deliciosa* was referred to as *A. chinensis* until 1984, but the species have since been separated. Ferguson (1990b) describes the geographic location of the two species; *A. chinensis* is found in eastern, warmer regions and *A. deliciosa* in western, colder regions. If the two species occur together in the same mountains then they are separated vertically, with *A. deliciosa* at higher altitudes. In the mountains, maximum summer temperatures seldom exceed 40C, and winter temperatures can be freezing, but there is a long frost-free period of 210 to 280 days. *A. deliciosa* would therefore be adapted to cool winters and warm

summers; this evolutionary background probably accounts for its current requirements for winter chilling and warm spring temperatures for budbreak, and perhaps also for the rapid rise in soluble solids concentration (SSC) with cool temperatures in autumn (see Section 1.4.2).

All *Actinidia* species are perennial and form climbing or straggling plants. Untrained, the stems can twist about and form tangles (Ferguson, 1990a). Shoots of the current season come from axillary buds of the previous season's growth; these buds overwinter in corky tissue above the leaf scar. Leaves are alternate, simple with a long petiole and the vines are deciduous. All members of the genus appear to be dioecious, that is, pistillate and staminate (female and male) flowers occur on separate plants (Hopping, 1990). Female flowers have stamens but viable pollen grains have never been found, while staminate plants have a reduced ovary with poorly developed styles. Occasionally *A. deliciosa* plants produce perfect flowers that are self-pollinating and self-setting. Vines with self-fertile bisexual flowers are being selected in the hermaphrodite kiwifruit breeding programme in New Zealand (McNeilage, 1992). Flowers, which are cup shaped with white petals, occur in the leaf axil, sometimes singly or in small inflorescences. The fruit is a berry with hundreds of small, dark seeds embedded in soft, juicy, bright green flesh. Details of vegetative growth and development, floral biology, fruit development (see also Section 1.2) and cultural practices can be found in Warrington and Weston (1990).

Missionaries in China were responsible for sending *A. deliciosa* seed to New Zealand in the early 1900s, the first plants being grown at a nursery near Wanganui (Ferguson and Bollard, 1990). These plants were propagated and distributed to other nurserymen and enthusiastic gardeners, both locally and further afield. The cultivars 'Hayward' and 'Bruno' were probably selected and developed from these propagated seedlings. Growing conditions in New Zealand, especially in the north, seemed more favourable for successful fruiting than those prevailing in Europe. During the next 25 years the original plants were propagated and the first commercial orchard planted at Wanganui. Plantings were

soon established in other parts of the country, such as the Bay of Plenty, Auckland and Kerikeri. From 1950 to 1970 kiwifruit plantings were slow to expand, and produced fruit largely to satisfy local requirements. From 1973 onwards plantings of kiwifruit were more extensive to fulfil the demand of the developing export industry. In 1973, about 90% of the total New Zealand plantings were in the Bay of Plenty and 72% of these were in Te Puke. By 1991, the Bay of Plenty contributed 65% to the total volume of kiwifruit exported, with the remaining 35% coming from seven other regions (Anon., 1992a).

The first shipment of kiwifruit (12.5 tonnes/3,500 trays) left New Zealand for the United Kingdom in 1952, followed by 13 tonnes (3,600 trays) of fruit to Australia in 1954 and exports of 30 to 40 tonnes (8,000 to 11,000 trays) in 1960. By 1964 the quantity of export fruit had doubled and 1976 marked the year when exports first exceeded local consumption. In 1991, 59 million trays (over 212,000 tonnes) were packed for export (M. Evans, 1992 New Zealand Kiwifruit Marketing Board Conference, Rotorua). Fruit are mainly packed in single layer trays of 3.6 kg (McDonald, 1990). These trays have eight designated fruit sizes of 25 to 46 fruit per tray. In the 1990 season, fruit in the mid sizes, 33 to 39, were most in demand in Europe, Japan and North America (Smith, 1991). Export value of unprocessed kiwifruit was NZ$539, 520 and 439 million (f.o.b.) for the year to 30 June 1990, 1991 and 1992, respectively (Anon., 1992b)

To cope with the growing demand for kiwifruit, researchers, growers and exporters had to determine suitable planting positions and spacings for female and male plants, pollination techniques, training and pruning methods, standardisation of cultivars (to 'Hayward'), development of a maturity standard for harvesting fruit (see Section 1.2.3), and storage, packaging and transport conditions.

Success of the industry in New Zealand encouraged other countries such as France, Italy, Japan and United States (California) to grow kiwifruit. Italy is the major producer of kiwifruit in Europe and in 1990 produced 210,000 tonnes from

the total European volume of 266,460 tonnes, Japan produced 54,720 tonnes and the United States 32,960 tonnes (R. Martin, New Zealand Kiwifruit Marketing Board, Auckland, pers. comm.). Probably of greater concern to the New Zealand industry is the quantity of fruit grown in South America (Chile) where labour is cheaper and fruit is produced and exported at the same time of year as in this country. In 1990, 39,300 tonnes were produced in Chile (Buzeta, 1992) compared to 281,520 tonnes in New Zealand. Estimated production in the northern hemisphere is expected to be 100,000 tonnes greater than that of the southern hemisphere by 1995. Predicted production of kiwifruit in 1995 is 75,000 tonnes in Chile, 262,800 tonnes in New Zealand and in Italy between 270,000 tonnes (R. Martin, New Zealand Kiwifruit Marketing Board, Auckland, pers. comm.) and 410,000 tonnes (Costa *et al.*, 1992b).

To survive, it is essential that the New Zealand industry in the future continues to supply a product that customers recognise as high quality. It is equally important that commercially viable new cultivars are developed. Over the last 10 years in New Zealand, considerable effort has been placed on plant breeding to broaden the range of cultivars available, and the selection of other varieties such as those that are early maturing (Seal, 1992). There is also a need to diversify into active marketing of the new and very different looking species and cultivars of kiwifruit being developed, before other countries do so. Production of a range of novel fruit types has been achieved. For example, a kiwifruit of similar size to 'Hayward' but with a distinctive green, hairless skin was obtained from crossing *A. arguta* to *A. deliciosa* and then by backcrossing to *A. deliciosa* (Beatson, 1992). Other species of interest are the smooth (brown) skinned *A. chinensis* and 'kiwigrapes', selections of *A. arguta* and hybrids with small, green skinned, hairless fruits with a rich, sweet flavour. Other important selections not yet evaluated are red-fleshed kiwifruit, large fruited hermaphrodites (bisexual vines) and easy-peel types (Seal, 1992).

1.2 Fruit growth, maturation and ripening

The processes of growth, maturation and ripening need to be defined and explained to give an understanding of the similarities and differences between various fruits. As it is a relatively new commercial crop, less research has been undertaken on kiwifruit than on many other species. It is therefore necessary to draw on information about other fruit in order to explain some processes in kiwifruit.

A fruit is the product of determinate growth from an angiospermous flower or inflorescence (Coombe, 1976). The Oxford English Dictionary (1990) defines fruit as "the usual sweet and fleshy edible product of a plant or tree, containing seed". The word fruit refers to the fleshy, edible portion of the inflorescence, irrespective of which part develops into the fruit flesh (see Coombe, 1976); for example a strawberry (*Fragaria ananassa* Duch.) develops from the receptacle and a peach (*Prunus persica* (L.) Batsch) from the mesocarp. After flowering there are periods of cell division and expansion when the developing fruit grows, leading to maturation, ripening and eventually senescence.

Maturation is the process of fruit development culminating in physiological maturity (Watada *et al.*, 1984). Maturity is the stage of physiological development which must be reached before a fruit may be removed from the plant, and yet still continue to develop until it is suitable for consumption (Beever and Hopkirk, 1990). Ripening refers to the composite processes that occur from the latter stages of maturation through the early stages of senescence to result in a characteristic aesthetic and/or food quality, as evidenced by changes in composition, colour, texture or other sensory attributes (Watada *et al.*, 1984). Watada *et al.* (1984) defined senescence as the processes that follow physiological maturity and lead to death of tissue. During senescence, eating quality of fruit deteriorates (Beever and Hopkirk, 1990). These definitions show how the processes described are linked biologically and cannot be easily segregated for scientific use. Fruits can be classified as being climacteric or nonclimacteric. Climacteric fruits are characterised by an increase in the rate of

respiration and ethylene production during ripening, whereas nonclimacteric fruits do not show any increase in either respiration or ethylene production (Biale and Young, 1981).

The work described in this thesis relates to the maturation of kiwifruit, that is to those processes and changes that lead to ripening. This study did not examine the climacteric, effect of ethylene or postharvest storage of kiwifruit. These points will, therefore, be briefly discussed in this review so the reader can comprehend the context of the biological processes of growth, maturation and ripening in which to place this work.

1.2.1 Fruit growth

Fruits that we eat are developed from many different parts of the inflorescence (Coombe, 1976) and we should expect, therefore, differences in growth and development. The pattern of fruit growth has been estimated for many species by measuring changes in fruit size using either linear dimensions or fluid displacement (Coombe, 1976). Some fruits display a pattern of growth that follows a single sigmoid growth curve. Here, an initial period of slow growth during rapid cell division after anthesis is followed by a period of major increase in size dominated by cell expansion, and a final period where growth rate decreases and ripening is initiated (Rhodes, 1980). Examples of fruit exhibiting single sigmoid growth curves are apple (*Malus domestica* Borkh) (Rhodes, 1980), pear (*Pyrus communis* L.) (Mann and Singh, 1988), guava (*Psidium guajava* L.) (Yusof and Mohamed, 1987) and muskmelon (*Cucumis melo* L.) (McCollum *et al.*, 1988). In contrast, other fruit exhibit double sigmoid curves where rapid growth phases are interspersed with one or two periods of little or no growth. Double sigmoid growth curves are exhibited by a number of different fruit. The initial lag phase is succeeded by a period of initial growth, followed in sequence by a period of little growth while the embryo develops and the pit (endocarp) hardens, then by rapid growth as the flesh (mesocarp) expands as in apricot (*Prunus armeniaca* L.) (Reid and Bieleski, 1974), cherry (*Prunus cerasus* L.)

(Pollack *et al.*, 1961), grape (*Vitis vinifera* L.) (Nii and Coombe, 1983), fig (*Ficus carica* L.) (Tsantili, 1990), feijoa (*Feijoa sellowiana* Berb) (Harman, 1987), and boysenberry (*Rubus* hybrid) (Given *et al.*, 1986). Kiwifruit were thought to be characterised by a triple sigmoid growth curve (Pratt and Reid, 1974), but Hopping (1976) and more recently Sawanobori and Shimura (1990) and Walton and de Jong (1990a) have described fruit growth as a single sigmoid curve.

1.2.2 Maturation and ripening

During maturation and ripening many changes occur in the fruit tissues. In kiwifruit these changes include, among others, changes in carbohydrate metabolism, cell wall metabolism and the patterns of ethylene production and respiration.

Carbohydrate metabolism

A common feature of most fruit is accumulation of carbohydrate during development. Some fruit store carbohydrate as starch which is later hydrolysed, while others accumulate all or some of the following: glucose, fructose, sucrose, sorbitol and inositol. In fruit containing starch as the storage carbohydrate, the concentration of starch increases during growth and then decreases as a result of hydrolysis to sugars during maturation and ripening, during which there is typically a concomitant rise in total sugar. This occurs in apple (Ohmiya and Kakiuchi, 1990), banana (*Musa* spp.) (Marriott *et al.*, 1981; Agravante *et al.*, 1990), kiwifruit (Okuse and Ryugo, 1981; Sawanobori and Shimura, 1990), pear (Mann and Singh, 1988) and tomato (*Lycopersicon esculentum* Mill.) (Dinar and Stevens, 1981; Garvey and Hewitt, 1991).

Fruit such as grape, melon and pineapple (*Ananas comosus* (L.) Merrill) accumulate sucrose, glucose and fructose and do not store starch (Tucker and Grierson, 1987). In addition, in the Rosaceae family (apple, apricot, nashi (*Pyrus pyrifolia* (Burm. f.)), peach) sorbitol is the major form of translocated

photosynthate; which is subsequently converted to other sugars in the fruit (Reid and Bieleski, 1974; Moriguchi *et al.*, 1990; Moriguchi *et al.*, 1992). In contrast, in kiwifruit sucrose is probably the major form of translocated photosynthate; although the fruit of kiwifruit are capable of photosynthesis, this is likely to provide a minor part of fruit carbohydrate requirement (Wegrzyn and MacRae, 1991).

In immature peach, Moriguchi *et al.* (1990) found that glucose and fructose were predominant, while the concentration of sucrose and sorbitol remained low. However, concentration of sucrose increased markedly to become the dominant compound in mature fruit (70% of total sugar). Similar patterns of sucrose contributions in mature fruit have been found in apricot (Reid and Bieleski, 1974; Nigam and Sharma, 1987). Grapes do not store starch, but at veraison (beginning of berry ripening) there is rapid accumulation of glucose and fructose, with very little sucrose in the flesh tissue (Nii and Coombe, 1983). This is similar to changes that occur in boysenberry (Given *et al.*, 1986). In all of these fruit, concentrations of sucrose, glucose and fructose increased during maturation and ripening due to accumulation from assimilate, but in starch-containing fruit, also to breakdown of stored carbohydrate.

In banana, Agravante *et al.* (1990) found that green fruit contained 10% starch and 1% sugar but that during ripening starch decreased to less than 1% and sugar concentration increased to 20% of fresh weight. Concentration of sucrose was equal to that of glucose and fructose 8 days before banana fruit were fully ripe (Marriott *et al.*, 1981), whereas in tomato, sucrose concentration remained low throughout maturation and ripening while glucose and fructose concentration increased (Garvey and Hewitt, 1991). However, results from Garvey and Hewitt (1991) should be treated with caution as the analysis procedure was unlikely to have inhibited invertase activity. Total sugar concentration in an eating-ripe kiwifruit ranges from 8 to 15% of the fresh weight; the major constituents are 2 to 6% glucose, 1.5 to 8% fructose and about 2% sucrose (Kawamata, 1977; Matsumoto *et al.*, 1983). Trace amounts of inositol have also been found (Kawamata, 1977; MacRae *et al.*, 1989a; Walton and de Jong, 1990a). The

absolute concentrations of sugars and acids, as well as the sugar:acid ratio, are important in the taste and therefore quality of ripe fruit (Rhodes, 1980). Pathways of sugar and starch synthesis are described more fully in Section 1.3. A trigger mechanism for conversion of starch to sugar has not been identified, but the process appears to be faster at cooler than warmer temperatures (see Section 1.3.2).

Carbohydrate concentration varies between different tissues in kiwifruit (MacRae *et al.*, 1989a). Similar concentrations of soluble sugars were found in the outer pericarp and core during fruit maturation. However, during the same period there was a gradient in SSC between distal and proximal ends of fruit with the distal end being highest. Soluble solids concentration of juice indicates the concentration of compounds in fruit that are soluble in water such as sugars, acids, vitamin C, amino acids, ions and some pectin, and is measured by refractive index (Harman and Watkins, 1986). Concentration of starch in the core was higher, but more variable, than in the outer pericarp. Numbers of starch grains in the outer pericarp and core of fruit with a firmness 75.5 N were estimated to be 380,000 and 1,280,000 grains mm^{-3} tissue, respectively (Wegrzyn and MacRae, 1991). In fruit near eating ripeness, where firmness was 14.7 N, the number of starch grains had decreased to 70,000 and 410,000 mm^{-3} tissue in the outer pericarp and core, respectively. These figures for changes in the number of starch grains in ripening kiwifruit correlated well with loss of starch measured by chemical methods. Hallett *et al.* (1992) showed that there were spherical and ellipsoidal cells of two size groupings in the outer pericarp: individual or small groups of large cells (0.5 to 0.8 mm cross-sectional diameter) in a matrix of smaller cells (0.1 to 0.2 mm cross-sectional diameter). Patterson *et al.* (1991) reported that the small cells were packed with starch grains and the larger cells were devoid of starch. Detailed examination of core tissue showed that it was composed of relatively uniform spherical/ellipsoidal cells of 0.1 to 0.2 mm cross-sectional diameter (Gould *et al.*, 1992; Hallett *et al.*, 1992). If the small cells in the core were the type that contained starch, then this may explain the higher concentration of starch in the core compared to the outer pericarp.

Cell wall metabolism

Changes in texture of fruit during ripening result from changes in the structure and composition of their cell walls (Rhodes, 1980). In addition, there are other biological changes occurring simultaneously such as pigment biosynthesis and production of volatiles (Fischer and Bennett, 1991). Modifications to cell walls are believed to be the major causes of softening and hence of textural change. The softening process is an integral part of ripening in almost all fruits. Decreases in fruit firmness during maturation and ripening have been demonstrated in many species including apple (Workman, 1963), fig (Tsantili, 1990), guava (Yusof and Mohamed, 1987), kiwifruit (MacRae *et al.*, 1989a), melon (Miccolis and Saltveit, 1991), peach (Maness *et al.*, 1992) and pear (Mann and Singh, 1988). In kiwifruit, the decrease was faster when fruit (especially immature fruit) were treated with ethylene (MacRae *et al.*, 1989b).

An understanding of cell wall composition is necessary before discussing the changes that occur during ripening. Carbohydrate polymers typically form 90 to 95% of the structural components of cell walls, the remaining 5 to 10% being glycoprotein (Tucker and Grierson, 1987). Cell wall material of kiwifruit contained 1.4, 2.2 and 2.7% protein in the outer pericarp, core and inner pericarp, respectively; the remaining 97.3 to 98.6% was carbohydrate (Redgwell *et al.*, 1988). Higher plants all contain the polysaccharides cellulose, hemicellulose and pectin, but in different proportions. Cellulose consists of linear chains of ß-1,4 linked D-glucan forming structures known as microfibrils. Hemicelluloses are composed of several polymers, the major ones in higher plants being xyloglucans, glucomannans and galactoglucomannans. Neutral pectins are arabinans, galactans or arabinogalactans, while acidic pectins have rhamnogalacturonan backbones (Tucker and Grierson, 1987).

Ultrastructural and chemical studies on fruit have demonstrated that compositional changes occur in the cell wall during ripening. These changes result from the action of cell wall degrading enzymes such as polygalacturonase

(PG), pectinmethylesterase (PE) and cellulase. Activities of such enzymes can be low or absent in unripe fruit but increase during the ripening process (Rhodes, 1980). Polygalacturonase is involved in solubilisation of pectin; both endo- and exo-PG are found in a wide range of fruits and this enzyme has been linked to tissue softening (Tucker and Grierson, 1987).

Polygalacturonase was thought to be the key enzyme in tomato fruit softening (Hobson and Harman, 1986), but recent work has indicated that it is probably not the primary agent (Smith *et al.*, 1988). Fruit softening has been studied extensively in tomato, as there are several wild type lines ('normal' fruit) and several ripening impaired mutant lines, for example, non ripening (*nor*), ripening inhibitor (*rin*) and neverripe (*Nr*) (Brady, 1987). Molecular biology has been used to alter the expression of PG in tomato such that the physiological and biochemical expression of this altered expression could be assessed in vivo (DellaPenna and Giovannoni, 1991). Two approaches have been used. Firstly, mutant complementation where the expression of PG is forced in a mutant background (such as *rin*) which does not normally express the protein (DellaPenna *et al.*, 1987; Giovannoni *et al.*, 1989). The greatly reduced level of PG mRNA in *rin* fruit is due to a near total block of PG gene transcription (DellaPenna *et al.*, 1989). Secondly, insertion of the PG gene into wild type fruit, but in its reverse (antisense) orientation reduced the level of endogenous PG expression (Smith *et al.*, 1988). The mutant complementation and antisense experiments have provided surprising and apparently conclusive evidence that the high level of PG activity expressed during tomato fruit ripening is both sufficient and necessary for pectin degradation and solubilisation, but not sufficient to cause softening of the fruit tissue (DellaPenna and Giovannoni, 1991). Similarly, in peach two PG enzymes increased in activity during the latter part of ripening when fruit were already very soft (Downs *et al.*, 1992). In kiwifruit, pectin solubilised early in the ripening process has a high molecular weight and is similar to that bound within the cell walls. Later, the solubilised pectin is degraded. Redgwell *et al.* (1992) suggested that PG may not be a major factor

in this solubilisation process, but that it is involved in depolymerising the solubilised pectin.

Pectinmethylesterase de-esterifies pectin and makes it more available to PG attack. Pectinmethylesterase is present in large amounts in unripe fruit, suggesting that there must be some mechanism in cells to prevent its activity (Tucker and Grierson, 1987). A 10% de-esterification of cell wall pectin was found early in kiwifruit softening, and this process continued throughout softening (Redgwell *et al.*, 1990; Redgwell *et al.*, 1992).

Cellulase levels are low in unripe fruit, but differ markedly between species in ripe fruit (Tucker and Grierson, 1987). Cell walls of kiwifruit swell during softening (after most of the pectin has been solubilised) and this may be due to the action of cellulase or hemicellulase. A decrease in xyloglucan has been measured (Redgwell *et al.*, 1992) and during later stages of ripening (after an ethylene treatment) a decrease occurs in the concentration of galactose from cell walls. The same processes occurred in non-ethylene and ethylene-treated fruit, but the relative timing of each process was different (Redgwell and Percy, 1992). Non-structural as well as structural carbohydrates may be involved in fruit softening. Starch degradation could be important in the early stages of kiwifruit softening (MacRae *et al.*, 1989b); the starch grains may not act as a physical barrier, but during their degradation affect the osmotic pressure and turgor within the cell (MacRae and Redgwell, 1992).

Climacteric and ethylene

Fruit of different species and genera can be classified as climacteric or nonclimacteric, the two groups differing in patterns of respiration and ethylene synthesis during ripening. Ethylene is a plant hormone which regulates many aspects of growth, development and senescence. As with other hormones, ethylene is thought to bind to a receptor to form an activated complex which triggers subsequent reactions leading to physiological responses (Yang, 1985).

All climacteric fruit, such as stonefruit and pipfruit, are characterised by transient increases in both respiration and ethylene synthesis at an early stage of ripening, but in kiwifruit this occurs at a later stage (Paterson V.J. *et al.*, 1991). In contrast, nonclimacteric fruit including citrus and grape do not show these increases, indeed there is a decrease in respiration rate throughout the ripening process, with a similar decrease in ethylene production (Tucker and Grierson, 1987).

Two systems of ethylene production have been proposed (McMurchie *et al.*, 1972); system I is common to nonclimacteric and climacteric fruit until ripening commences, while climacteric fruit also operate system II which results in a massive increase in ethylene production by the tissues, followed by ripening and senescence. System I is the low level of ethylene present in fruit before the onset of ripening, while system II is the autocatalytic burst of ethylene production accompanying the ripening process. In preclimacteric fruit, resistance to ripening (or resistance to ethylene action) is so high that the ripening process is not initiated. During maturation there is a progressive decrease in resistance to ethylene action (or an increase in sensitivity to ethylene action) and this process is thought to be controlled by endogenous ethylene (Yang, 1985). When resistance to ethylene action decreases to a point at which fruit tissues become responsive to their endogenous ethylene levels, the ripening process is initiated, resulting in the autocatalytic burst of ethylene production (system II). The important factor that triggers the onset of ripening is the decrease in resistance (or an increase in the sensitivity) to ethylene action (Yang, 1985).

Climacteric and nonclimacteric fruit also differ in their response to exogenous ethylene (Tucker and Grierson, 1987). In nonclimacteric fruit, application of exogenous ethylene increases the rate of respiration in proportion to the concentration of ethylene applied, and the rate of respiration decreases to a basal level when ethylene is removed. Applying ethylene to climacteric fruit hastens the onset of the respiration peak, the magnitude of the response being independent of the concentration of applied ethylene. The difference between the response of climacteric and nonclimacteric fruit to added ethylene is due to autocatalytic

synthesis of ethylene in climacteric fruit. Once synthesis is triggered ethylene concentrations will increase so that the final respiration rate is independent of the original ethylene concentration (Tucker and Grierson, 1987).

Tissues are exposed to low levels of ethylene (system I) throughout development. Exposure to higher concentrations of ethylene produces, in fruit tissues, a response that varies between species and with fruit maturity (Brady, 1987). Some climacteric fruit, such as banana, are more sensitive to applied ethylene and ripen faster than less sensitive species such as tomato or apple.

Two key enzymes involved with ethylene synthesis are 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (which catalyses the conversion of S-adenosylmethionine (SAM) to ACC and ACC oxidase (which catalyses the conversion of ACC to ethylene) (Tucker and Grierson, 1987). The concentration of ACC and activity of ACC synthase are very low in preclimacteric fruit. The rate limiting enzyme in preclimacteric fruit is ACC synthase, as ACC oxidase has a capacity in excess of ACC synthase at all maturities (Brady, 1987).

Kiwifruit behave as climacteric fruit as they ripen. When the respiration rate rises towards the end of ripening, the concentration of ethylene also rises before falling as fruit reach senescence (Pratt and Reid, 1974). In kiwifruit these increases occurred after the flesh had softened considerably and when it was at eating ripeness (Paterson V.J. *et al.*, 1991), that is, after the hydrolysis of starch and increase in sugar concentration that occurred during maturation. These results are in contrast to other climacteric fruit (such as banana) where starch degradation occurs at the same time as the climacteric peak (Rhodes, 1971). Addition of ethylene to kiwifruit resulted in rapid softening (ripening) of the fruit at 20C (Pratt and Reid, 1974; Matsumoto *et al.*, 1983; MacRae *et al.*, 1989b) and in fruit stored at 0C with 0.1 $\mu l\ l^{-1}$ ethylene, compared to ethylene-free control fruit (maximum ethylene 0.02 $\mu l\ l^{-1}$) (Harris, 1981). However, fruit cells must be ready to ripen before they can respond to ethylene (Romani, 1987) so that factors other than ethylene may determine whether a fruit will ripen (Romani, 1984). Some

ripening changes in tomato are apparently independent of ethylene stimulation (Jeffery *et al.*, 1984) and ripening changes can still occur in pear even though ethylene production was blocked (Romani *et al.*, 1983). It is likely that many changes occur at the genetic level during maturation and are independent of the ethylene concentration.

1.2.3 Maturity indices

A maturity index is an arbitrary measurement used to determine whether a particular crop is at an appropriate maturity for harvest. The index should be objective rather than subjective and preferably nondestructive (Reid, 1992). Maturity measurements to be made by growers, exporters and quality control personnel must be simple and require relatively inexpensive equipment. Potential indices should be highly correlated to fruit quality and be consistent between seasons (Underhill and Wong, 1990). Fruit should be harvested once they are physiologically mature to ensure that they are of an acceptable quality to the consumer, since immature fruit do not maintain metabolic homeostasis and are likely to senesce rapidly (Huber, 1987). If kiwifruit are harvested too early they will not develop their full flavour and aroma; flesh softens more rapidly than in mature fruit and may have a water soaked appearance, whilst after storage the core may still be hard and the flesh have a bitter taste (Beever and Hopkirk, 1990). Peach should be harvested when skin colour changes from green to yellow, earlier harvests result in incomplete maturation and poor quality ripe fruit (Zucconi, 1986).

There are many changes that occur concurrently in fruit during maturation and some of these have been used to provide estimates of maturity (Reid, 1992). Generally a single indicator, or combination of several indicators, is used as the maturity index to determine the time of harvest of a crop. For example, number of days from full bloom to harvest in apple and pear, firmness in stonefruit, starch pattern index in apple, SSC in kiwifruit and sugar:acid ratio in citrus (*Citrus* spp.).

Skin ground colour was a feasible maturity index for peach (Delwiche and Baumgardner, 1985). Measurement of skin colour of dark sweet cherries (*Prunus avium* L.) for canning was proposed as the maturity index using reflectance colour (Drake *et al.*, 1982). Another maturation index is moisture content, used for harvesting guava (Yusof and Mohamed, 1987). Criteria evaluated as indicators of maturity in apple included calendar date, number of days from full bloom, accumulated 'heat-units' (see Section 1.4.3), skin ground colour, seed colour, flesh firmness, respiration rate, SSC, acid concentration, starch concentration, ethylene concentration and sugar:acid ratio (Dennis, 1986). Ethylene production was used as an indicator of harvest maturity for 'Delicious' apples (Smith *et al.*, 1969). The starch iodine test was evaluated on apple in New Zealand and found to be useful for predicting harvest dates and assessing maturity at harvest for some cultivars (Reid *et al.*, 1982b). Hesse and Hitz (1938) found that none of the indices they used proved wholly successful in forecasting maturity of 'Grimes' and 'Jonathon' apples. Flesh firmness was used as the maturity index for pear (Chen *et al.*, 1993). Often several indices are used rather than one. In apple, Truter and Hurndall (1988) used three or more maturity indices, measured weekly, to determine the optimum date of picking. More than one maturity index was recommended for rockmelons, where the melon should appear mature when inspected both externally and internally and SSC should be at least 8% (Wade, 1981).

In citrus the total soluble solids:total acid ratio is extremely sensitive as the acids decline and sugars increase towards ripeness; the ratio is considered the most reliable index of ripeness for citrus (except lemon (*Citrus limon* (L.) Burm f.) and lime (*Citrus* sp.)) (Monselise, 1986). A similar index (SSC:acid ratio) was recommended for determining lychee (*Litchi chinensis* Sonn.) harvest date in Queensland (Underhill and Wong, 1990).

A need for a suitable indicator of maturity was recognised for kiwifruit to ensure that fruit reached an appropriate stage of development before harvest. In the 1960s, harvest season for kiwifruit commenced on a specific date (1 May) and

fruit harvested before that date were not allowed to be exported (Beever and Hopkirk, 1990). This procedure was generally satisfactory and eliminated export of very immature fruit, but 1 May was an inappropriate date in some seasons. In 1979, some fruit harvested soon after 1 May and exported from New Zealand were considered to be of poor quality as the fruit showed symptoms characteristic of fruit harvested when immature (Beever and Hopkirk, 1990). Other maturation characteristics were investigated, but those such as external appearance and flesh colour do not change during kiwifruit maturation as they do in many other fruit (Beever and Hopkirk, 1990). Another unsatisfactory indicator was ethylene production, as it generally reached a maximum rate well after fruit softened (Pratt and Reid, 1974). During the period of final maturation in kiwifruit, changes in flesh firmness and SSC were found to occur with reasonable consistency (Harman, 1981). Flesh firmness decreased, but the pattern of decrease varied from year to year and did not relate to storage quality. As fruit matured SSC increased due to hydrolysis of starch to sugar, and SSC did relate well to fruit quality after storage. In addition, SSC has a consistent positive relationship with sugar concentration in kiwifruit (Harman, 1981) and can be easily measured, even in the field, by using a hand held refractometer calibrated to a sugar solution of known concentration (Harman and Hopkirk, 1982). The standard starch iodine test was unsuitable for kiwifruit because the purple staining was difficult to detect on the green flesh, and sugar concentration was not easy to measure (Beever and Hopkirk, 1990).

The kiwifruit industry in New Zealand has adopted 6.2% SSC at harvest as the minimum level for export fruit. The 6.2% SSC maturity index was selected on the basis of eating quality after storage and is approximately when hydrolysis of starch commences. The maturity index of a crop such as kiwifruit must be taken from a sample of fruit from different vines, because there are differences in SSC not only between vines but also between fruit from different positions on the same vine (Hopkirk *et al.*, 1986; Smith *et al.*, 1992). A similar problem was also found in the sampling of cherries (Drake and Fellman, 1987). Harman and Hopkirk (1982) showed that fruit from the same orchard block matured at different

rates in different years and that there were also differences among regions. This may have been due to the different growing conditions, for example temperatures experienced by vines between years or regions. The effect of temperature on rate of kiwifruit maturation has not yet been clearly defined (see Section 1.4.2). Recently, Hopkirk (1992) reviewed the development of the maturity standard for New Zealand kiwifruit and suggested that fruit should be picked at 7% SSC to ensure high quality fruit after long-term storage.

Maturity indices suitable in one country may not be appropriate to another country. There was no evidence that the maturity index used in New Zealand was the most appropriate for fruit grown in the warmer Australian climate, or for other cultivars such as 'Bruno' and 'Dexter' (Scott *et al.*, 1986). These workers related potential maturity tests to eating quality of the fruit; SSC at harvest did not predict the quality of kiwifruit grown in New South Wales. Instead they suggested using total solids (dry matter concentration) as a more relevant measure of fruit maturity. The main perceived advantage of the dry matter test is that it is a measure of total carbohydrate and is less influenced by the conversion of starch to sugars than the SSC (Hopkirk, 1991). Hopkirk (1991) states that the relationship between dry matter and the storage and eating quality of fruit from a range of sources must be evaluated thoroughly before the test could be used by the New Zealand kiwifruit industry.

1.3 Carbohydrates

Carbohydrates are important in plants in both structural and non-structural roles. In the context of this study the focus was on the role of non-structural carbohydrates, that is, those 'available' in the fruit cells as glucose, fructose, sucrose and also starch as a storage form of carbohydrate. The synthesis and degradation of sucrose and starch is discussed, as is the effect of temperature on cell metabolism (enzyme regulation of the biochemical pathways) and the function of carbohydrates in respiration. Of special interest is the effect of low

temperature on starch hydrolysis. Kiwifruit is a relatively new crop (see Section 1.1) so that, until recently, little research had been undertaken on its carbohydrate metabolism. As a result, much of the literature refers to other crops.

Carbohydrates form 60 to 90% of a plant's dry matter. Monosaccharides are the simplest sugars made up of a carbon chain with oxygen and hydrogen e.g. 5 carbon (pentose) or 6 carbon (hexose) sugars, the most common in fruits being the hexose, D-glucose. Complex sugars are a series of monosaccharides linked by glycosidic bonds and are called oligosaccharides. Sucrose, commonly found in plants, is a disaccharide composed of one unit of glucose and one of fructose. Maltose is comprised of two units of glucose. Polysaccharides are composed of more than ten monosaccharide units and tend to be variable in molecular weight and complex in terms of the constituent monosaccharides and the types of linkages (Hall *et al.*, 1974). Starch is a polysaccharide made up of glucose. Hexose phosphates, formed in photosynthesis, gluconeogenesis and breakdown of storage carbohydrates in higher plants, are used in glycolysis, the oxidative pentose phosphate pathway and in the synthesis of oligo- and polysaccharides (ap Rees, 1980). Details of the glycolytic pathway, the oxidative and reductive pentose phosphate pathways and gluconeogenesis are beyond the scope of this review but can be found in many texts and papers (e.g. Hall *et al.*, 1974; Dennis and Miernyk, 1982; Copeland and Turner, 1987). However, specific aspects, such as the effect of temperature on enzymes involved in the glycolytic and gluconeogenic pathways are discussed in Section 1.3.2.

Starch is the major storage polysaccharide in both photosynthetic and non-photosynthetic tissues. Many storage organs in plants contain starch, such as cereal grains, potato tubers (*Solanum tuberosum* L.), and fruit such as apple, banana and kiwifruit. The accumulation and subsequent use of storage carbohydrates or reserve substances are fundamental processes in living cells that enable them to maintain metabolic activities when exogenous sources are unavailable (Steup, 1988). Starch accounts for over 70% of the calorific value of the diet of the human race, but relatively little progress has been made towards

understanding its metabolism (ap Rees, 1988). Starch occurs as water insoluble granules. The size and shape of these granules depends on their botanical origin, for example pollen starch granules are 2 μm in diameter and those from barley (*Hordeum vulgare* L.) grains are 15 to 35 μm (Banks and Muir, 1980). Starch grains from kiwifruit are, on average, 5.5 μm in diameter, but most range in size from 3.0 to 7.0 μm (Fuke and Matsuoka, 1984). There are two structurally different polysaccharides in the granules. Amylose is mainly composed of long linear chains of 1,4-linked α-D glucopyranose residues and accounts for 20 to 30% of normal starch. In kiwifruit, the amylose content of starch grains has been estimated to range from 10.8% (Fuke and Matsuoka, 1984) to 20% (E. MacRae, HortResearch, Auckland, unpublished data). The other main component is amylopectin (α1,6-linkages), which is a macromolecule consisting of short amylose chains linked into a branched structure (Kainuma, 1988). Starch in banana contains about 80% amylopectin (Garcia and Lajolo, 1988), similar to kiwifruit (E. MacRae, HortResearch, Auckland, unpublished data). Development of starch granules is similar in many plant species. As the organ develops the amylose content of starch increases in potato, pea (*Pisum sativum* L.), barley, wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) (Banks and Muir, 1980). In pea, increases in granule diameter corresponded to an increase in starch concentration.

1.3.1 Synthesis and degradation of starch

Photosynthesis is the dominant process responsible for hexose phosphate formation in plants. The reductive pentose phosphate pathway is the only pathway known to be capable of forming sugars from carbon dioxide in plants (ap Rees, 1980). This pathway is a starting point for many biosynthetic sequences, but conversion of hexose phosphate to sucrose or starch is the most important. The initial source of hexose phosphate in non-photosynthetic cells are compounds translocated from leaves via the phloem; these are mainly sucrose but include sugar alcohols in some species (ap Rees, 1988). Phloem transport

of sucrose depends on a decreasing gradient of sucrose from source (leaves) to sink (fruit) tissue.

The enzyme sucrose 6-P synthase (SPS) catalyses formation of sucrose. However, there are three enzymes connected with sucrose degradation: acid invertase, alkaline invertase and sucrose synthase (Fig. 1.1). Invertases catalyse the conversion of sucrose to glucose and fructose, where alkaline invertase is present in the cytoplasm and acid invertase in the vacuole. Nielsen *et al.* (1991) studied carbohydrate metabolism during fruit development in sweet pepper (*Capsicum annuum* L.), where in young fruits the invertases were active in sucrose breakdown, in contrast to sucrose synthase, which was more active during maturation and ripening than the invertases. In cultivated tomato (*L. esculentum*) invertase activity was high during final stages of ripening, resulting in high concentrations of glucose and fructose compared to sucrose (Yelle *et al.*, 1988; Stommel, 1992). In contrast, in wild type tomato *L. peruvianum* (Stommel, 1992) and *L. chmielewskii* (Yelle *et al.*, 1988) invertase activity was minimal, resulting in a higher concentration of sucrose than glucose and fructose in ripe fruit. The activity of acid invertase reflected the changes in sugar concentration in potato tubers, whereas there was no evidence of a specific alkaline invertase (Richardson *et al.*, 1990). In potato tubers (Morrell and ap Rees, 1986a) and tomato (Wang *et al.*, 1993) sucrose synthase was more active than the invertases in sucrose breakdown. When sucrose synthesis exceeds the capacity of the leaf to either transport or store sucrose, a regulatory mechanism operates to reduce photosynthesis and divert sucrose into starch. Similar conversion of sucrose to starch may occur in fruit tissue.

There are two forms of soluble starch synthase specific for ADP-glucose, and a starch granule-bound starch synthase is also active with UDP-glucose (Preiss, 1988). The enzyme ADP-glucose phosphorylase catalyses the rate limiting step of starch synthesis (G1P to ADPG). Degradation occurs when oligo- or polysaccharides are converted into products having a lower degree of

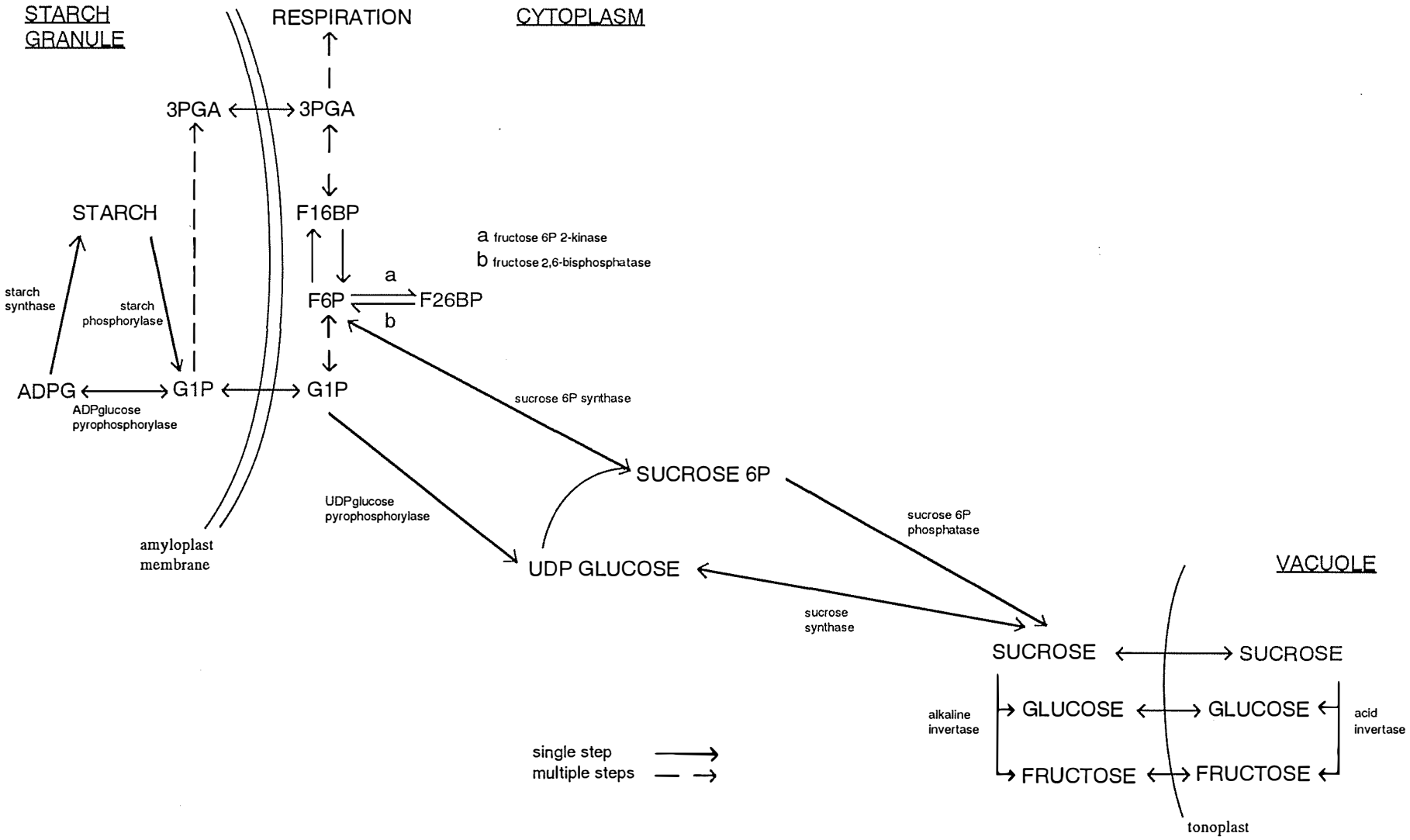


Fig. 1.1 A theoretical scheme for the partitioning of carbon in potato tubers (adapted from Sowokinos, 1990b).

polymerisation (Steup, 1988). Starch can be degraded by amylase, debranching enzymes and phosphorylase.

Amylases are a group of three different enzymes that hydrolyse starch. The enzyme α-amylase cleaves internal α-1,4 linkages of linear or branched starch into a mixture of linear malto-oligosaccharides and branched oligosaccharides which are hydrolysed further, for example to glucose or maltose. The enzyme ß-amylase catalyses successive hydrolysis of alternate α-1,4 linkages from the non-reducing end of amylose and amylopectin to release ß-maltose. The action of both enzymes is blocked by α-D(1,6) branch linkages. α-glucosidase cleaves both α-1,4 and α-1,6 linkages and can convert branched and unbranched chains to glucose (Steup, 1988). Debranching enzymes hydrolyse only α-1,6 bonds but require α-1,4 linkages to be present in the starch (Preiss and Levi, 1980; Kainuma, 1988; Steup, 1988). Degradation of starch in banana was hydrolytic, that is, as a result of amylase action rather than phosphorolytic cleavage of starch (Iyare and Ekwukoma, 1992). Phosphorolysis is the other major pathway of starch breakdown, as shown in potato tubers (Sowokinos, 1990a) and tomato fruit (Robinson *et al.*, 1988). Phosphorylases attack the α-1,4 glucan chains from the non-reducing end to give glucose 1-phosphate (G1P) (Hall *et al.*, 1974). Starch hydrolysis may occur firstly by action of α-amylase, secondly by debranching enzymes followed by phosphorolysis of the remaining fragments. However, in this thesis the term hydrolysis has been used as a general term to indicate degradation of starch.

In vitro experiments have indicated that the initial attack on a storage starch granule could be by α-amylase, the rate depending on source and size of the starch granule and properties of the respective α-amylase (Steup, 1988). Degradation often commences at a few sites on the starch granule, forming cavities and allowing rapid degradation of the inner region, leaving a hollow shell. Alternatively, surface erosion can be dominant. The entire reaction sequence of starch degradation has not been defined (Steup, 1988), but reserve starch degradation often begins after a large increase in the amount and activity of

α-amylase. Seven amylases (3 α- and 4 ß-amylase) were detected in banana and their activity increased during fruit maturation (Garcia and Lajolo, 1988). These researchers examined not only the concentration of starch in banana during maturation and ripening, but also the starch granule sizes. In green bananas (18.4% starch), 60% of the granules were 10 to 20 μm diameter and 15% were smaller than 10 μm. Granules in bananas with 3.2% starch were smaller with about 60% being less than 10 μm.

Coordination of starch synthesis and degradation must be finely regulated; if both systems operated simultaneously it would result in a futile cycle (Preiss, 1988). Metabolic pathways are often located in subcellular compartments, allowing different and often opposing metabolic processes to occur simultaneously (ap Rees, 1980). Transport mechanisms are required to 'carry' metabolites from one subcellular compartment to another if these metabolites do not diffuse freely. Many experiments have been undertaken to determine the location of the enzymes involved in synthesis and degradation of starch, in order to identify which processes occur in the amyloplasts (chloroplasts are similar and can transform into amyloplasts and vice versa) and the cytosol (Preiss, 1988). A diagrammatic scheme of the suggested pathways involved in carbohydrate oxidation in chloroplasts was put forward by ap Rees (1985).

1.3.2 Effect of temperature

Temperature affects the rate of chemical reactions not only in vitro but also in vivo. One aim of this study was to investigate the effect of temperature, especially low temperature, on the rate of kiwifruit maturation. In New Zealand, kiwifruit maturity is determined by the SSC of juice from the fruit. Fruit can be harvested for export once the SSC exceeds a minimum of 6.2% (commercial maturity). There is a difference in timing of commercial maturity in the different kiwifruit production areas in the country and from year to year; these variations may be due to different temperatures prevailing during the growing season.

There is very sparse information on the conversion of starch to sugar in relation to kiwifruit, but it has been extensively studied in other crops, such as potato, where temperature effects on this process have been studied. In particular, much reference is made to the cold induced sweetening in harvested potato tubers. This process is important to the potato processing industry which requires tubers after storage to have a low sugar concentration, thereby avoiding a non-enzymatic browning reaction between reducing sugars and amino acids during processing which will lower potato chip quality (Shallenberger *et al.*, 1959).

It is assumed in this study that similar biochemical processes are occurring at cold temperatures in both the harvested potato tuber and in fruit of kiwifruit that are growing on the vine. Products of starch degradation in isolated storage tissue, such as the potato, are relatively straightforward to determine. In kiwifruit however, starch degradation during the later stages of fruit maturation will account for only part of the increase in sugars, as some will be translocated from the leaves via photosynthesis.

As early as 1882, Müller-Thurgau detected accumulation of reducing sugars in potato tubers stored at low temperatures, and their subsequent disappearance when tubers were returned to higher temperatures. Many experimenters have shown that 'cold sweetening' occurs in potato at low temperatures is due to increase in sugar as a result of hydrolysis of starch (Pressey and Shaw, 1966; Isherwood, 1973; Pollock and ap Rees, 1975; Dixon and ap Rees, 1980b; Morrell and ap Rees, 1986a; Sowokinos, 1990a). Isherwood (1973) found that concentration of total sugar in potato increased when temperature was changed from 10 to 2C and was almost entirely due to conversion of starch to sugar (when an allowance was made for respiration). The decrease in sugar and increase in starch which occurred when tubers were transferred from 2 to 10C was accounted for by resynthesis of starch from sugar. Pressey and Shaw (1966), Pollock and ap Rees (1975) and Morrell and ap Rees (1986a) all demonstrated an increase in sugar when potato tubers were stored at 2, 4 or 5C compared to 10, 18 or 10C, respectively. The concentration of glucose increased when potato

tubers were transferred from 9 to 3C and decreased when placed at 18C (Sowokinos, 1990b). Unfortunately none of these authors measured starch concentrations during the experiments.

Conversion of starch to sugars at low temperatures has also been recorded in tomato fruit (Walker and Ho, 1977) and in tulip (*Tulipa gesneriana* L.) bulbs (Haaland and Wickstrøm, 1975). The enhanced accumulation of sucrose in cooled (5C) tomato fruit compared to those at 25C was thought to result from both inhibition of sucrose hydrolysis and increased hydrolysis of starch (Walker and Ho, 1977). The concentration of starch in scales of tulip bulbs stored at 5C was lower, but the sugar concentration was higher than in bulbs stored at 21C for 15 weeks (Haaland and Wickstrøm, 1975). Similar patterns of a decrease in starch and increase in sugar concentration were seen in tulip bulbs cool-stored for 6 weeks at 9C compared to those stored at 18C (Davies and Kempton, 1975). Not only was the concentration of sugar (glucose and fructose) in tulip bulbs higher after 6 weeks storage at 5 than 21C, but there were differences in glucose and fructose concentrations between cultivars (Moe and Wickstrøm, 1973).

Why are these changes occurring with temperature? What are the metabolic processes involved? These are difficult questions to answer because little is currently known. Experiments have been undertaken to measure enzyme concentrations of potato tubers stored at different temperatures (Pressey and Shaw, 1966; Pollock and ap Rees, 1975; Morrell and ap Rees, 1986a), while others have postulated possible mechanisms (Dixon and ap Rees, 1980b). New information is being generated and only recently a new sugar (fructose 2,6 - bisphosphate) was discovered to be an important regulator of carbohydrate metabolism (Copeland and Turner, 1987).

Regulatory enzymes

All of the enzymes in a metabolic pathway will contribute to the magnitude of the flux, but there are also certain steps that are catalysed by regulatory enzymes

and these have particular significance for control. A summary of the reactions of glycolysis and gluconeogenesis is shown in Fig. 1.2. Rates of all enzymes in the pathway will be affected by an increase or decrease in the activity of an enzyme that has a regulatory function. The first committed step in a metabolic pathway, and its branch points, are strategic points for control, often catalysed by a regulatory enzyme (Copeland and Turner, 1987).

Available evidence suggests that phosphofructokinase and pyruvate kinase are the main control points of glycolysis in plant tissues. Reactions catalysed by hexokinase (glucose to glucose 6-phosphate), phosphofructokinase (fructose 6-phosphate to fructose 1,6-bisphosphate) and pyruvate kinase (phosphoenolpyruvate to pyruvate) are strongly exergonic and effectively irreversible. These reactions have a negative free energy change and release energy to their surroundings. Hall *et al.* (1974) give the standard free energy changes in the glycolytic conversion of 1 mole of glucose to 2 moles of pyruvate as -3.4, -3.4 and -7.5 kcal $mole^{-1}$ for hexokinase, phosphofructokinase (PFK) and pyruvate kinase, respectively. Alternative reverse reactions are necessary to overcome the thermodynamically unfavourable steps; for example, fructose 1,6-bisphosphatase (F16BP) catalyses the reverse reaction of PFK and is considered a regulatory enzyme in the gluconeogenic pathway (Hall *et al.*, 1974). Dixon and ap Rees (1980a), from work on mature potato tubers, concluded that entry into glycolysis is regulated by PFK, while pyruvate kinase controls the movement of carbon out of glycolysis and into the oxidative pentose phosphate pathway.

Pollock and ap Rees (1975) found that the temperature coefficients of PFK and pyruvate kinase in the range 2 to 10C were higher than in the range 10 to 25C. Activities of these enzymes were reduced by lowering the temperature, suggesting that PFK and pyruvate kinase were cold labile. This instability may create a favourable environment for the reverse reactions to occur; that is, for gluconeogenesis to proceed as starch is converted to sugar. If glycolysis was inhibited at low temperatures, then hexose phosphates would be expected to

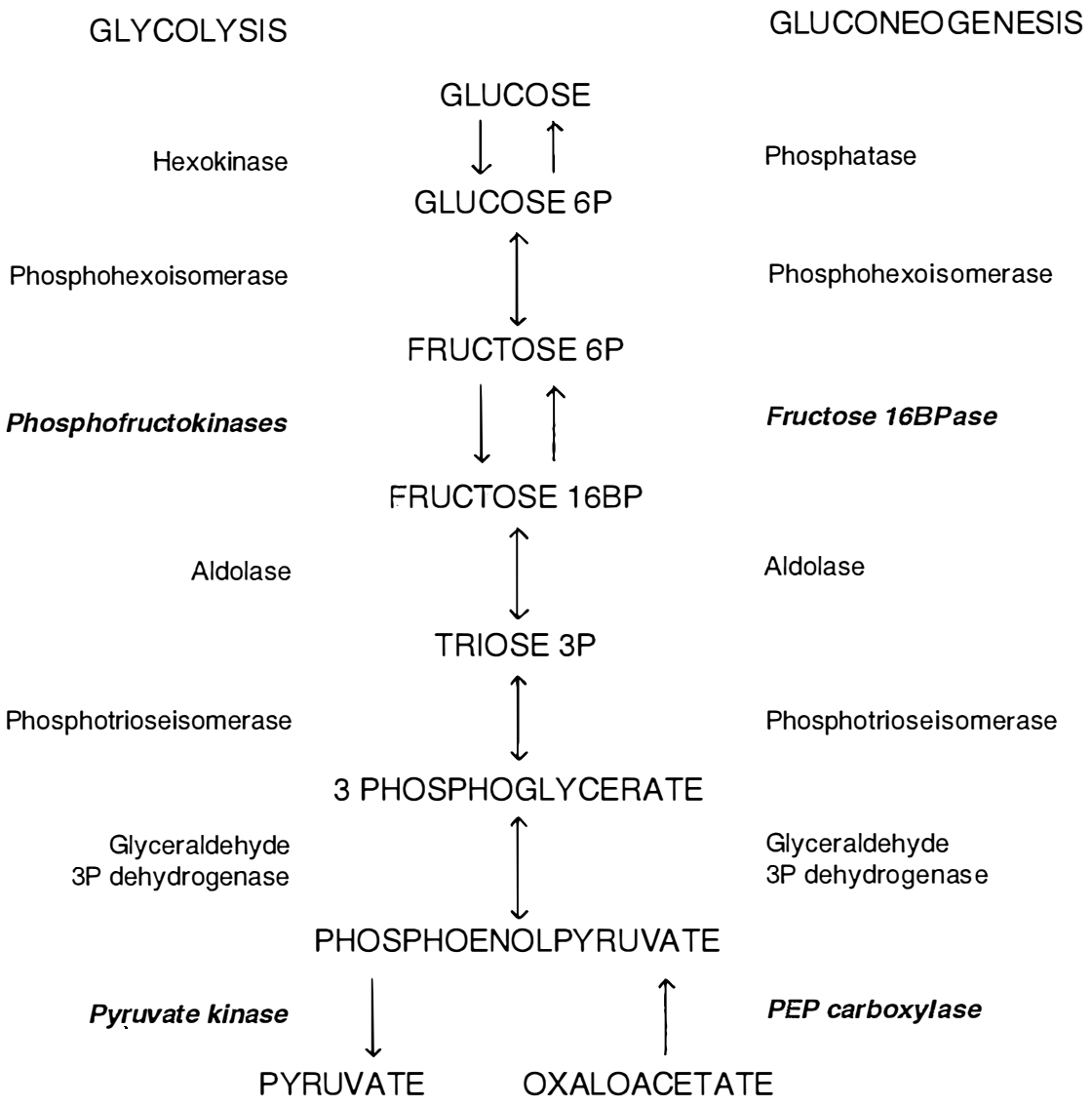


Fig. 1.2 Simplified summary of some reactions in glycolysis and gluconeogenesis, with controlling enzymes in ***italics*** (Hall *et al.*, 1974).

accumulate (Pollock and ap Rees, 1975). Similar conclusions were drawn by Dixon and ap Rees (1980b) from an experiment using a 2 hour pulse of labelled glucose followed by an 18 hour chase with unlabelled glucose. At 2C, the labelled hexose phosphate concentration decreased as these compounds were converted to free sugars (sucrose, glucose and fructose) during the chase. At 25C there was a much smaller increase in the sucrose and hexose concentration after the chase, while that of starch nearly doubled. This again indicated that the enzymes controlling glycolysis were sensitive to the cold.

Starch was the only significant carbon source for sucrose synthesis during cold induced sweetening in potato (Isherwood, 1973). The role that invertase plays in regulating the content of hexose in potato is still unclear (Sowokinos, 1990b). Once the initial postharvest rise in acid invertase had taken place, there was little evidence that invertase activity alone regulated the balance between sucrose versus glucose and fructose during subsequent storage at either 3 or 10C (Richardson *et al.*, 1990). Determination of in vivo acid invertase activity is complicated by the presence of an endogenous protein inhibitor (Pressey and Shaw, 1966). Activities of a range of degradative enzymes were measured in potato tubers stored at 5 or 10C for 17 weeks (Morrell and ap Rees, 1986a). The activities of sucrose synthase, acid and alkaline invertase were low and neither α- nor ß-amylase could be detected. However, starch breakdown in isolated tubers is probably phosphorolytic as there was high activity of α-glucan phosphorylase, a starch degrading enzyme, and this was significantly higher at 5 than at 10C. Sucrose 6-P synthase concentration was highly correlated to the glucose concentration of potato tubers when stored at low temperatures (Sowokinos, 1990b).

Biochemical pathways

An understanding is required of the changes in sugars that are occurring at the subcellular level. This section investigates the biochemical pathways that are likely to be involved (refer to Fig. 1.1).

The new hexose, fructose 2,6-bisphosphate (F26BP) which modulates sugar metabolism was discovered at the beginning of the 1980s (Stitt, 1987) At about the same time it was found that plants contain two sets of PFKs that catalyse the interconversion of F6P to F16BP (Black *et al.*, 1985). The phosphorylation of F6P is catalysed by an ATP-dependent enzyme (ATP-phosphofructokinase or PFKase) as described earlier, or a PPi-dependent enzyme (pyrophosphate: fructose 6-phosphate phosphotransferase or PFPase) which is stimulated by F26BP. The reactions are:

$$\text{F6P} + \text{ATP} \xrightarrow{\text{PFKase}} \text{ADP} + \text{F16BP}$$

$$\text{F6P} + \text{PPi} \underset{\text{PFPase}}{\rightleftharpoons} \text{Pi} + \text{F16BP}$$

$$\leftarrow \text{Gluconeogenesis} \qquad \text{Glycolysis} \rightarrow$$

There are differences in the way these two enzymes operate. The reaction above shows that PFPase is reversible. It is located in the cytoplasm, in contrast to PFKase which is in both the cytoplasm and the chloroplast. PFPase uses PPi as an energy source and is sensitive to the presence of F26BP, while PFKase is insensitive to F26BP. F16BPase is also sensitive to and inhibited by F26BP. The ability to inhibit this gluconeogenic enzyme indicates a regulatory site in triose to hexose conversions. PFPase was found to catalyse both the glycolytic and gluconeogenic flow of carbon between hexoses and trioses (Black *et al.*, 1985). PFPase catalyses a near-equilibrium reaction, but the role of PFPase and F26BP in plant metabolism is complex and not fully understood (Stitt, 1990). There was thought to be two interconvertible molecular forms of the enzyme, one sensitive to F26BP or high levels of PPi while the other form was insensitive (Wu *et al.*, 1983). The large form of PFPase is more active in glycolysis and the small one in gluconeogenesis. F26BP regulates the interconversion of PFPase into the

large and small forms. However, Stitt (1990) concludes that evidence for a change in molecular mass of PFPase was inconclusive.

Black *et al.* (1985) demonstrated that there is a plentiful supply of PPi in plant cells and the F6P to pyruvate step in glycolysis is driven by PPi, without ATP, and stimulated by F26BP. PFPase may contribute to gluconeogenesis, but cytosolic F16BP often plays a major role (Stitt, 1990). As the concentration of F26BP decreases, the inhibition of F16BPase is relieved. Morrell and ap Rees (1986b) measured a decrease in the concentration of F26BP and an increase in glucose in potato tubers stored at 4C compared to 20C, therefore indicating an inhibition of glycolysis. In sweet green pepper the concentration of F26BP was markedly lower when tissue slices were placed at 3C compared to 30C (Phelps and McDonald, 1989). It is likely that F26BP may play a role in increased conversion of starch to sugar at low temperatures.

Effect of warm temperatures

What happens to starch synthesis under warm temperatures? The corollary of much of the work on cold sweetening in potato is that the concentration of starch increases in tubers stored at warm temperatures. Incorporation of ^{14}C sucrose into starch in discs of potato tuber reached an optimum at 21.5C compared to incorporation over the temperature range 9 to 31C (Mohabir and John, 1988). Increased starch concentration was found at warm temperatures in tulip bulbs (Haaland and Wickstrøm, 1975) and in apical and basal kernels of maize (*Zea mays* L.) ears (Ou-Lee and Setter, 1985). The apical regions of maize ears were heated to 25C from 7 days after pollination to maturity to obviate the effects of low night temperatures and periods of cool weather. Tip heating hastened some of the developmental events in apical kernels. ADP-glucose phosphorylase and starch synthase reached peak levels and the starch concentration began rising earlier in heated than unheated apical kernels. The effect of tip heating on dry matter accumulation patterns could largely be attributed to differences in the starch accumulation rate.

Starch synthesis in chilling sensitive plants such as avocado (*Persea americana* Mill.), maize and sweet potato (*Ipomea batatas* Lam.) was inhibited in the chilling range of 0 to 12C but not inhibited in the chilling resistant potato (*S. tuberosum*) (Downton and Hawker, 1985). Asaoka *et al.* (1984) investigated the effects of temperature on the structure, amylose content and other properties of endosperm starch during development of rice plants grown in controlled environments. High temperatures (30C) decreased amylose and short-chained amylopectin content compared to plants grown at 25C, but increased the amount of long-chained amylopectin. Therefore, temperature affects not only the concentration of starch in plant tissue but also the molecular composition of that starch.

There are specialised tissues where starch degrades rapidly under hot temperatures. In Arum (*Arum* spp.) lilies there is a period of heat generation (thermogenesis) soon after the floral chamber opens and volatilisation of insect attracting compounds occurs (Halmer and Bewley, 1982). Starch, which comprises 40% of the spadix dry weight, is degraded completely within a few hours, generating a maximum temperature increase of 9C above ambient in 6 hours. The major pathway of mobilisation of starch is by α-amylase and glycolysis. There are increases in the activities of hexokinase, phosphoglucomutase, PFK (presumably PFKase) and amylase 6 to 18 hours before thermogenesis, to a sufficient concentration to accommodate the rate of carbohydrate consumption during the heating.

Rate of change

How fast can the metabolic systems respond to a change in temperature? Is there an immediate rise in sugars when the temperature is dropped, or a period of time before an increase in sugar is recorded?

Pressey and Shaw (1966) and Isherwood (1973) both carried out experiments on potato stored initially at cool and warm temperatures, and then reversed the treatments. Unfortunately, Pressey and Shaw (1966) did not measure starch

concentration, but total sugar concentration increased during the cold periods (4C) and decreased at 18C. Measurements were taken at 2 week intervals, which does not give a good indication of the rapidity of change. However, Isherwood (1973) took starch and sugar measurements at 2 to 8 day intervals and found changes in total sugar similar to those found by Pressey and Shaw (1966). In both mature and immature tubers there appeared to be a 4 day delay after the tubers were transferred from 10 to 2C before there was any noticeable change in total sugar concentration. There was no delay when the tubers were transferred back to 10C (measured 3 days after transfer), as the sugar concentration decreased immediately. At 10C, starch concentration did not change during the experiment (56 days), whereas at 2C it decreased immediately (measured 2 days after transfer) and then reached a plateau for the final 14 days. When tubers were transferred back to 10C starch concentration increased after a 2 day delay.

A similar type of transfer experiment has not previously been undertaken with kiwifruit. During maturation, one cold night was thought to result in an increase in SSC (G. Costa, University of Udine, Italy, pers. comm.), however the effect of cold temperature on the rate of increase in SSC has never been quantified. As fruit are approaching commercial maturity, it is essential to determine the effect of a period of cool or warm weather on the rate of kiwifruit maturation. This information is of value scientifically, for the grower and necessary for derivation of a mathematical model that relates temperature to the increase in SSC during maturation.

1.3.3 Respiration

Respiration involving uptake of oxygen and release of carbon dioxide, is the process providing energy for many components of growth. Respiration encompasses both complete oxidation of substrate to carbon dioxide so providing high energy compounds, and partial oxidation to provide intermediates for biosynthesis. In plants, carbohydrate is the main respiratory substrate, via

glycolysis and the oxidative pentose phosphate pathway. Respiration can be separated into growth and maintenance components (Penning de Vries, 1975). Growth respiration is the energy source for synthesis of new biomass, whereas maintenance respiration supplies energy for maintenance of current biomass (including protein turnover, maintenance of ion gradients, acclimatisation of the plant to environmental changes especially temperature).

Temperature sensitivity of respiration has been shown in kiwifruit (Wright and Heatherbell, 1967; Walton and de Jong, 1990a) and in harvested potato tubers (Isherwood, 1973; Dixon and ap Rees, 1980b). Each of these authors showed that rate of respiration decreased with a decrease in temperature. Walton and de Jong (1990b) measured fruit gas exchange on attached kiwifruit in the orchard throughout the season at temperatures of 15 to 40C at 5C intervals. They found that rate of respiration increased with temperature at all times during the growing season, but declined with time at a given temperature. In addition, there was a seasonal trend in respiration, where respiration rate of (detached) kiwifruit was highest early in the season and declined with time (Pratt and Reid, 1974).

1.4 Phenology and modelling

Experiments in plant science are usually designed to alter plant growth and development in some way to determine the effect of a treatment on the plant. Experiments described in this thesis were designed to investigate the effect of temperature on rate of fruit maturation. Data collected from such experiments can be used to develop mathematical models. There are many types of models that can be used, depending on the aim of the experiment and/or the data collected.

Phenology is the study of the effects of environmental factors on plant growth and development, and these studies often lead to formulation of mathematical models. Such models can predict the effects of temperature on plant growth, e.g. the chilling requirements for budbreak and subsequent flowering. Environmental

conditions (temperature, rainfall, irradiation, wind) will influence plant growth and development. For example, soybean (*Glycine max* (L.) Merrill) growth and development is primarily determined by temperature and photoperiod (George *et al.*, 1990). Floral initiation in soybean is primarily a photoperiodic response, whereas floral growth is regulated mainly by temperature, and both temperature and photoperiod influence post-flowering development. Separation of temperature and photoperiod effects in field trials is inherently difficult because, as planting date is altered through the season to give different temperature treatments, there will be a corresponding difference in photoperiod. George *et al.* (1990) circumvented the problem and isolated temperature as the major variable by using elevations within the same latitude which received similar photoperiod, rainfall and irradiance.

Many studies with fruit crops have shown differences in harvest date among sites, and at the same site among years. Kronenberg (1988) studied the effects of temperature on flowering and picking dates of ten apple cultivars at four different sites over several years. There was variation in mean flowering date and mean picking date both between sites and between years. The physical and chemical indicators of maturity in apple (e.g. SSC, starch pattern index, seed colour index) showed variation in concentration or index, respectively among seasons (Krishna Prakash *et al.*, 1988). In addition, there were differences in dry matter and sugar concentration of apple fruit measured at the same site but in two different years (Marguery and Sangwan, 1993). Apricots showed a 30 day difference in the date of ripening between two sites only 22 km apart (Lilleland, 1936).

Kiwifruit in British Columbia flowered several weeks later than in California, but fruit were picked at similar times (Kempler *et al.*, 1992). In New Zealand, SSC in the final maturation period before harvest varied in one orchard among seasons (Harman, 1981) and also among orchards at different geographical locations (Harman and Hopkirk, 1982; MacRae *et al.*, 1989a; H. McPherson, HortResearch, Auckland, pers. comm.; see also Section 1.2.3). In Japan, two orchards at

different locations were measured for 2 consecutive years and differences between the orchards and years in timing of maturity, final SSC and rate of starch breakdown were determined (Sawanobori and Shimura, 1990). One orchard was cooler than the other. At the cooler orchard, increase in SSC and rate of starch degradation were both faster than at the warmer orchard. These differences indicate that environmental conditions, especially temperature, may affect harvest date of kiwifruit between seasons and orchards.

1.4.1 Controlled environment facilities

Controlled environment facilities, such as growth rooms, in which temperature, daylength, light intensity and humidity can be controlled, are used to distinguish between the effects of environmental factors that are normally closely inter-related in natural environments. Different artificial climates can be made and even altered at various times during the growth period or at specific stages of growth. Thorne (1969) studied grain growth in wheat both in controlled environments and in the field and concluded that the good agreement between field and growth room results gave increased confidence to both sets of results. In discussing the use of controlled environment rooms, Thorne (1969) admitted that there are many differences between controlled environments and the field in factors other than those tested that will affect plant growth. "If results from experiments in controlled environments are to be incorporated into models of field growth, it is important to test that the responses to changing climatic factors in artificially lit environments also occur in natural light and in the field. This should be possible by using experimental designs and mathematical techniques once the qualitative and quantitative nature of the phenomena have been identified in the fully controlled conditions".

The work described in the remaining chapters of this thesis followed this approach. Temperature was isolated as probably being a major influence on kiwifruit maturation and these changes were quantified in the controlled environment treatments (Chapter 2). Accurate temperature records were also

made in four orchards in different growing regions in New Zealand during maturation (Chapter 3) and attempts to mathematically link these two approaches are discussed in Chapter 5.

Two research institutions in New Zealand have controlled environment facilities where temperature, light, humidity and carbon dioxide concentration can be controlled to study their influences on plant growth (Warrington and Rook, 1989). Both of these facilities were used to study the responses of conifers (e.g. *Pinus radiata* D. Don) to temperature in order to provide a better understanding of growth and development (Rook, 1989). Results obtained from the research, for example, seasonal differences in frost tolerance of seedlings, were found to be applicable to the forest environment.

Vegetative growth of 'Hayward' kiwifruit has been studied at different temperatures and photosynthetic photon flux densities using controlled environments (Morgan *et al.*, 1985); growth was more sensitive to temperature over the range 10 to 30C than to a reduction in photosynthetic photon flux density from 650 to 280 μmol s^{-1} m^{-2}. Growth, measured by leaf number and area, shoot length, shoot and total dry weight, was lowest at 10C and highest between 20 and 30C. 'Winter' and 'spring' temperatures were manipulated in controlled environments to determine the influence of pre- and post-budbreak temperatures on flowering in kiwifruit (Warrington and Stanley, 1986). Percentage budbreak was highest in treatments with the coolest pre-budbreak temperature (13/3C day/night), but the proportion of fruitful shoots was higher when the post-budbreak temperature was 21/11 rather than 17/7C (day/night). This type of interaction between temperature and seasons would be very difficult to determine without the steady-state conditions of the controlled environment.

1.4.2 Controlled temperature experiments

In this section, examples have been given of the methods researchers have used to control temperature around plants in an orchard, and the results obtained from such experiments. For perennial plants, temperature treatments have largely involved 'limb chambers' where one branch of the tree is warmed or cooled in relation to the remainder, or the whole plant has been covered.

Early work using limb chambers was rather primitive compared to modern facilities. Lilleland (1936) had no thermostatic control on the chambers used, and there was no information given about temperature variations within each treatment. Tukey (1952, 1956) attempted to control night temperatures in chambers containing apples, to ambient, 5.5, 11 and 14C above ambient: "The temperature settings of the thermostats were determined each night from the temperatures of the previous night and personal observations of the weather conditions; nightly adjustments of the thermostats were required frequently". Perhaps we take for granted the equipment available today!

Snelgar *et al.* (1988) developed relocatable greenhouses to cover one half of a kiwifruit vine. The greenhouses had an automatic ventilation system and an accuracy of ±1C when the temperature was raised to 5C above ambient. Different responses (number of flowers on apical shoot) to the temperature treatment were recorded on the treated and untreated halves of the vine. Kiwifruit have been shown to translocate carbohydrates over distances of several metres (Snelgar *et al.*, 1986) so that an enhanced supply of carbohydrate from the heated half of the vine may have accounted for the increased flowering on the other half. Long distance translocation of carbohydrate may also be possible in apricot (Reid and Bieleski, 1974). This does cast some doubt on validity of results from limb chamber experiments. Mellenthin and Bonney (1972) obviously had not considered this problem for their work with limb chambers on fruit trees, because they concluded that the technique allowed for pairing of limbs on a single tree to

reduce tree to tree variability. Greenhouses that covered the whole vine were used in future work by Hopkirk *et al.* (1989) and that of Kempler *et al.* (1992).

Blankenship (1987) placed apple trees grown in 19 l containers into controlled environment rooms during fruit development and maturation. Snelgar *et al.* (1988) were critical of the use of container-grown kiwifruit vines, suggesting that vines were small, not trained and pruned in an orthodox manner and insufficient fruit would be available for postharvest assessments. Although some of these criticisms are valid, use of container-grown vines in controlled environments where temperatures can be manipulated to give a steady-state environment and a wide range of temperature treatments is more precise than relying on ambient temperature, as in the work of Snelgar *et al.* (1988), Hopkirk *et al.* (1989) and Kempler *et al.* (1992). The benefits of controlled environments, where a factor such as temperature is controlled throughout maturation, give an excellent data base for mathematical modelling and far outweighs any potential disadvantage. The use of controlled environments in conjunction with field work was also justified by Thorne (1969) and other researchers. Controlled environment studies showed that a 1C drop in mean daily temperature between budbreak and flowering in kiwifruit resulted in a 7.5 day delay in flowering, similar to observed differences in field grown fruit (Warrington and Stanley, 1986). Working with annual plants, Warrington and Kanemasu (1983) used controlled environments to derive a heat-sum model (see Section 1.4.3) for the time from sowing to anthesis in maize. This model was considered applicable to sweetcorn development over the range of temperatures experienced in New Zealand growing regions (Brooking and McPherson, 1989).

There is a plethora of literature relating to the effects of temperature on fruit maturation by the use of limb chambers through to large chambers covering the whole plant, and controlled environment facilities. Increasing night temperature relative to the ambient temperature accelerated fruit growth and enhanced maturity in apricot (Lilleland, 1936), cherry (Tukey, 1952), grape (Tukey, 1958) and peach (Batjer and Martin, 1965). Decreasing temperature delayed the

harvest date of muskmelons grown at different night temperatures in a glasshouse, and affected the maturation period more than the SSC of the fruit (Welles and Buitelaar, 1988). In contrast, Wang *et al.* (1971) has demonstrated that cool temperatures increased rate of maturation in pear. One hypothesis for the difference between these two effects could be the types of carbohydrate present in the fruit. Stonefruit, melon and grape contain little or no starch (see Section 1.2.2), whereas apple and pear contain starch, the breakdown of which is enhanced at cool temperatures (see Section 1.3.2).

Timing of temperature treatment can affect fruit response. Night temperatures lower than the day temperature were necessary to increase the size of apples grown in limb chambers from 38 days after petal fall; high temperatures (14C above ambient) were detrimental to fruit size (Tukey, 1956). The period of one month before harvest was the most critical time for the induction of enhanced ripening in pears given a cool temperature treatment (Wang and Mellenthin, 1972). At this stage degradation of starch, accumulated in the fruit during growth, would already be occurring, and this process would be hastened by cool temperatures.

Container-grown apple trees were placed in controlled environment treatments 20 days after full bloom at different temperature treatments, where the day temperature was 26C and night temperatures were either 22 or 11C (Blankenship, 1987). In contrast to results with pear, there was no difference in SSC, flesh firmness, ethylene production or ACC concentration between the two treatments, although reddening of skin colour was increased at the cooler night temperature. These results seem surprising, but the author does comment that the cool night fruit abscised and by the end of the experiment (149 days after full bloom) there were no fruit from the cool night treatment remaining on the trees. Temperatures below 11C are not common until after the peak harvest times in many of the large United States apple producing areas. Perhaps the unseasonal low temperatures affected fruit development and therefore maturation.

There is circumstantial evidence that cool temperatures accelerate kiwifruit maturation, although field data can be masked by year-to-year variations in temperatures. Sawanobori and Shimura (1990) studied fruit maturation at two different sites and recorded earlier maturation at the cooler site in one of the two years. The cooler temperatures in British Columbia than California (Kempler *et al.*, 1992) could account for fruit maturing at the same time even though flowering was several weeks earlier in California. Also in California, kiwifruit sampled from the coolest of three sites reached harvest maturity earlier than those from the warmer sites (Walton and de Jong, 1990a). Kiwifruit grown in relocatable greenhouses under increased temperatures had a lower SSC, but a higher starch concentration than in untreated control fruit (Hopkirk *et al.*, 1989). Fruit harvested in Nelson consistently mature 3 to 4 weeks earlier than fruit harvested from the Bay of Plenty (Hopkirk, 1986) and this may be due to the cooler temperatures experienced by vines in Nelson compared to those in Te Puke. In New Zealand in 1990, commencement of kiwifruit harvesting was delayed over the whole country probably due to warm temperatures, especially those at night (Kelly, 1990).

Use of controlled environment facilities to quantify the effect of temperature on rate of kiwifruit maturation under controlled conditions appeared to be the next logical step to define further the processes involved in maturation. Data from Hopkirk *et al.* (1989) make a contribution to our understanding of the effect of temperature on kiwifruit maturation, but there are some drawbacks. Temperatures used in their experiments were only a few degrees above or below ambient, which is insufficient to be able to effectively define the effect of temperature on fruit maturation. Hence, controlled environments allow a much wider range of temperatures to be studied and data can be used effectively in a mathematical model.

1.4.3 Modelling

There are many different types and applications of models in relation to fruit production and prediction of harvest times. A few examples are given here, but it should be noted that models are very versatile and can be adapted for specific purposes (see Chapter 5). Mathematical models are used in many fields, from economics and demography to nuclear physics and astronomy (Thornley, 1976). Although the problems investigated using this approach may be very diverse, the methods and underlying philosophy are similar.

What is a mathematical model? A model should provide a representation of a system, that is, resemble and be capable of simulating the system. The most important feature of a model according to Thornley (1976) is that "it should be possible to understand it more readily, or to describe it more fully than the real system". Thus a model is usually a simplification of the real system, but essential characteristics of the system should appear in the model. A mathematical model consists of an equation or set of equations that quantitatively represent the assumptions or hypotheses made about the real system. These equations are then solved to give predicted values to those measurements from the real system. The equations in the model express and interpret the hypotheses quantitatively, but they do not provide its biological or scientific content. The model should be validated with observed data to give confidence in the model.

Types of models

'Modelling' is a term to describe use of these mathematical models (Thornley, 1976). Progress can be made towards a quantitative understanding of plants and their responses to the environment when there is a mathematical basis to the hypothesis. There are two types of model used in research. Firstly, the mechanistic model is used when responses of a biological system are understood, right down to individual components and their interactions. Parameters of the model have biological meaning and give some understanding

of the system. Such models are developed before undertaking the experiments. Secondly, the empirical model is developed where data are collected and an equation or set of equations are derived and fitted to form the model. In effect, an empirical model simply redescribes data and is often a convenient method to summarise a large quantity of data. There is no clear dividing line between these two methods and most models contain both to varying degrees.

Attempts to relate the timing of plant developmental events, such as flowering, to environmental conditions have been made. Models constructed to link physiological mechanisms to environmental factors are difficult because developmental processes are complex and responses to environmental conditions subtle and diverse. Therefore, empirical models mainly have been used to relate development to environmental variables (McNaughton *et al.*, 1985). These authors split empirical models into two groups. The first type are not physiologically based as input variables (temperature, photoperiod) are related to outputs (predicted intervals between developmental events) for example, 'growing degree-day' and 'heat-sum' models. The second type allows plant development to progress continuously in time. In theory, development can be observed at any instant, but in practice it is measured by the occurrence of externally recognised developmental events. The discrete rate analysis (McNaughton *et al.*, 1985) is useful because it is physiologically based and relates actual rate of development to temperature, rather than choosing a base temperature from some preconceived idea of how a plant ought to respond.

Uses of models

The heat-sum model mentioned above is not a new concept; Reaumur described this model in 1740 (Waggoner, 1977), and it is still the centre of plant phenology. The heat-sum concept assumes a linear response between the rate of development and mean temperature between defined minimum and maximum temperature limits, and that no development occurs below a certain base temperature (e.g. Warrington and Kanemasu, 1983; Brooking and McPherson,

1989). Timing of a particular phenological event can be determined by integration of daily heat-sums to some critical value. Another assumption made in these models is that temperature is the sole factor affecting plant development, and that plant environment is adequately described by daily maximum and minimum air temperatures (New Zealand Meteorological Service, 1983; Brooking and McPherson, 1989). Sometimes other factors are included in a heat-sum model such as water stress or daylength (McPherson *et al.*, 1979).

The heat-sum model has been used in a variety of ways for different crops. A simple linear degree-day model with a 6C base temperature accounted for 89% of the variation in crop duration for 489 sweetcorn crops grown in three regions of New Zealand (Brooking and McPherson, 1989). Use of this model enabled crop duration to be predicted more accurately than by using calendar days. A minimum temperature of 11C and maximum temperature of 28C was used for prediction of tasselling and anthesis in maize (Warrington and Kanemasu, 1983). A growing degree-day model (base temperature 15C) was used in the Hawaiian islands to predict the beginning of harvest and 50% of harvest completion for guava (Bittenbender and Kobayashi, 1990).

Predictions with perennial plants are probably more difficult than with annuals, because there may be carry over effects from the previous year. However, the heat-sum model concept has been used to develop 'chill-units' for determining budbreak after winter dormancy in peach (Richardson *et al.*, 1974), apple (Ebert *et al.*, 1986) and sour cherry (Anderson *et al.*, 1986). A period of cold is required to overcome winter dormancy and to enable the plant to resume normal growth when external conditions become favourable. These models are based on the accumulation of chill-units below a pre-selected optimum temperature (about 5 to 7C depending on species). The chilling contribution becomes less as temperatures drop below or rise above the optimum temperature, with very low or high temperatures resulting in a negative contribution to the model. The amount of winter chilling required to 'break' dormancy varies with each crop. The 'Richardson chill-units' were developed for peach trees and this is probably the

reason why the model does not work satisfactorily for kiwifruit (H. McPherson, HortResearch, Auckland, pers. comm.). No specific models for kiwifruit are currently available.

There are other different types of mathematical models that can be used in plant physiology, depending on the aim of the experimenter and/or modeller. Here, other examples of models are given to show the variety of types that have been used. A recent trend appears to be the determination of the carbon balance in fruit. A model of this type is very detailed because all inputs and outputs of fruit during development have to be known (or determined). Carbon budgets offer a useful tool for understanding plant growth and use of resources. Models of partitioning processes that divide the plant into supply and demand components can be used to investigate the interactions between them. Carbon balance models have been determined for apple (Seem *et al.*, 1986), sour cherry (Kappes and Flore, 1986) and kiwifruit (Walton and de Jong, 1990b).

Techniques are required which allow the kiwifruit industry to plan for handling 50 to 70 million trays efficiently during the harvesting and marketing season. Kiwifruit are harvested at different times according to the season and to location within the country. The judgment of when to harvest is based on conversion of starch to sugar, as seen by the rise in SSC. Differences in timing of harvests may be due to temperature differences between the growing regions and seasons. Kiwifruit quality and storage life are influenced by the maturity of the fruit at harvest, so that picking at the correct stage of maturity is critical. The prediction of harvest date in commercial orchards would benefit the New Zealand Kiwifruit Marketing Board as well as growers, packaging companies, shippers and allied support industries who currently operate with considerable uncertainty regarding the timing of the harvest period. Prediction of harvest date for perennial crops has not often been attempted. However, Perry *et al.* (1987) used ten different methods of accumulating heat-units to predict harvest date in apples from early season temperature measurements. No one method was superior and the predictions missed the harvest date by 1 day for 'Delicious' and 8 days for 'Golden

Delicious', although the authors decided that these values were acceptable for an early season prediction. Perhaps a different model would be more appropriate for the prediction of harvest date in fruit crops.

Salinger and Morley-Bunker (1988) suggested that SSC in kiwifruit increased linearly with a decrease in temperature once the SSC was greater than 5.0%. Snelgar *et al.* (in prep.) tested this model using kiwifruit plants exposed to a range of temperatures between 11 to 16C. They found that this linear model lacked precision. It was realised that the change in SSC with temperature may be curvilinear rather than linear as proposed by Salinger and Morley-Bunker (1988). Another problem in kiwifruit is predicting when SSC becomes greater than 5%, as a rapid increase in SSC occurs soon after. Nevertheless, the relationship determined by Salinger and Morley-Bunker (1988) and used by Snelgar *et al.* (in prep.) is an indication that harvest date can be predicted, but the model has limitations, lacks precision and needs to be further developed.

1.5 Rationale for this study

The work in this thesis was designed to quantify the effect of temperature on the rate of kiwifruit maturation by using controlled environments (Chapter 2) and field sites (Chapter 3). Maturation of kiwifruit from four field sites located at the geographical extremes of the kiwifruit growing regions was recorded and accurate temperature records were obtained for each site (Chapter 3). Concentrations of soluble and insoluble carbohydrates were determined at different stages of maturation in both the controlled environment and field work. The carbohydrate status of fruit from field-grown vines was manipulated by girdling in an attempt to determine the effect of carbohydrate status on fruit maturation (Chapter 4). Data from the controlled environment treatments were mathematically related to the field data to derive a model to relate changes in SSC to temperature based on meteorological temperature data in kiwifruit growing regions (Chapter 5).

In summary, the aims of this work were:

- to determine the effect of temperature on carbohydrate changes and the rate of kiwifruit maturation.
- to obtain accurate temperature records and details of kiwifruit maturation in four orchards at different geographical locations, hence providing data that would enable the development of a mathematical model.
- to alter the carbohydrate status of field grown fruit and determine the effect on rate of SSC accumulation and fruit maturation.
- to help develop and use a mathematical model to relate changes in SSC to temperature, and to use this model to predict harvest date.

CHAPTER 2

MATURATION OF KIWIFRUIT GROWN AT DIFFERENT TEMPERATURES IN CONTROLLED ENVIRONMENTS

2.1 Introduction

Maturation is the stage of development leading to the attainment of physiological maturity when fruit will continue ontogeny even if detached from the parent plant (Watada *et al.*, 1984). The processes that occur from the latter stages of growth and development through to the early stages of senescence are called ripening. Changes in composition, colour, texture or other sensory attributes result in the characteristic aesthetic quality of a ripe fruit (Watada *et al.*, 1984). Many changes occur in fruit tissue during maturation and ripening and these may be affected by, for example, temperature throughout the growing season. A maturity index is an arbitrary measurement used to determine whether a particular crop is mature. Potential indices should be easy to measure (Reid, 1992), highly correlated to fruit quality and be consistent between seasons (Underhill and Wong, 1990).

Maturation is the result of several simultaneous processes. Apart from changes in carbohydrates, there is also a concurrent decrease in flesh firmness. Fruit softening during maturation has been observed in many fruit including kiwifruit (Pratt and Reid, 1974), apple (Workman, 1963), fig (Tsantili, 1990), guava (Yusof and Mohamed, 1987), melon (Miccolis and Saltveit, 1991), peach (Maness *et al.*, 1992) and pear (Mann and Singh, 1988). The pattern of decrease in flesh firmness varied from year to year in kiwifruit and did not relate consistently to fruit quality after storage (Beever and Hopkirk, 1990). For this reason flesh firmness was not used as a maturity index for kiwifruit.

Kiwifruit harvested before they are physiologically mature do not store and ripen satisfactorily (Harman, 1981). A suitable indicator of maturity for kiwifruit had to

be developed to ensure that fruit reached physiological maturity before harvest (Beever and Hopkirk, 1990). In the 1960s, the harvest season for kiwifruit commenced on 1 May; fruit harvested before that date were not permitted to be exported (Beever and Hopkirk, 1990). This index was not entirely satisfactory because, in some seasons such as 1979, fruit were immature at harvest. Instead, SSC was adopted as the maturity index for kiwifruit in New Zealand and fruit can be harvested for export once fruit SSC exceeds a minimum of 6.2% (Beever and Hopkirk, 1990). Components of SSC in kiwifruit include water soluble compounds, such as sugars, acids, vitamin C, amino acids and some pectins (Harman and Watkins, 1986). Changes in SSC during maturation reflect changes in carbohydrates and are positively related to sugar concentration during maturation (Harman, 1981). The time of reaching 6.2% SSC can vary in the same orchard by up to 3 weeks among years (Harman, 1981). In addition to variation in the timing of reaching 6.2% SSC among seasons, there are differences in SSC among fruit harvested from different positions on the same vine (Hopkirk *et al.*, 1986; Smith *et al.*, 1992) and gradients in carbohydrate concentration within the fruit. For example, within each fruit during maturation, SSC increased more rapidly at the distal end of the fruit than at the proximal end (Hopkirk *et al.*, 1989; Cotter *et al.*, 1991) and concentration of starch in the core was higher than in the outer pericarp (MacRae *et al.*, 1989a).

Total non-structural carbohydrate concentration is composed of starch and sugars, predominantly glucose and fructose, with some sucrose and traces of inositol (Kawamata, 1977). Starch is the main storage form of carbohydrate in kiwifruit, and in many other fruit including apple, banana (Tucker and Grierson, 1987) and tomato (Dinar and Stevens, 1981; Garvey and Hewitt, 1991). In such fruit, the starch concentration typically increases during growth and then decreases during maturation and ripening as a result of hydrolysis to sugars, with a concomitant rise in total sugar concentration. Total carbohydrate concentration in kiwifruit was assumed to be similar to the dry matter concentration of fruit (Hopkirk, 1991). In fact, Scott *et al.* (1986) recommended the use of dry matter as an appropriate maturity index for harvesting kiwifruit in Australia.

Kiwifruit from the same orchard block have been shown to mature at different rates in different years (Harman, 1981) and timing of commercial harvest has been shown to vary among orchards at different geographical locations (Harman and Hopkirk, 1982). Soluble solids concentration was measured in two orchards at different locations in Japan; at the cooler orchard increase in SSC and decrease in starch concentration were each faster than for fruit at the warmer orchard (Sawanobori and Shimura, 1990). In British Columbia, kiwifruit flowered several weeks later than in California, but fruit were picked at a similar time in both locations (Kempler *et al.*, 1992). These differences indicate that environmental conditions, especially temperature, may affect the harvest date of kiwifruit between seasons and orchards.

The effect of temperature on maturation of many different fruit species has been studied using 'limb chambers', where one branch of a tree was warmed or cooled in relation to the remainder. This approach was used with apricot (Lilleland, 1936), cherry (Tukey, 1952), grape (Tukey, 1958), peach (Batjer and Martin, 1965) and pear (Wang *et al.*, 1971). By using limb chambers, it was found that warm temperatures enhanced fruit maturation in stonefruit and grapes, whereas cool temperatures enhanced maturation in pear. The differing response may be due to the different carbohydrate metabolism of the different species. Stonefruit and grape contain little or no starch, whereas pear accumulates starch as a storage carbohydrate. In an alternative approach, relocatable greenhouses were used in an orchard to modify the temperature around entire mature kiwifruit vines (Snelgar *et al.*, 1988; Hopkirk *et al.*, 1989; Kempler *et al.*, 1992). Kiwifruit grown in relocatable greenhouses under increased temperatures (3C above ambient) contained lower SSC but higher starch concentration compared to control (unheated) fruit (Hopkirk *et al.*, 1989). A disadvantage of relocatable greenhouses used to test the effect of temperature on the rate of kiwifruit maturation in the study by Hopkirk *et al.* (1989) was that temperature in the greenhouses could only be raised or lowered a few degrees from ambient. However, use of controlled environments allows accurate control of temperature (Warrington and

Rook, 1989) and a greater choice of temperature treatments throughout fruit maturation.

In New Zealand, the warmer than average night temperature in autumn 1990 was thought to have resulted in the delayed harvest that year (Kelly, 1990). This implied that cool night temperatures were required to enhance maturity. Hopkirk *et al.* (1989) also suggested that the marked increase in SSC after cold nights, observed over several seasons, altered the time of reaching 6.2% SSC (commercial maturity). There have been no published studies that establish whether it is minimum (night) temperature *per se*, mean temperature, or magnitude of the difference between maximum (day) and minimum temperature that affects kiwifruit maturation. Indeed, there has been very little work undertaken to determine the effect of temperature on any aspect of kiwifruit maturation. In particular, there is a lack of detailed analyses of changes in starch and sugar concentrations with advancing maturation under different temperature conditions.

The controlled environment facilities used in this study allowed temperature to be accurately controlled around container-grown kiwifruit vines. Placement of vines in controlled environment rooms at different temperatures during late summer allowed temperature to be isolated from the impact of other environmental factors and its effect on fruit maturation to be studied. In particular, study was made of the effects of minimum temperature, mean temperature, and the magnitude of the difference between the maximum and minimum temperature on fruit maturation. The effect of a temperature perturbation and its timing were also studied. In addition to recording changes in SSC, firmness, fruit size and respiration, detailed measurements of starch and total sugar concentrations were made during fruit development to determine the effect of temperature on the partitioning of carbohydrate, and to attain a more complete understanding of the processes occurring at the cellular level. An analytical method for the estimation of starch and sugar concentrations was specifically adapted for kiwifruit to allow the rapid analysis of a large number of samples (Seager and Haslemore, 1993).

2.2 Effect of different day and night temperatures

2.2.1 Materials and Methods

Mature 'Hayward' kiwifruit vines were grown in 28 l containers (Plate 2.1). The soil medium in the container was composed of 40 parts sterilised soil (Opiki humic silty clay loam; a fine illitic, mesic Histic Humaquepts), 20 parts crusher dust (4 mm gravel), 20 parts coarse pumice (mixture of 5 and 10 mm particles) with added minerals of 133 g m^{-3} dolomite (lime) and 69 g m^{-3} Nitrophoska® (10:2:6:1 N:P:K:S). Vines were trained to have a 1 m high stem with a short permanent leader and were pruned to have three or four one-year-old canes (Plate 2.2). Before treatment, vines were grown outdoors in Palmerston North under polyethylene-covered shelters (sheltered site). An automated irrigation system distributed either modified Hoagland's A solution (Brooking, 1976) or water as required. A regular programme of chemical sprays ensured that vines were kept free of pests and diseases. Plants were hand pollinated during flowering.

Experiments were carried out in walk-in controlled environment rooms at the National Climate Laboratory, HortResearch, Palmerston North (Anon., 1981). Changes in temperature and relative humidity between the daily maximum and minimum were programmed to occur in 16 discrete steps, with each step being maintained for about 45 minutes. Temperature treatments were representative of the range of field conditions occurring in autumn months (March, April, May), encompassing the geographical extremes of the main kiwifruit growing regions in New Zealand (Table 2.1).

Lighting was provided by 4x1000 W 'Metalarc' high-pressure metal halide lamps (M1000 BU Sylvania) and 4x1000 W quartz halogen lamps (Philips type 12012 R) giving a photosynthetic photon flux density of approximately 700 μmol m^{-2} s^{-1} at cane height (Warrington *et al.*, 1978). The lights were switched on 6 hours before the maximum day temperature was reached and off 6 hours before the minimum

Plate 2.1 Container-grown vines being maintained in the sheltered site during early spring growth. The 1 m high permanent stem and one-year-old canes supporting the flowering laterals are visible (see also Plate 2.2).

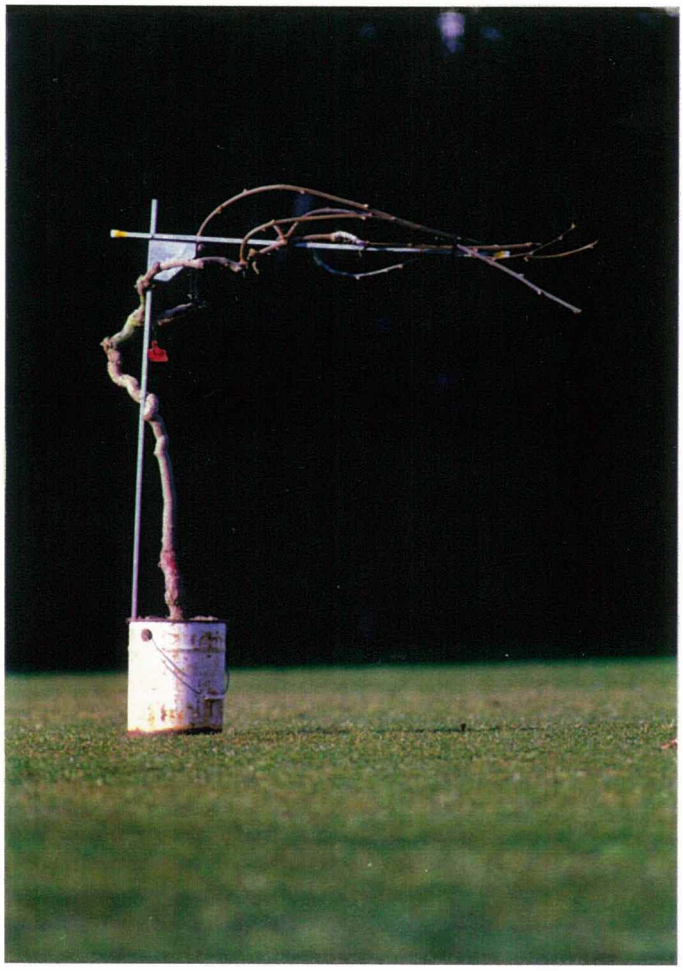


Plate 2.2 Container-grown vine after winter pruning showing five one-year-old canes tied to the supporting strut.

Table 2.1 Mean daily maximum and minimum temperatures (C) at four sampling locations in March, April and May. Meteorological site codes are shown (New Zealand Meteorological Service, 1983).

	Kerikeri (A52391)		Te Puke (B76835)		Palmerston North (E05363)		Riwaka (C12191)	
Month	Max. temp. (C)	Min. temp. (C)	Max. temp. (C)	Min. temp. (C)	Max. temp. (C)	Min. temp. (C)	Max. temp. (C)	Min. temp. (C)
March	23.5	13.1	22.5	12.7	20.9	11.7	21.5	10.5
April	21.2	11.1	20.0	10.5	18.2	9.6	18.7	7.6
May	18.4	9.1	16.7	7.2	15.0	6.8	15.7	4.5

night temperature was reached giving a daylength of 12 hours. The CO_2 concentration was monitored via a central infra-red gas analyser and maintained close to 340 µl CO_2 l^{-1} air. Airflow through the canopy was 0.5 m s^{-1}.

Each temperature treatment was placed in a different controlled environment room, and treatments were not replicated within a season. An independent estimate of variability between rooms was therefore difficult to determine. However, one temperature treatment (18/8C) was repeated in Experiment 1 and Experiment 2 using different controlled environment rooms. In this instance, differences between rooms was also confounded with differences in ages of the vines between the two years. Alternatively, in Experiment 3 (Section 2.2) vines were placed in two different rooms but at the same temperature with two temperature regimes (11/7 and 26/20C) for the first 37 days of the experiment. To test whether there was a difference between rooms programmed to be the same temperature, a nonlinear regression was used to fit an exponential equation for both treatments, either separately or together. Comparison of the data using an F ratio showed that there was no significant difference in the rate of increase in SSC at the 5% ($P \leq 0.05$) level for the two rooms tested in 1991. In another check, there was no difference in SSC from vines placed in two different rooms maintained at 26/20C for 37 days (Fig. 2.14). An assumption was made that a

similar relationship applied between other controlled environment rooms in the same, or different years.

Five experiments were undertaken to investigate the effect of temperature on the rate of kiwifruit maturation. The first two experiments separated the effects of minimum temperature (Experiment 1) and maximum/minimum temperature difference (Experiment 2, Section 2.2) and the final three experiments determined the effect extremes of temperature (Experiment 3, Section 2.3) and of a perturbation in temperature (Experiments 3, 4 and 5).

Minimum temperature treatments (Experiment 1)

The temperature treatments and respective relative humidities at 0.6/0.3 kPa vapour pressure deficit (maximum/minimum temperature, respectively) are shown in Table 2.2. Temperature and relative humidity in controlled environment rooms were controlled to within 0.5C and 5% of programmed conditions, respectively, for all studies in this chapter.

Table 2.2 Temperatures (C) and relative humidities (%) in controlled environment rooms during different minimum temperature treatments (Experiment 1).

Maximum temperature (C)	Minimum temperature (C)	Mean temperature (C)	Relative humidity (%)
18	4	11	71/63[1]
18	8	13	71/72
18	12	15	71/76
18	16	17	71/84

[1] Relative humidity at maximum and minimum temperature, respectively

Twenty vines bearing fruit were transferred from the sheltered site on 23 March 1989 to the controlled environment treatments (rooms) for a period of 56 days. Five vines were selectively placed in each treatment to give a similar number of

fruit per room. Each week, 10 randomly selected fruit per temperature treatment (2 fruit per vine) were sampled for SSC, flesh firmness and fruit growth measurements. Fresh weight of each harvested fruit was recorded and a method of water displacement was used to measure fruit growth non-destructively by changes in volume (Lai, 1987). The concentration of soluble solids was measured with juice squeezed from a 15 mm section cut from each of the distal and proximal ends of each fruit (Fig. 2.1). An Atago N-20 hand-held refractometer was used to measure SSC, following an initial calibration with 6% sucrose solution and the zero baseline was frequently checked with distilled water (Harman and Hopkirk, 1982). Measurements were made at laboratory room temperature. Flesh firmness was recorded with an Effegi penetrometer (FT327) with an 8 mm head. Skin was removed to a depth of 2 mm on opposite sides of the fruit in the equatorial region (Fig. 2.1) and the penetrometer plunged into the flesh. To remove operator variability, all measurements were taken by the same person. Multiplication of the reading by 9.807 was necessary to convert the measurements from kilograms force (kgf) to newtons (N).

Maximum/minimum temperature combination treatments (Experiment 2).

Temperature combinations were chosen to allow the separate effects of maximum, minimum, daily mean and maximum/minimum temperature differential to be evaluated (Table 2.3). A common maximum/minimum vapour pressure deficit of 0.5/0.3 kPa was maintained in each treatment.

Thirty vines were chosen from the sheltered site and placed in the controlled environment treatments on 13 February 1990 for a period of 73 days. Five vines were selectively placed in each room to give a similar number of fruit per treatment. At each of 10 harvests, twenty randomly selected fruit from each temperature treatment were picked and used to determine SSC, flesh firmness, fresh weight and fruit growth as described previously. In addition, a 3 mm section through the equatorial region (Fig. 2.1) of some fruit was taken and dried at 40C for 48 hours to determine dry matter concentration. Tissue from the outer pericarp

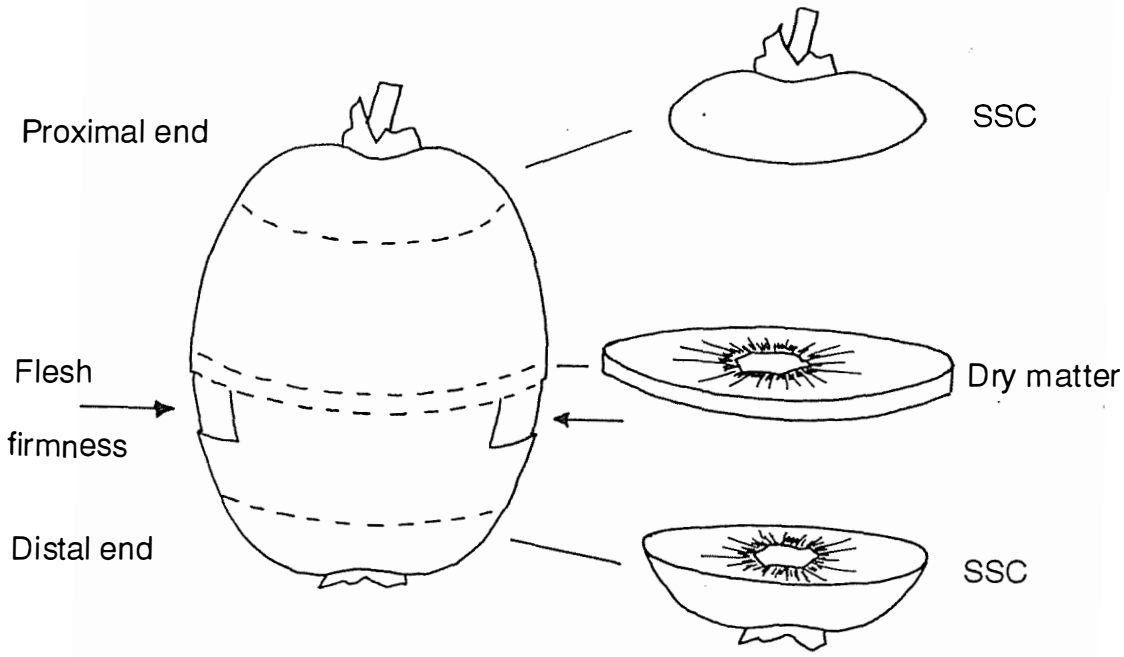


Fig. 2.1 Sampling positions for analysis of soluble solids concentration, dry matter and flesh firmness of kiwifruit.

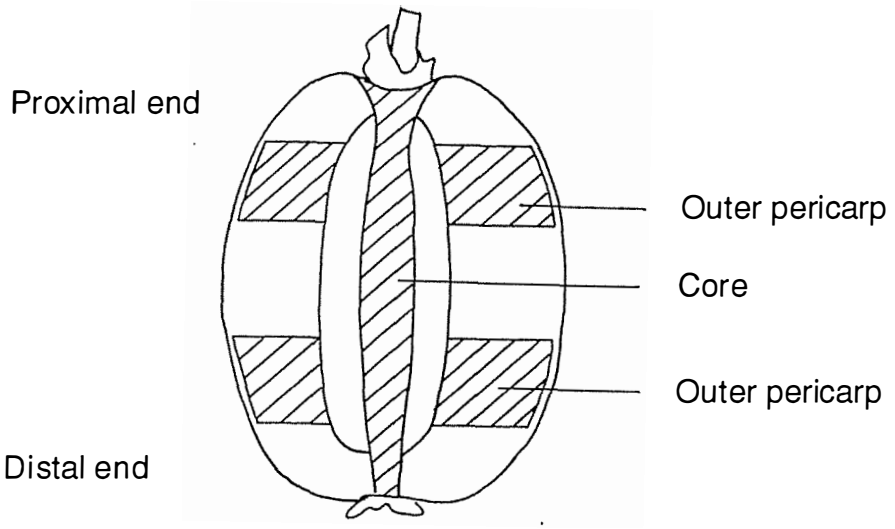


Fig. 2.2 Sampling positions for chemical analyses from outer pericarp and core tissue of kiwifruit.

Table 2.3 Temperatures (C) and relative humidities (%) in controlled environment rooms during different maximum and minimum temperature combination treatments (Experiment 2).

Maximum temperature (C)	Minimum temperature (C)	Mean temperature (C)	Max./min. temperature difference (C)	Relative humidity (%)
14	8	11	6	69/72[1]
18	8	13	10	76/72
14	12	13	2	69/79
22	8	15	14	81/72
26	8	17	18	85/72
22	12	17	10	81/79

[1] Relative humidity at maximum and minimum temperature, respectively

and core (Plate 2.3) was stored for subsequent analysis of starch and total sugar concentration. To provide sufficient tissue for analysis, two fruit were harvested from the same vine and tissues from the same sampling region (Fig. 2.2) were combined. Tissue samples from three regions were collected: outer pericarp tissue from each of the proximal and distal ends of the fruit, and core tissue. Between 4 to 5 g of the respective tissue was weighed into a vial, covered with 20 ml methanol/water/formic acid (ratio 12:2:1) and stored at 3C. Samples were later analysed for starch and total sugar concentration by methods specifically developed for kiwifruit during this work (Seager and Haslemore, 1993, see Appendix 1).

Statistical analyses

The statistical packages used for all numerical analyses were SAS, version 6.0 (Statistical Analysis Systems, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA) and Minitab, version 7.2 (Minitab Statistical Software, 3081 Enterprise Drive, State College, PA 16801, USA).

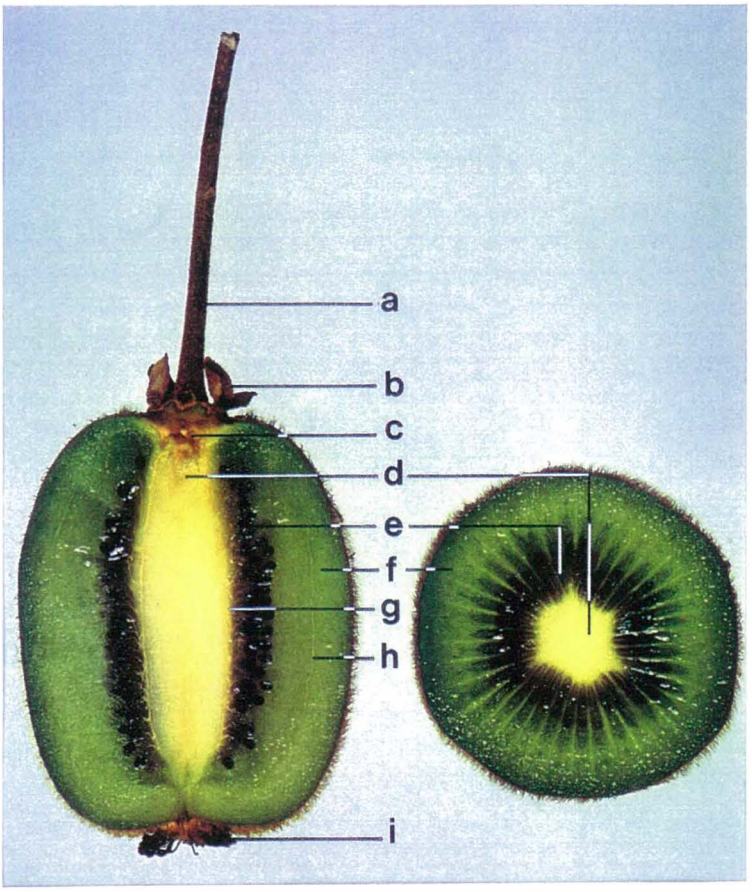


Plate 2.3 Nomenclature of the different tissues in a mature kiwifruit (from Beever and Hopkirk, 1990). a) stalk, b) sepals, c) sclerified plug, d) core, e) inner pericarp containing seeds, f) outer pericarp, g) ventral carpellary bundle system, h) dorsal carpellary bundle system, i) withered stamens and styles.

A logistic curve was used to fit the changes in SSC over time in each temperature treatment. The equation used in Fig. 2.3 was:

$$S = a + \frac{c}{1 + e^{-h(t - m)}}$$

where S = SSC (%)

a = initial asymptote (% SSC)

c = final asymptote - a (% SSC)

$a+c$ = final asymptote (% SSC)

h = rate constant (dimensionless)

t = time (days)

m = point of inflection (days).

Data were linearised by taking the log of the response variable. To determine the value for the upper asymptote, the linear model was fitted with different values for the upper asymptote until the lowest residual sum of squares was found. This provided a value of c=16% SSC for Experiments 1, 2 and 3, except the 11/7C temperature treatment in Experiment 3 where c=14% SSC. It should be noted that the initial value on each graph is not the intercept as the method results in a rescaling of the lower asymptote, allowing the curves in some treatments to be different at day 0 (see Figs 2.5, 2.7 and 2.13). The standard error of treatments was compared at the point of inflection (m), that is, the mid-point between the lower and upper asymptote.

Starch and total sugar concentration data were fitted using a smoothing routine because a parametric model was not suitable. In Minitab the smoothing routine obtains running medians of 3 data points (Experiment 2) and medians of 4, 2, 5 and 3 consecutive data points (Experiment 3). Starch and total sugar concentrations were similar between samples taken from the proximal and distal ends of the fruit, as found previously by Bowen *et al.* (1988). The mean value of the two samples was therefore used to present results for Experiments 2 and 3.

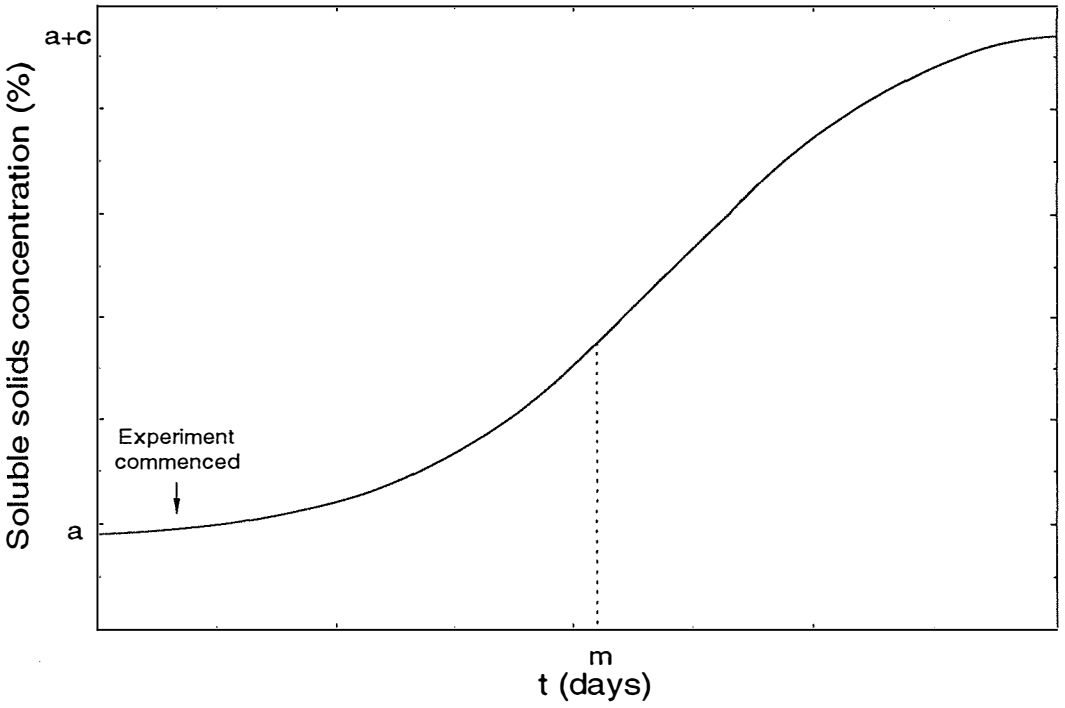


Fig. 2.3 Format of logistic curve (used in Chapters 2 and 3) showing assumed change in soluble solids concentration (%) of kiwifruit during maturation. a = lower asymptote, a+c = upper asymptote, m = point of inflection, t = time (days).

Linear regressions were fitted to flesh firmness data. To compare SSC or flesh firmness between the fixed temperature treatments (11/7 and 26/20C) and respective temperature perturbation treatments in Experiment 3, a 95% confidence interval was calculated for the fixed temperature treatment (Figs 2.12 and 2.14).

2.2.2 Results

Minimum temperature treatments (Experiment 1)

Fruit weighed 67 ±1.6 g at the beginning of the experiment and increased to 74 ±2.6 g by the end of the experiment. The increase in fruit volume from the beginning to the end of the experiment was 134 mm^3 for all treatments, an increase of 2.4 mm^3 day^{-1}.

Flesh firmness decreased during the experiment, but there was no consistent relationship with minimum temperature (Fig. 2.4). Initial firmness was 98.9 N and the estimate of the rate of change in firmness was -0.54, -0.72, -0.89 and -0.92 N day^{-1} for the temperature treatments of 18/12, 18/16, 18/4 and 18/8C, respectively. The fastest decreases in firmness were in the treatments of 18/4 and 18/8C, but differences between these treatments were not significant ($P \leq 0.01$). However, firmness in the 18/12 and 18/16C treatments decreased at a slower rate than in the 18/4 and 18/8C temperature treatments ($P \leq 0.01$). The treatment by time interaction was significant. There was a significant negative correlation ($r=0.92$) between flesh firmness and SSC (pooled for all treatments) ($P \leq 0.05$).

Initial SSC (Fig. 2.5) in fruit from container-grown vines was approximately 1.5% higher than that for field-grown fruit (Fig. 3.2) at the start of treatment. Nevertheless, fruit from container-grown vines appeared to be typical of field fruit and substantial changes in SSC occurred subsequent to vines being placed in each treatment.

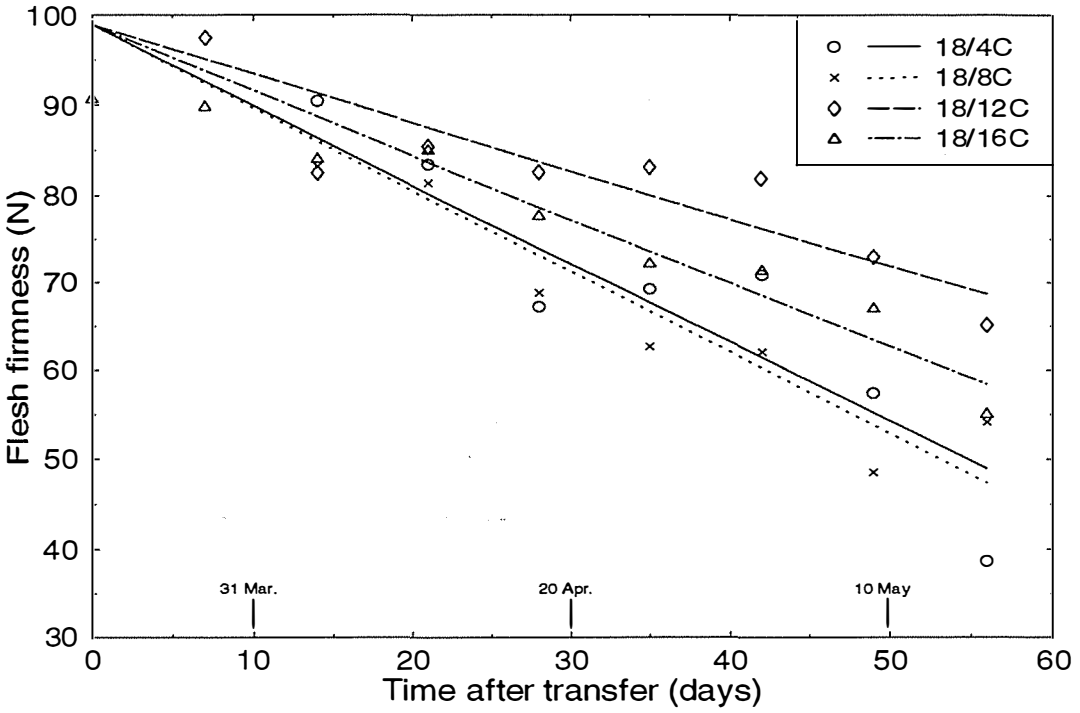


Fig. 2.4 Flesh firmness (N) of kiwifruit grown under different minimum temperatures (C) (Experiment 1). Regression coefficients in A.3.1.

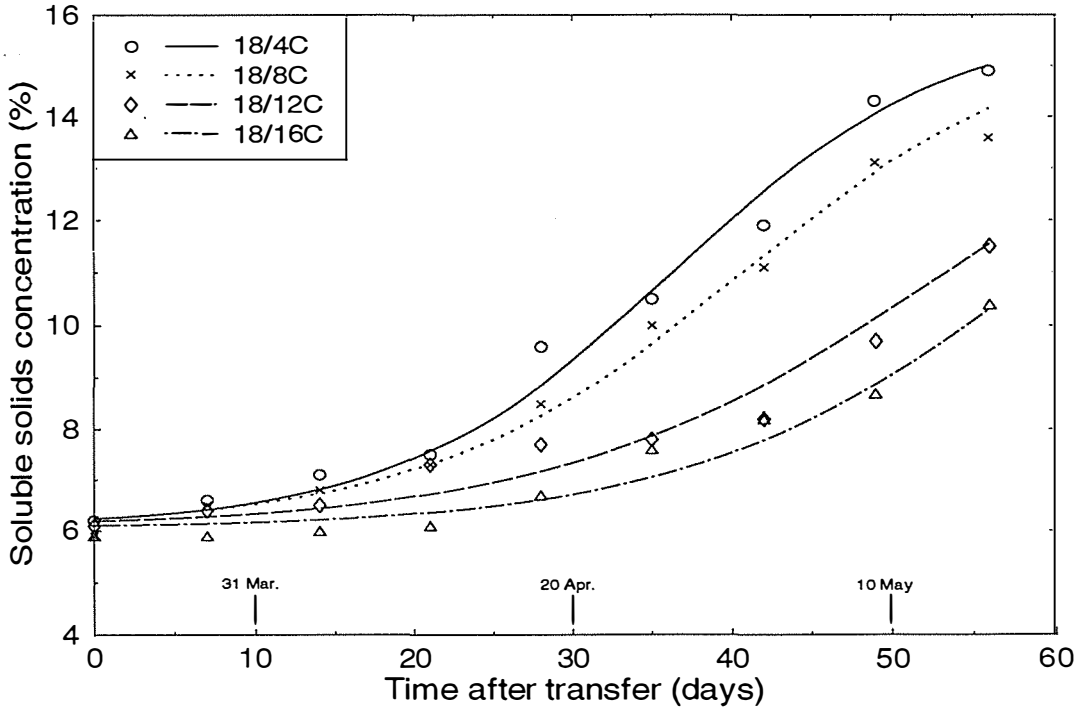


Fig. 2.5 Soluble solids concentration (%) of kiwifruit grown under different minimum temperatures (C) (Experiment 1). Regression coefficients in A.3.2.

Concentration of soluble solids increased in all treatments but at a faster rate when exposed to the cooler minimum temperatures (Fig. 2.5). In addition, there were differences among treatments in the shape of SSC curves. Increase in fruit SSC was 15.6, 13.8, 9.6 and 7.9% day^{-1} ($x10^{-2}$) from fruit grown in the 18/4, 18/8, 18/12 and 18/16C temperature treatments. Time taken to reach the inflection point varied with temperature; fruit from the temperature treatment of 18/4C reached the inflection point 5, 18 and 23 days before fruit from the 18/8, 18/12 and 18/16C treatments, respectively (Table 2.4). Final SSC at day 56 was 14.9, 13.6, 11.5 and 10.4% for fruit harvested from the treatments of 18/4, 18/8, 18/12 and 18/16C, respectively. Details of SSC at both proximal and distal ends of the fruit are given in Appendix 2. Soluble solids concentration at the distal end of fruit was greater than or equal to SSC at the proximal end from the beginning of the experiment and remained higher throughout.

Table 2.4 Comparison of the maximum rate of change in soluble solids concentration (slope) and number of days to the inflection point of kiwifruit grown under treatments of different minimum temperatures (C) (Experiment 1). Values determined from fitted logistic curves where a maximum soluble solids concentration of 16% is assumed.

Temperature (max./min.) (C)	Mean temperature (C)	Slope (% day^{-1})	Time to inflection point (days)
18/4	11	0.112 (0.006)[1]	36
18/8	13	0.098 (0.007)	41
18/12	15	0.081 (0.010)	54
18/16	17	0.091 (0.011)	59

[1]±SE (standard error)

Maximum/minimum temperature combination treatments (Experiment 2)

There were no significant differences in fruit growth among treatments. Mean fruit weight for all treatments at the beginning and end of the experiment was 41 ±0.9 g and 58 ±1.1 g, respectively (measured from destructive harvests). For vines exposed to the different maximum/minimum temperature combinations, the increase in volume was 163 mm^3 as a mean for all treatments; that is, an average rate of 2.2 mm^3 day^{-1} (measured non-destructively).

Fruit softened fastest at the cooler mean temperatures and slowest at the warmer mean temperatures (Fig. 2.6). Initial firmness was 86.5 N and estimates of the rate of change in firmness were -0.76, -0.65, -0.61, -0.53, -0.49 and -0.45 N day^{-1} for the temperature treatments of 14/8, 18/8, 14/12, 22/12, 26/8, and 22/8C, respectively. Rates of change in flesh firmness were not different among the treatments of 22/8, 26/8 and 22/12C but were different between these and the remaining temperature treatments ($P \leq 0.05$). The treatment by time interaction was significant.

Dry matter increased throughout the experiment in all treatments; at day 0 the dry matter of fruit tissue was 17.6 ±0.72% and at the end of the experiment was 20.6 ±1.22% (mean of all six treatments). Differences among treatments did not appear to be related to temperature as the rates of change in dry matter were 2.1, 2.5, 3.0, 4.2, 5.5 and 6.6 (% day^{-1} $x10^{-2}$) for the treatments of 22/12, 26/8, 18/8, 14/8, 14/12 and 22/8C, respectively.

Soluble solids concentration of fruit from vines exposed to the different minimum/ maximum temperature combinations increased fastest with the coolest mean daily temperatures, irrespective of the maximum and/or minimum temperature (Fig. 2.7). Increase in SSC was 12.8, 10.3, 9.1, 6.4, 5.5 and 4.8% day^{-1} ($x10^{-2}$) for fruit from the 14/8, 18/8, 14/12, 22/8, 22/12 and 26/8C temperature treatments, respectively. The number of days taken to reach the inflection point varied with the temperature treatment (Table 2.5). In the treatment of 14/8C, fruit

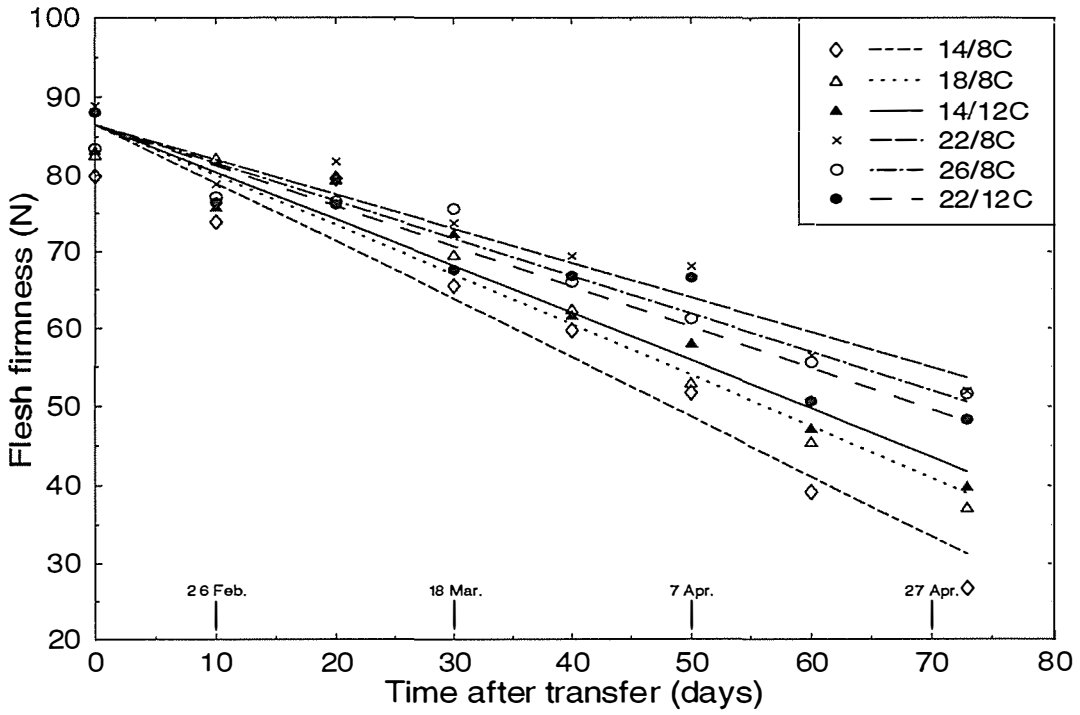


Fig. 2.6 Flesh firmness (N) of kiwifruit grown under different maximum and minimum temperatures (C) (Experiment 2). Regression coefficients in A.3.3.

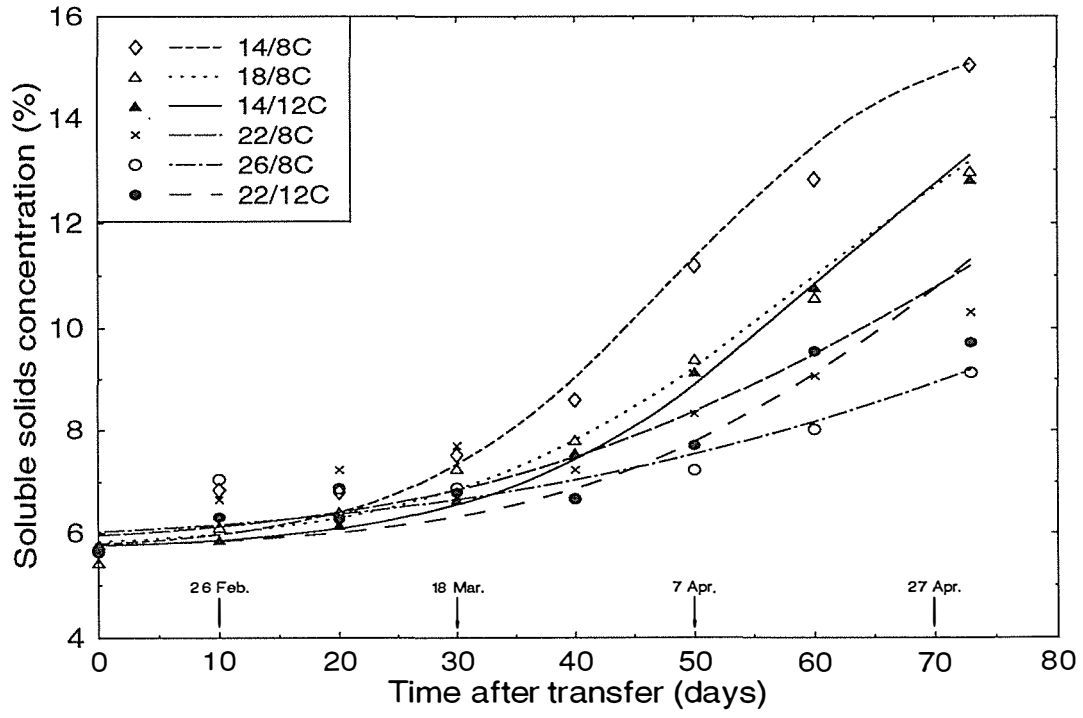


Fig. 2.7 Soluble solids concentration (%) of kiwifruit grown under different maximum and minimum temperatures (C) (Experiment 2). Regression coefficients in A.3.4.

reached the inflection point by day 48, whereas fruit from the treatments of 18/8, 14/12, 22/8 22/12, and 26/8C reached the inflection point 11, 12, 22, 22 and 43 days later. Final SSC was 15.0, 13.0, 12.8, 10.3, 9.7 and 9.1% for fruit in the treatment of 14/8, 18/8, 14/12, 22/8, 22/12 and 26/8C, respectively. Soluble solids concentration was recorded at the proximal and distal ends of the fruit; it was higher in the proximal than distal end for fruit from the first harvest in all treatments except 22/12C, where SSC was highest in the proximal end from day 10 (Appendix 2).

Table 2.5 Comparison of the maximum rate of change in soluble solids concentration (slope) and number of days to the inflection point of kiwifruit grown under treatments of different minimum temperatures (C) (Experiment 2). Values determined from fitted logistic curves where a maximum soluble solids concentration of 16% is assumed.

Temperature (max./min.) (C)	Mean temperature (C)	Slope (% day^{-1})	Time to inflection point (days)
14/8	11	0.092 (0.013)[1]	48
18/8	13	0.070 (0.005)	59
14/12	13	0.079 (0.003)	60
22/8	15	0.051 (0.013)	70
26/8	17	0.037 (0.012)	91
22/12	17	0.067 (0.016)	70

[1]±SE (standard error)

Concentration of starch in fruit decreased fastest in treatments with the coolest mean temperatures (Fig. 2.8). For example, in the 14/8C temperature treatment starch concentration in the outer pericarp decreased rapidly after 32 days. In comparison, starch concentration increased in the outer pericarp in 26/8C treatment up to day 20 without further change during the remainder of the study. Decreases in starch concentration in the outer pericarp in fruit from 18/8, 14/12 and 22/8C temperature treatments were between the change in starch

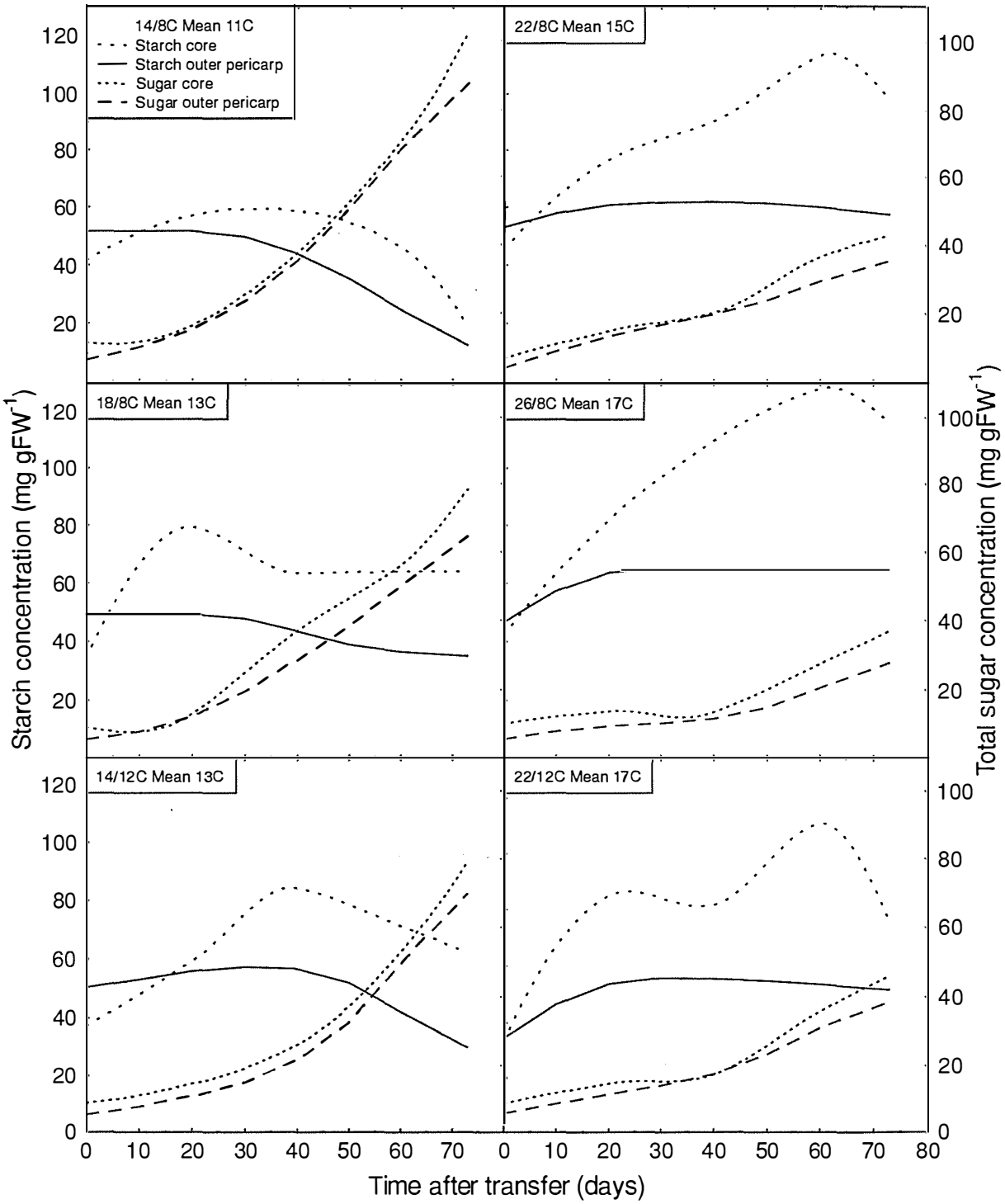


Fig. 2.8 Starch and total sugar concentrations (mg gFW^{-1}) of outer pericarp and core tissue of kiwifruit grown under different maximum and minimum temperatures (C) (Experiment 2). Day 0 = 13 February.

concentration in the extreme temperature treatments described above. The concentration of starch was higher in the core than in the outer pericarp, except at the early harvests. This difference was especially noticeable towards the end of the experiment in the warmer temperature treatments (e.g. 26/8C), where starch accumulated in the core throughout the duration of the experiment. In contrast, in the cooler temperature treatments (e.g. 14/8C), starch concentration in the core declined to a value similar to that in the outer pericarp tissue. In most temperature treatments the concentration of starch in core tissue reached a peak and then decreased; the peak tended to be reached later in warmer temperature treatments (cf. 18/8 and 26/8C). The peak was not as evident in outer pericarp tissue. Increases in total sugar concentration were similar to those in SSC; that is, faster increases occurred at the cooler mean temperatures (Figs 2.7 and 2.8). Concentration of total sugar was similar in outer pericarp and core tissue (Fig. 2.8). Changes in total sugar concentration were highly correlated with changes in SSC ($r=0.98$) ($P \leq 0.05$) (Fig. 2.9). Changes in starch and sugar concentration in outer pericarp and core tissue are shown in 3D graphs to enable comparison of all temperature treatments (Fig. 2.10).

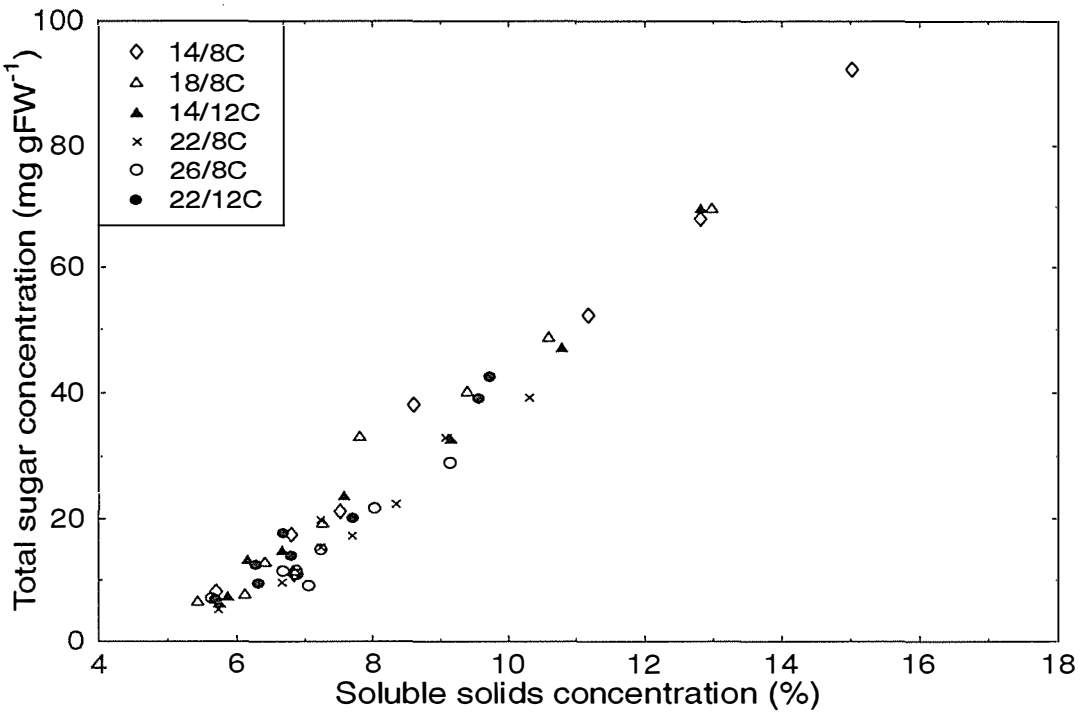


Fig. 2.9 Correlation between total sugar concentration (mg gFW^{-1}) and soluble solids concentration (%) of kiwifruit (Experiment 2). Y = - 45.4 + 8.8% SSC (0.24 standard deviation) (R^2 = 0.97).

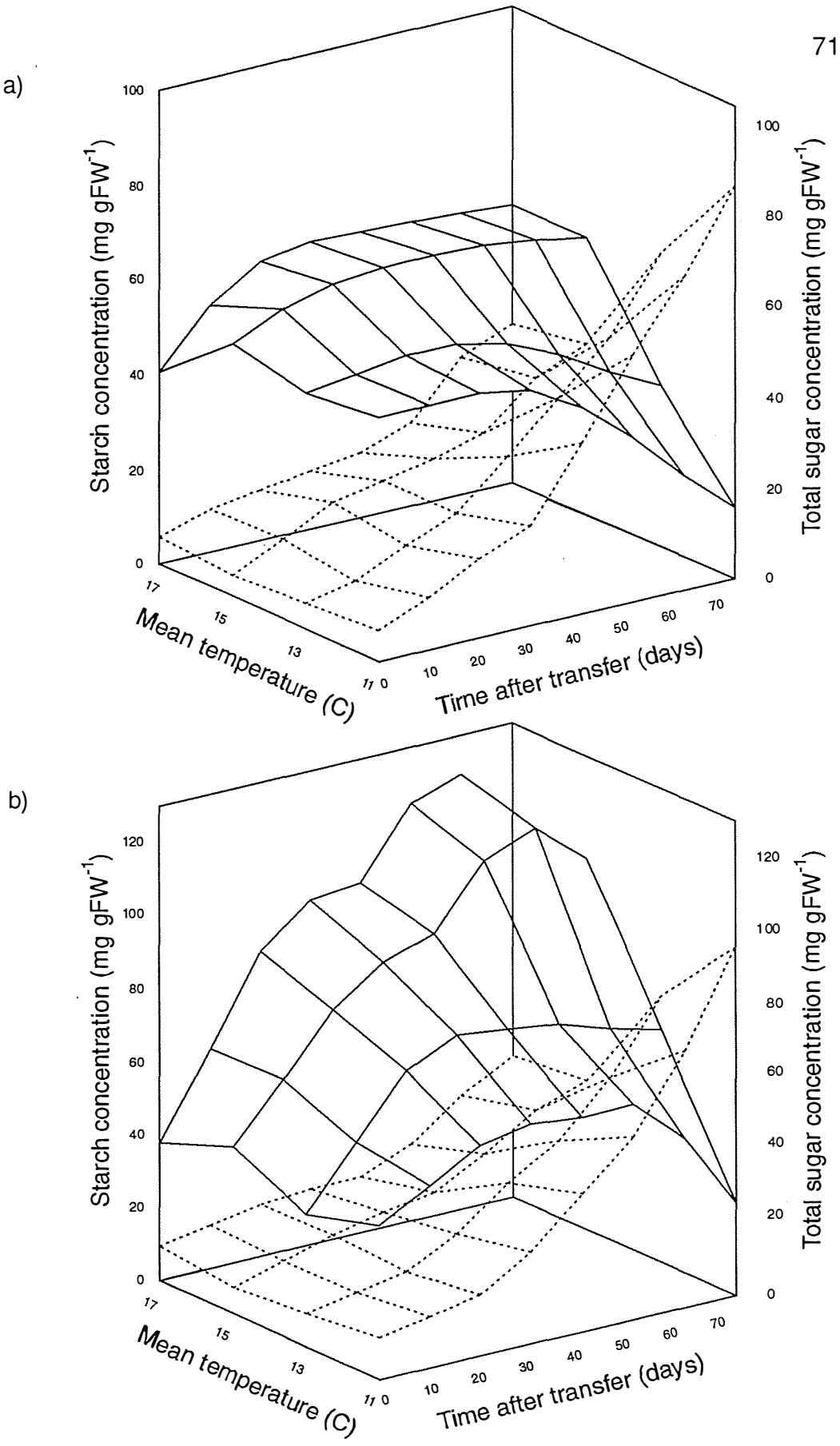


Fig. 2.10 Starch (——) and total sugar (·····) concentration (mg gFW^{-1}) of kiwifruit grown under different temperatures (C) (Experiment 2). a) outer pericarp tissue, b) core tissue. Temperature treatments of 18/8 and 14/12C and 26/8 and 22/12C were combined.

2.3 Effect of a perturbation in temperature

2.3.1 Materials and methods

Temperature extremes and perturbation treatments (Experiment 3)

Vines and controlled environment rooms were used as described in Section 2.2.1. Temperature treatments (Table 2.6) were chosen to extend the range of temperatures beyond that used previously (Section 2.1) and to examine the effect of a perturbation in temperature on fruit maturation. A unit matrix (Table 2.7) was used to determine the frequency of temperature for each treatment. The four treatments where conditions remained the same throughout are referred to as the fixed temperature treatments. The two treatments where the temperature perturbation occurred at day 37, and then reverted back to the original temperature at day 54, are the temperature perturbation treatments.

Changes in temperature and relative humidity between the maximum and minimum (also minimum to maximum) temperatures were programmed to occur within 120 minutes. This programme resulted in 10 hours at both the maximum and minimum temperatures, except the treatment of 15/11C where the temperature change took 240 minutes so that time spent at the maximum and minimum temperatures was reduced accordingly. The maximum/minimum water vapour pressure deficit for each treatment was 0.5/0.4 kPa and the corresponding relative humidities are given in Table 2.6.

Thirty vines were selected from the sheltered site and transferred to the controlled environment treatments on 4 March 1991 for a period of 75 days. Five vines were selectively placed in each treatment to give a similar number of fruit per treatment. Randomly selected fruit were harvested every 3 days in the fixed temperature treatment and in the temperature perturbation treatment every 5 days at the beginning of the experiment, but every 2 days following the temperature change. At each harvest, six fruit from each temperature treatment

Table 2.6 Temperatures (C) and relative humidities (%) in controlled environment rooms during a) fixed temperature and b) temperature perturbation treatments (Experiment 3).

Maximum temperature (C)	Minimum temperature (C)	Mean temperature (C)	Relative humidity (%)
a) Fixed temperature treatments (4 treatments)			
11	7	9	60/62[1]
15	11	13	70/71
15	15	15	77/71
26	20	23	83/85
b) Temperature perturbation treatments (2 treatments)			
11/7→20/13→11/7C treatment			
11	7	9	60/62
20	13	16.5	73/79
11	7	9	60/62
26/20→15/11→26/20C treatment			
26	20	23	83/85
15	11	13	70/71
26	20	23	83/85

[1] Relative humidity at maximum and minimum temperature, respectively

were sampled for SSC, flesh firmness, fresh weight, dry weight and fruit growth measurements. Cortex and core tissue from these fruit was stored for subsequent analysis of starch and sugar concentration as described in Section 2.2.1.

A portable gas-exchange system (McPherson *et al.*, 1983) using an infrared gas analyser (Binos; Leybold Heraeus G.m.b.H., Haneau, Germany) in a closed system with a darkened chamber (model 6000-10; LiCor, Nebraska, USA) was used to measure fruit respiration. The chamber contained a fan at both ends to assist air movement. Respiration from individual fruit attached to the vine was

Table 2.7 Unit matrix showing the frequency distribution of temperature (C) for the fixed temperature and temperature perturbation treatments (Experiment 3).

Temperature (max./min.) (C)	Temperature (C)						
	7	11	13	15	20	26	Total
11/7	0.50	0.50	0	0	0	0	1.00
15/11	0	0.33	0.33	0.33	0	0	1.00
15/15	0	0	0	1.00	0	0	1.00
26/20	0	0	0	0	0.50	0.50	1.00
11/7→20/13→11/7	0.39	0.39	0.11	0	0.11	0	1.00
26/20→15/11→26/20	0	0.11	0	0.11	0.39	0.39	1.00

recorded five times in each temperature treatment during the experiment. At each recording time, two fruit from each of three vines were measured at the maximum temperature in each temperature treatment. Three consecutive measurements, each of 120 seconds, were made on each fruit.

Subsamples from selected sugar extracts (11/7 and 26/20C fixed temperature treatments) were prepared for analysis by high performance liquid chromatography (HPLC). The subsamples (1 ml) were evaporated to dryness, reconstituted to half the original volume with 80% acetonitrile and filtered through a 0.2 μm nylon membrane. At ambient temperature, samples were injected into a Waters HPLC fitted with an amino Bio-rad column (220 x 4 mm) and eluted with solvent (80% acetonitrile). The flow rate was 1 ml min^{-1} and the guard column was a 300 mm Carbo-C column. The reference standard contained 300 μl l^{-1} each of glucose, fructose and sucrose.

Unfortunately, data from the 26/20C temperature treatment were not obtained after day 56 due to a small leak of ethylene gas (3 ppm for 10 hours) into the controlled environment room, and this was sufficient to cause an extremely rapid rise in SSC in the fruit. The remaining controlled environment rooms were checked for the presence of ethylene, but were unaffected. To check the effects

of ethylene gas on kiwifruit attached to the vine, at the end of the experiment, 3 ppm ethylene was injected into the controlled environment room containing the 26/20C fixed temperature treatment and maintained at that concentration for about 6 hours. Measurement of SSC in the fruit over the following few days showed a rapid rise in SSC similar to that seen in the temperature perturbation treatment. These results indicated that it was ethylene that triggered the rapid rise in SSC and not the change in temperature that had been imposed 2 days previously.

Temperature perturbation treatments (Experiment 4)

A further experiment was undertaken to determine the effect of a temperature perturbation. However, in this experiment the temperature perturbation treatments were imposed for different periods of time, in order to determine the physiological response of fruit to short and long periods at cool temperatures.

The vines and controlled environment rooms used were described in Section 2.2.1. The treatments were either a fixed temperature of 22/18C or a perturbation in temperature to 14/10C (Table 2.8). The first temperature perturbation commenced on day 14 and the second on day 56, for periods of 2, 6 or 13 days on both occasions before reversion back to 22/18C. On day 56, each of the three temperature perturbation treatments were programmed to occur in the same controlled environment room as those used for treatments imposed on day 14. Changes in temperature and relative humidity from the maximum to the minimum took 120 minutes for all treatments, resulting in 10 hours at both the maximum and minimum temperature. A common maximum/minimum vapour pressure deficit of 0.4/0.3 kPa was maintained in each treatment (Table 2.8).

Twenty vines bearing fruit were transferred from the sheltered site on 24 February 1992 to the controlled environment treatments for 75 days. Five vines were selectively placed in each treatment to give a similar number of fruit per treatment. At each harvest, ten randomly selected fruit from each temperature

Table 2.8 Temperature (C) and relative humidities (%) in controlled environment rooms during temperature perturbation treatments (Experiments 4 and 5).

Maximum temperature (C)	Minimum temperature (C)	Mean temperature (C)	Relative humidity (%)
14	10	12	75/76[1]
22	18	20	85/86

[1] Relative humidity at maximum and minimum temperature, respectively

treatment were sampled for determination of SSC (Section 2.2.1). Fruit were harvested at intervals of 2 to 10 days depending on the treatment and stage of the experiment.

Temperature perturbation treatments on immature fruit (Experiment 5)

An experiment was undertaken to determine the minimum SSC at which fruit became responsive to a period of cool temperatures.

The vines and controlled environment rooms used were described in Section 2.2.1. The treatments were either a fixed temperature of 22/18C or a perturbation in temperature to 14/10C, as in Experiment 4 (Table 2.8). The first temperature perturbation commenced on day 2 and the second on day 22, for periods of 7 days on both occasions before reversion back to 22/18C. Different controlled environment rooms were used for each of the two temperature perturbation treatments. Changes in temperature and relative humidity from the maximum to the minimum took 120 minutes for all treatments, resulting in 10 hours at both the maximum and minimum temperature. A common maximum/minimum vapour pressure deficit of 0.4/0.3 kPa was maintained in each treatment (Table 2.8).

Twenty four vines bearing fruit were transferred from the sheltered site on 15 March 1993 to the controlled environment treatments for 36 days. Six vines were selectively placed in each treatment to give a similar number of fruit per

treatment. At each harvest, eight randomly selected fruit from each temperature treatment were sampled for determination of SSC (Section 2.2.1). Fruit were harvested at intervals of 7 days.

2.3.2 Results

Temperature extremes and perturbation treatments (Experiment 3)

There was little difference in fruit growth (measured by volume) between treatments, except the 26/20C fixed temperature treatment where fruit were larger, but also grew at a faster rate during the experiment. Growth rate of fruit was 3.3 mm^3 day^{-1} in all treatments except for the 26/20C fixed temperature treatment where it was 7.2 mm^3 day^{-1}. Fruit weighed 62 ±1.8 g and 70 ±4.0 g at the beginning and end of the experiment, respectively (mean of all treatments except 26/20C treatment). Fruit from the 26/20C temperature treatment were 8 g heavier than the average of all the other treatments at the beginning of the experiment, but this margin had increased to 35 g by the end of the experiment.

Flesh firmness decreased during the experiment for all treatments, with the fastest decrease occurring in treatments with the coolest mean temperatures (Fig. 2.11). Initial firmness was 89.3 N and estimates of the rate of decrease in firmness were -0.59, -0.88, -0.91 and -1.13 N day^{-1} for the temperature treatments of 26/20, 15/15, 15/11 and 11/7C, respectively. Flesh firmness was different between each temperature treatment ($P \leq 0.01$). The treatment by time interaction was significant. The decrease in flesh firmness of fruit in the perturbation treatment that changed temperature from 11/7C to 20/13C and from 20/13 to 11/7C was similar to that exhibited by fruit from vines exposed to 11/7C throughout the experiment (Fig. 2.12). The change in temperature from 26/20 to 15/11C did not alter the rate of decrease in flesh firmness compared to the fixed temperature treatment of 26/20C.

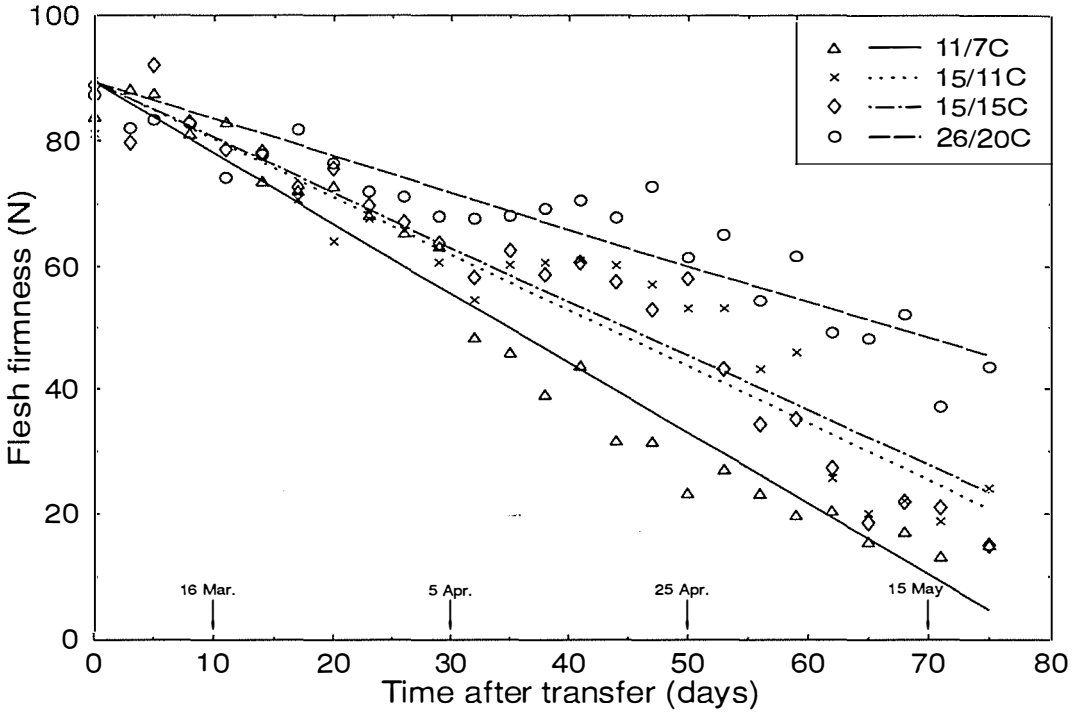


Fig. 2.11 Flesh firmness (N) of kiwifruit grown under fixed temperature (C) treatments (Experiment 3). Regression coefficients in A.3.5.

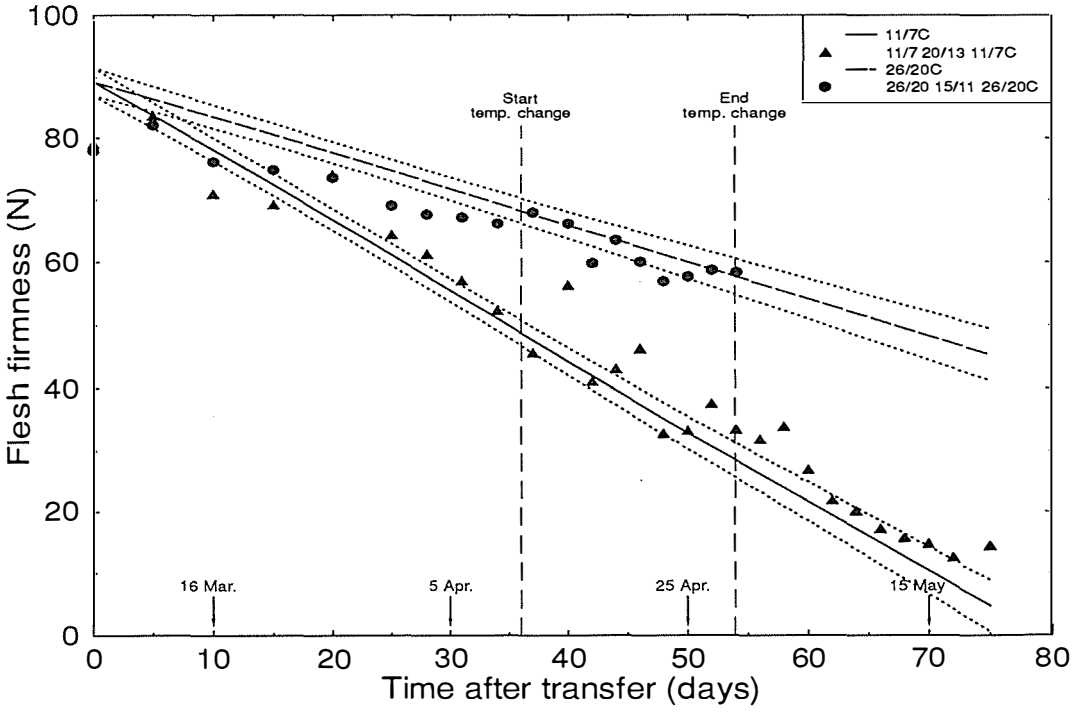


Fig. 2.12 Flesh firmness (N) of kiwifruit grown under fixed temperature (C) and temperature perturbation treatments (Experiment 3). Linear regressions from 11/7 and 26/20C fixed temperature treatments (as Fig. 2.11) are shown with 95% confidence limits. Vertical lines indicate time of temperature perturbation.

Fruit dry matter increased during the experiment and was higher in the warmer than in the cooler temperature treatments after 75 days (Table 2.9). Increase in dry matter was 1.1, 1.3, 2.9 and 6.4% day^{-1} ($x10^{-2}$) for the 15/15, 11/7, 15/11 and 26/20C treatments, respectively. Perturbation in temperature did not affect dry matter of fruit from the 11/7C temperature perturbation treatment compared to the 11/7C fixed temperature treatment.

Table 2.9 Dry matter (%) at the beginning and end of the treatments for kiwifruit grown under a) fixed temperature (C) and b) temperature perturbation treatments (Experiment 3).

Temperature (max./min.) (C)	Time after transfer (days)	
	0	75
	Dry matter (%)	
a) Fixed temperature treatments		
11/7	16.9 (1.2)[1]	17.9 (1.6)
15/11	16.6 (0.8)	18.7 (2.4)
15/15	18.5 (0.9)	19.4 (1.0)
26/20	16.0 (0.7)	20.8 (0.9)
b) Temperature perturbation treatments		
11/7→20/13→11/7	17.8 (0.6)	18.5 (0.9)
26/20→15/11→26/20	18.5 (0.2)	-

[1]±SE (standard error)

Soluble solids concentration increased very rapidly in fruit exposed to the coolest mean temperature (Fig. 2.13). Increase in SSC was 13.1, 10.4, 9.7 and 4.3% day^{-1} ($x10^{-2}$) for fruit from the 15/11, 11/7, 15/15 and 26/20C temperature treatments, respectively. The inflection point was reached in 43 days by fruit in the 11/7C fixed temperature treatment, and 7, 8 and 46 days later for fruit in the 15/15, 15/11 and 26/20C treatments, respectively (Table 2.10). Final SSC was

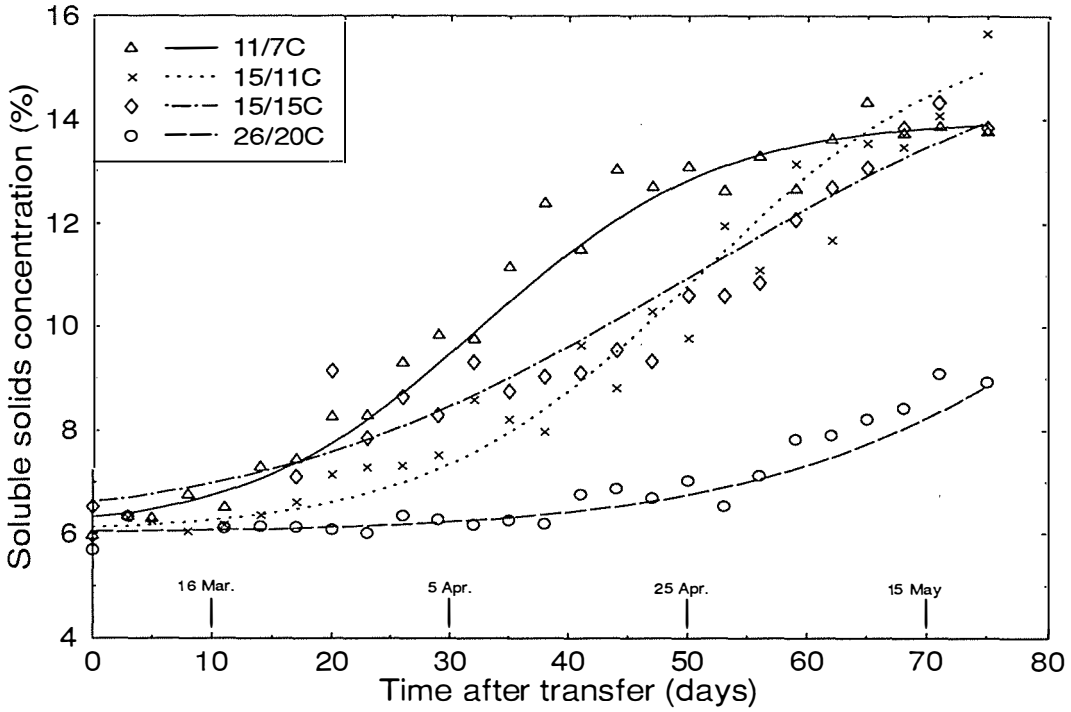


Fig. 2.13 Soluble solids concentration (%) of kiwifruit grown under fixed temperature (C) treatments (Experiment 3). Regression coefficients in A.3.6.

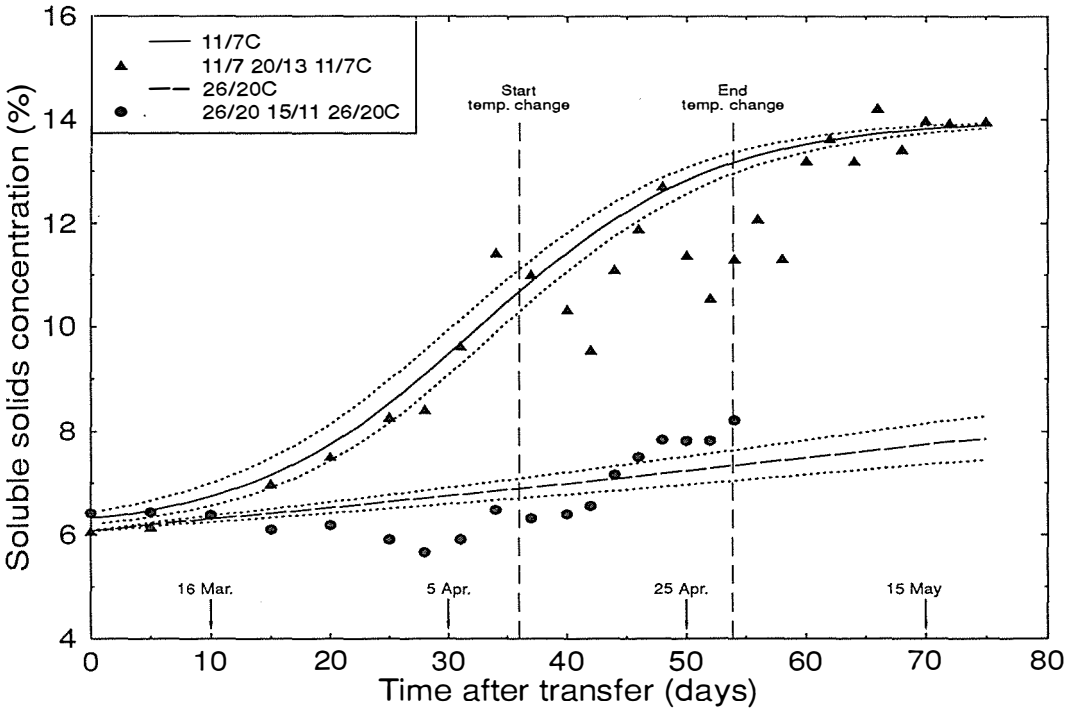


Fig. 2.14 Soluble solids concentration (%) of kiwifruit grown under fixed temperature (C) and temperature perturbation treatments (Experiment 3). Logistic fit from 11/7 and exponential fit from 26/20C fixed temperature treatments are shown with 95% confidence limits. Vertical lines indicate time of temperature perturbation.

15.7, 13.8, 13.8 and 10.0% for fruit in the treatments of 15/11, 11/7, 15/15 and 26/20C, respectively. The difference between maximum and minimum temperatures in Experiment 3 was obtained by a square-wave curve compared to a sine wave curve in Experiments 1 and 2 (Sections 2.2.1, 2.3.1). Timing of the temperature change between maximum and minimum did not affect the rate of increase in SSC. Soluble solids concentration at the distal end of fruit was greater than or equal to that at the proximal end after day 20 in the 11/7, 15/11 and 15/15C treatments, but after day 23 in the 26/20C temperature treatment (Appendix 2).

Table 2.10 Comparison of the maximum rate of change in soluble solids concentration (slope) and number of days to the inflection point of kiwifruit grown under treatments of different minimum temperatures (C) (Experiment 3). Values determined from fitted logistic curves where a maximum soluble solids concentration of 16% is assumed (except 11/7C treatment where maximum soluble solids concentration was 14%).

Temperature (max./min.) (C)	Mean temperature (C)	Slope (% day^{-1})	Time to inflection point (days)
11/7	9	0.072 (0.006)[1]	43
15/11	13	0.089 (0.005)	51
15/15	15	0.055 (0.004)	50
26/20	23	0.064 (0.014)	89

[1]±SE (standard error)

Initial rise in SSC in the first temperature perturbation treatment was similar to that in the fixed temperature treatment of 11/7C (Fig. 2.14). After the temperature changed from 11/7 to 20/13C on day 37, there was an immediate decrease in SSC followed by marked fluctuations, but although the variation was large, the rate of change in SSC was slower at 20/13C than under the 11/7C conditions. Upon reversion to 11/7C, SSC increased over a period of 6 days to a maximum concentration of 14%, similar to that attained by fruit in the fixed temperature treatment of 11/7C.

There was no difference in SSC between the fixed temperature treatment and second temperature perturbation treatment of 26/20C until the temperature change (Fig. 2.14). After the temperature change to 15/11C, there was a 5 day delay before any marked increase in SSC occurred. The difference in SSC between the temperature perturbation and fixed temperature treatment was enhanced over the following 10 days, resulting in a final SSC (at day 54) of 8.3 and 6.5% in the temperature perturbation and fixed temperature treatments, respectively.

Degradation of starch and subsequent increase in total sugar concentration was fastest in the coolest temperature treatments (Fig. 2.15). In the 11/7C temperature treatment starch concentration decreased rapidly in the outer pericarp and core from approximately day 22 to 8 mg gFW^{-1} or less from day 48. In this treatment there was an inversely proportional increase in total sugar concentration in fruit. However, although total sugars rose after day 48 by 10 mg gFW^{-1} (Fig. 2.15), there was little further increase in SSC in this period (Fig. 2.13). In the 26/20C fixed temperature treatment there was an initial decrease in starch concentration in the outer pericarp followed by little further change, while starch concentration in core tissue increased and reached a plateau at about day 30 (Fig. 2.15). Total sugar concentration increased slowly in this treatment after day 30. Decrease in starch and increase in total sugar concentration in the 15/11 and 15/15C temperature treatments were between the extremes described for the 11/7 and 26/20C treatments (Fig. 2.15). Correlation of total sugar concentration and SSC ($r=0.98$) was identical to Experiment 2. Changes in starch and total sugar concentration for all the fixed temperature treatments in the outer pericarp and core tissue are shown in 3D graphs (Fig. 2.16).

In the first temperature perturbation treatment, when the temperature was changed from 11/7 to 20/13C, the rate of decrease in starch concentration in the outer pericarp and core was slower during the 20/13C period compared to that in the fixed temperature treatment of 11/7C (Fig. 2.15). However, the starch

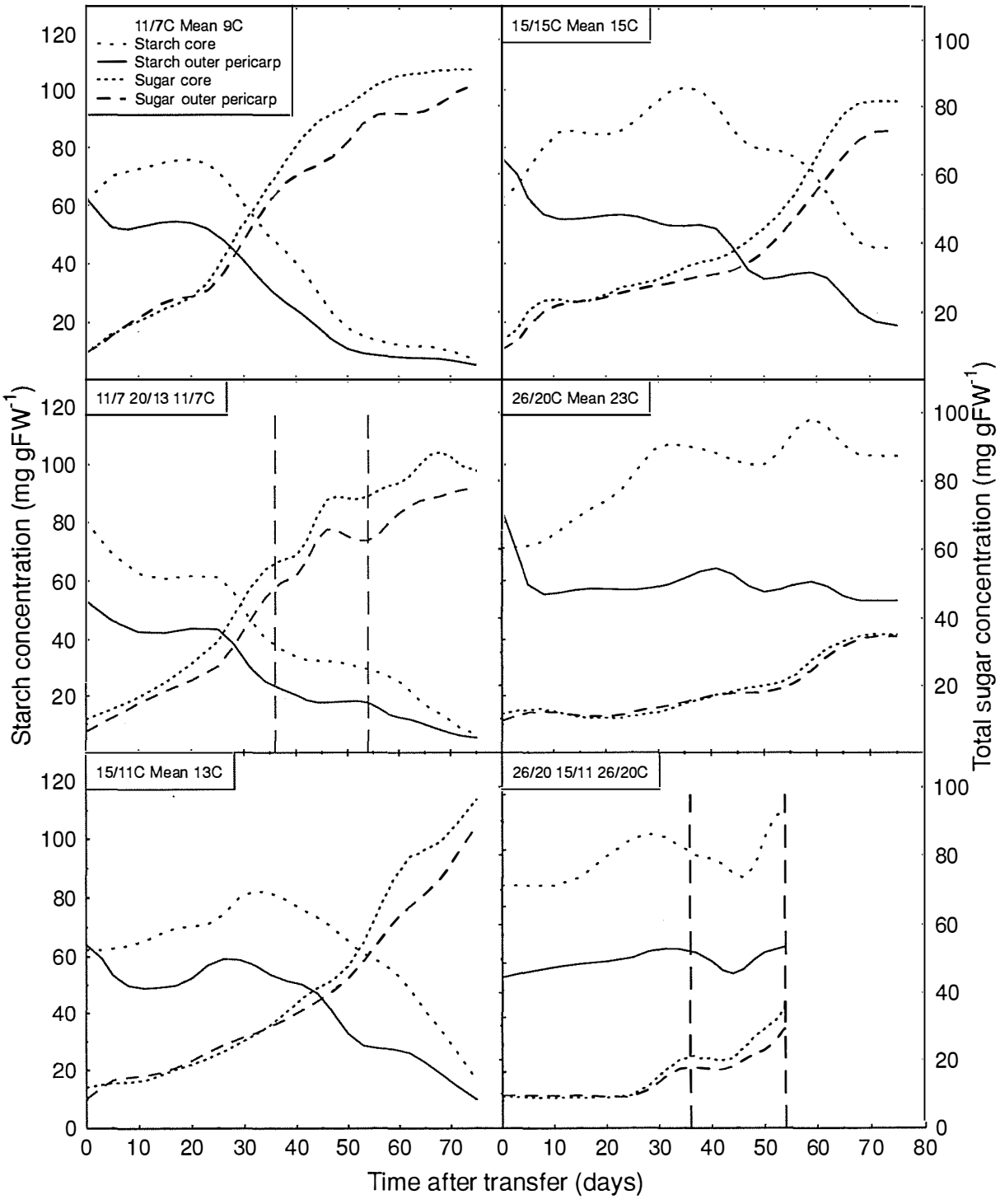


Fig. 2.15 Starch and total sugar concentrations (mg gFW^{-1}) of outer pericarp and core tissue of kiwifruit grown under fixed temperature (C) and temperature perturbation treatments (Experiment 3). Vertical lines indicate time of temperature perturbation. Day 0 = 4 March.

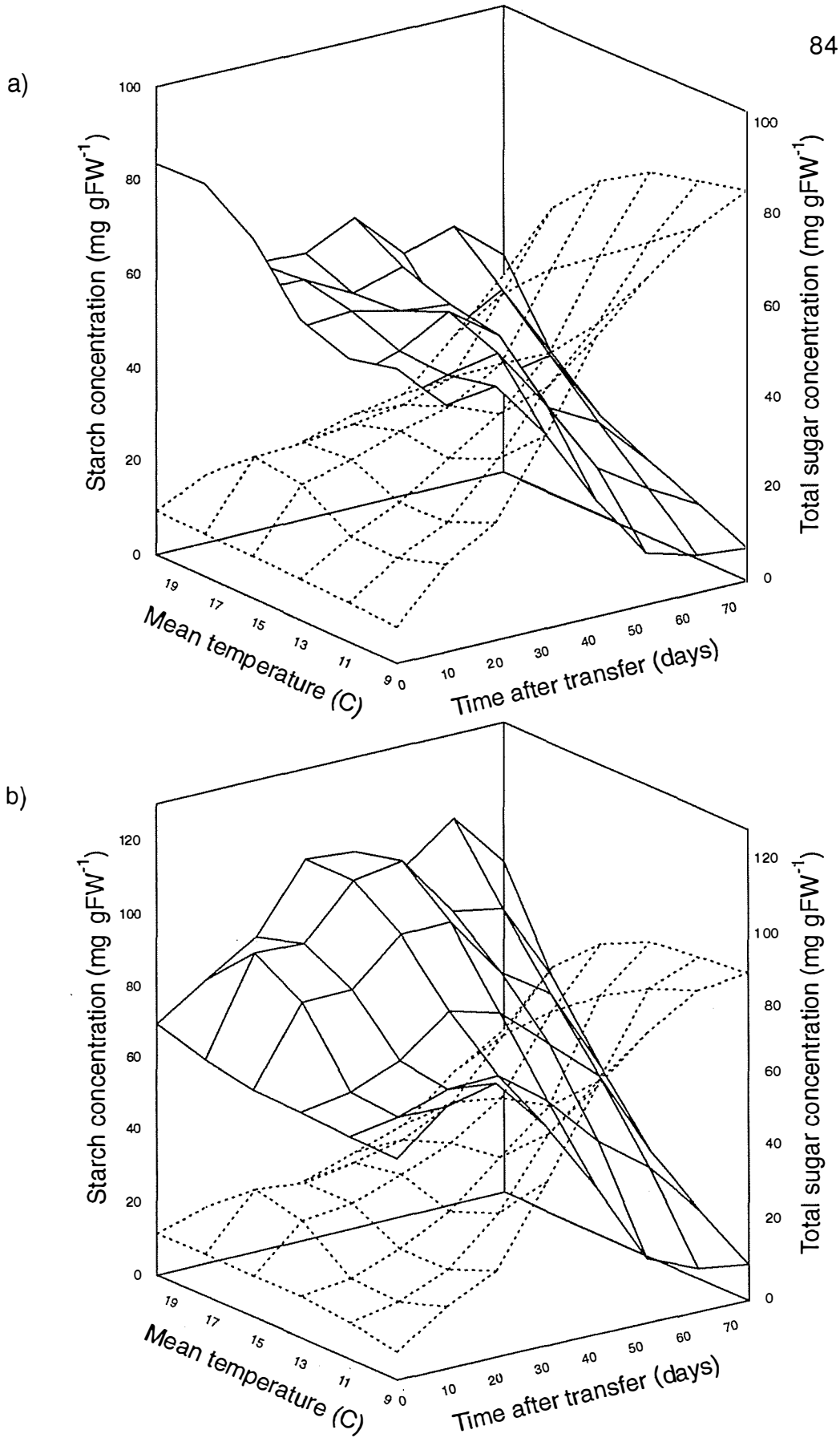


Fig. 2.16 Starch (——) and total sugar (·····) concentration (mg gFW^{-1}) of kiwifruit grown under different fixed temperature (C) treatments (Experiment 3). a) outer pericarp tissue, b) core tissue.

concentration decreased again upon reversion to 11/7C and final starch concentrations in the two treatments were similar. After the temperature was changed from 11/7 to 20/13C total sugar concentration did not change for 2 days, then increased in the outer pericarp and core but later decreased slightly in the outer pericarp (Fig. 2.15). Final sugar concentration was approximately 10 mg gFW^{-1} lower in the temperature perturbation treatment than it was in the fixed temperature treatment. In the second temperature perturbation treatment, when the temperature was changed from 26/20 to 15/11C, starch concentration in the outer pericarp and core decreased slightly, then increased again to become similar to the fixed temperature treatment of 26/20C (Fig. 2.15). However, concentration of sugar increased after the temperature changed to 15/11C, akin to the rise in SSC (Fig. 2.14). In both temperature perturbation treatments starch concentration in core tissue was over 20 mg gFW^{-1} higher than that in outer pericarp tissue at the beginning of the experiment, the reasons for this difference are unclear as all vines came from the same population.

Concentrations of glucose, fructose and sucrose in kiwifruit were variable, but increased during the experiment and to a greater extent at 11/7 than at 26/20C (fixed temperature treatments) (Table 2.11). The concentration of fructose was either higher or similar to the glucose concentration in fruit, while sucrose concentration was either similar to or lower than that of either fructose or glucose. The concentrations of glucose, fructose and sucrose in the core were usually similar to or higher than those in the outer pericarp. However, in the 26/20C temperature treatment after 71 days, the concentrations of glucose and fructose were lower in core tissue than outer pericarp. The (glucose plus fructose)/sucrose ratio was lower in fruit from 11/7 than 26/20C temperature treatments in both the core and outer pericarp tissues at all measurement times.

Respiration rate was highest in fruit from the 26/20C temperature treatment and lowest in the coolest temperature treatment of 11/7C (Table 2.12). Throughout the experiment, respiration decreased in fruit from the fixed temperature treatment of 11/7C, in contrast to those in the 26/20C temperature treatment

Table 2.11 Concentrations of glucose, fructose and sucrose (mg gFW^{-1}) and (glucose plus fructose)/sucrose ratio in fruit from the fixed temperature (C) treatments (Experiment 3).

Temperature (max./min.) (C)	Tissue type	Time after transfer (days)	Sugar concentration (mg gFW^{-1})			
			Glucose	Fructose	Sucrose	$\frac{(G+F)}{S}$[1]
11/7	Outer	11	11.7 (1.8)[2]	16.2 (2.5)	7.3 (1.5)	3.8
	pericarp	38	17.1 (1.3)	19.8 (1.2)	12.1 (2.3)	3.1
		71	23.3 (0.3)	25.8 (0.7)	16.3 (2.2)	3.0
	Core	0	1.4 (0.2)	3.4 (0.5)	7.7 (2.3)	0.6
		11	8.2 (2.2)	18.6 (1.2)	11.6 (3.6)	2.3
		38	18.2 (2.4)	22.3 (2.8)	23.4 (1.3)	1.7
		53	20.2 (3.7)	19.2 (2.6)	18.9 (4.2)	2.1
		71	26.1 (2.6)	28.9 (4.4)	34.4 (9.2)	1.6
26/20	Outer	11	7.3 (2.9)	10.3 (3.0)	4.3 (1.1)	4.1
	pericarp	38	3.7 (0.5)	5.5 (0.7)	1.7 (0.4)	5.4
		71	12.4 (1.8)	13.1 (1.5)	5.8 (0.9)	4.4
	Core	0	2.2 (0.7)	3.3 (0.7)	1.7 (0.1)	3.2
		11	5.3 (2.8)	14.2 (2.9)	7.3 (2.0)	2.7
		38	3.8 (0.2)	6.7 (2.4)	3.7 (0.9)	2.8
		53	4.3 (1.6)	6.4 (1.6)	2.8 (1.5)	3.8
		71	8.0 (0.9)	11.1 (1.3)	11.8 (2.6)	1.6

[1](glucose plus fructose)/sucrose ratio
[2]±SE (standard error)

Table 2.12 Respiration (nmol CO_2 $kgFW^{-1}$ s^{-1}) of attached kiwifruit grown under a) fixed temperature and b) temperature perturbation treatments (Experiment 3).

Temperature (max./min.) (C)	Time after transfer (days)				
	29	43	50	55	70
	Rate of respiration (nmol CO_2 $kgFW^{-1}$ s^{-1})				
a) Fixed temperature treatments					
11/7	71 (4)	85 (4)	39 (4)	24 (2)	18 (2)
15/11	94 (9)	97 (16)	81 (11)	50 (9)	62 (16)
15/15	45 (5)	64 (3)	55 (5)	85 (5)	48 (7)
26/20	126 (16)	173 (9)	172 (7)	199 (9)	170 (8)
b) Temperature perturbation treatments					
11/7→20/13→11/7 treatment					
11/7	87 (5)			40 (5)	22 (3)
20/13		77 (11)	72 (9)		
26/20→15/11→26/20 treatment					
26/20	150 (12)			203 (10)	-
15/11		58 (6)	97 (7)		

[1]±SE (standard error)

where respiration remained high. Respiration rate of fruit was lower in the 15/15 than 15/11C temperature treatment for all measurements, except those on day 55. There was no change in the rate of respiration following change in temperature from 11/7 to 20/13C. Respiration decreased upon reversion to 11/7C, but it took a further 15 days until rates similar to those in the fixed temperature treatment were attained - that delay is similar to the length of the temperature perturbation period itself. In the second temperature perturbation treatment, when the temperature was changed from 26/20 to 15/11C, there was a marked decrease in respiration. Following reversion to 26/20C, respiration returned to a high rate, similar to the rate in the fixed temperature treatment of 26/20C (Table 2.12).

Temperature perturbation treatments (Experiment 4)

There was no effect of the temperature perturbations on SSC in any treatment, irrespective of the duration or timing of the temperature change (Fig. 2.17). Soluble solids concentration did not increase before day 56 in any treatment. Initial SSC was lower than in any of the three previous experiments (Figs 2.5, 2.7 and 2.13). Soluble solids concentration at the distal end of the fruit was lower than that at the proximal end for the majority of measurements in each treatment (Appendix 2).

Temperature perturbation treatments on immature fruit (Experiment 5)

Initial SSC was lower (Fig. 2.18) than that in Experiments 1, 2 and 3, but similar to that in Experiment 4 (Fig. 2.17). In the fixed temperature treatment (22/18C), SSC increased by 0.9% during the experiment. After the temperature changed to 14/10C on day 2, SSC decreased but was not different to SSC of fruit remaining at 22/18C. However, when the same temperature perturbation was imposed on day 24, SSC increased during the cool temperature period compared to that in the fixed temperature treatment (Fig. 2.18). Final SSC was 7.3 and 6.4% in the temperature perturbation and fixed temperature treatments, respectively. Soluble solids concentration at the distal end of fruit was lower than that at the proximal end of fruit for the majority of measurements in each treatment (Appendix 2).

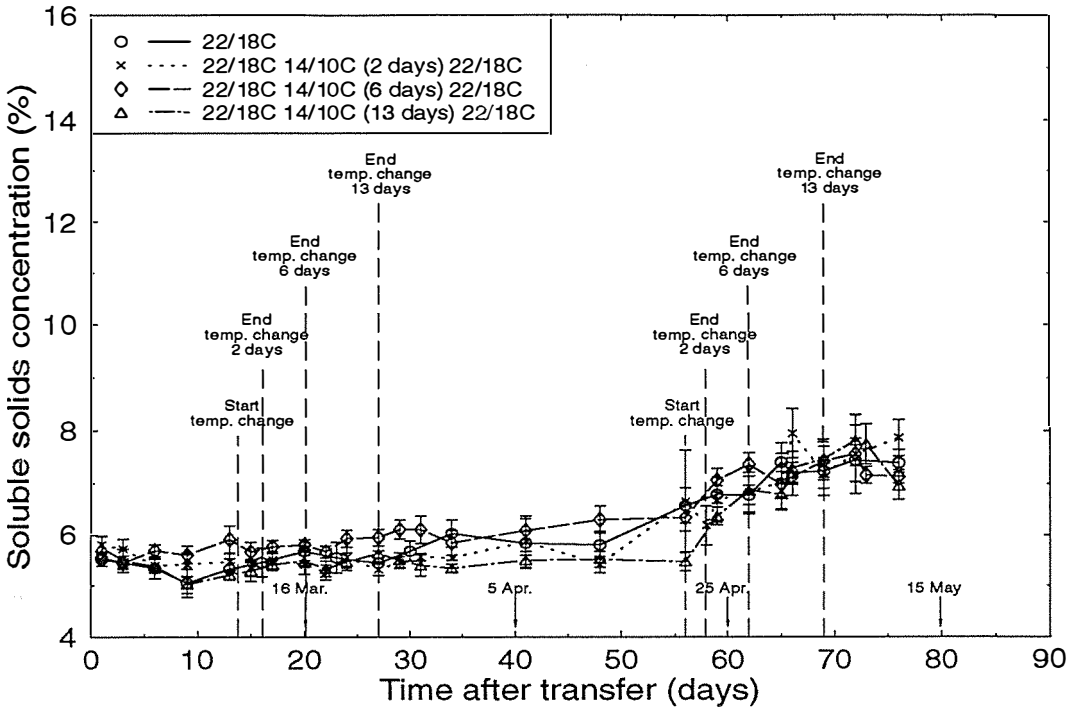


Fig. 2.17 Soluble solids concentration (%) of kiwifruit grown under different temperature (C) perturbation treatments (Experiment 4). Bars = SE. Vertical lines indicate time of temperature perturbation.

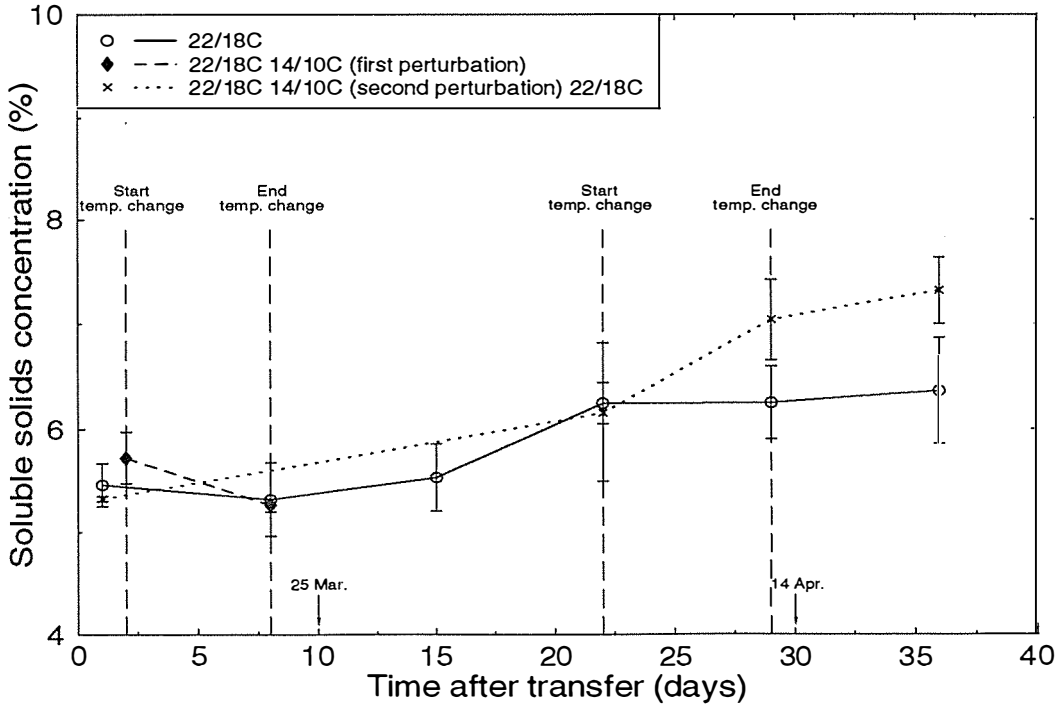


Fig. 2.18 Soluble solids concentration (%) of kiwifruit grown under different temperature (C) perturbation treatments (Experiment 5). Bars = SE. Vertical lines indicate time of temperature perturbation.

2.4 Discussion

Fruit growth in container-grown vines followed the accepted sigmoidal pattern as ascertained for field-grown kiwifruit (Pratt and Reid, 1974; Okuse and Ryugo, 1981). Fruit from container-grown vines were smaller than those from field-grown vines, but were similar in other respects, for example dry matter and changes in SSC, starch and sugar concentration during maturation (Pratt and Reid, 1974; Okuse and Ryugo, 1981; MacRae *et al.*, 1989a). However, initial SSC was 1 to 1.5% higher than that for field-grown fruit (e.g. Wright and Heatherbell, 1967; Reid *et al.*, 1982a; Chapter 3). This may be due to the effect of root restriction by growing plants in containers. Peach seedlings grown in small containers developed a reduced number and shorter roots than unrestricted plants and hence, production of hormones from root apices may be affected (Richards and Rowe, 1977a, 1977b).

The rate of increase in fruit growth was similar between treatments in Experiments 1, 2 and 3, except the 26/20C fixed temperature treatment in Experiment 3. The reason for the large increase in fruit growth in the 26/20C treatment is not fully understood but they were, by chance, larger to start with than fruit from other treatments. Also, this treatment encouraged growth as there was visibly more active vegetative growth throughout the experiment and therefore, a greater quantity of photosynthate available for fruit growth compared to other treatments.

Decreases in firmness of kiwifruit occur during maturation and ripening of fruit on the vine (MacRae *et al.*, 1989a; Beever and Hopkirk, 1990). This is characteristic of several other fruit including apple (Workman, 1963), peach (Maness *et al.*, 1992), pear (Mann and Singh, 1988), fig (Tsantili, 1990) and guava (Yusof and Mohamed, 1987). However, there are differences among species and between years in the rate and extent of softening. For example, decrease in firmness during maturation was mostly linear in apple and kiwifruit, but quadratic in some years (Harman, 1981; Ingle and D'Souza, 1989). Immature guava were very firm

(>295 N), but flesh firmness decreased rapidly within a 14 day period to 98 N at eating ripeness (Yusof and Mohamed, 1987). In contrast, at physiological maturity in peach, flesh firmness was between 4 and 8 N, decreasing to 0.5 to 5 N when fully mature, depending on cultivar (Maness *et al.*, 1992). Kiwifruit had higher flesh firmness than that in peach at physiological maturity, but flesh firmness in both species was similar at eating ripeness (Beever and Hopkirk, 1990). Flesh firmness decreased in all experiments regardless of temperature treatment. However, there was a tendency for the greatest decrease in firmness to occur in treatments with the coolest mean temperature, but separation of treatments was not as clear as shown with SSC. Hopkirk *et al.* (1989) showed that whilst there were differences in SSC among fruit at ambient temperature compared to those 3C above ambient, there were no differences in fruit firmness. Similarly, there was no marked change in flesh firmness in either temperature perturbation treatment after the temperature change, compared to the equivalent fixed temperature treatment, in contrast to the marked changes in SSC, especially in the 26/20 to 15/11C temperature perturbation treatment.

During maturation, decrease in firmness is one of many changes taking place. Coombe (1976) described three important contributors to ripening: expansion, solute accumulation and softening. Each has its own timing and control which may be interrelated, but the events are not necessarily coincident. Degradation of starch was thought to affect the early stages of fruit softening (MacRae *et al.*, 1989b). Starch granules do not affect firmness *per se*, but may do so during degradation by affecting the osmotic pressure and turgor within the cell (MacRae and Redgwell, 1992). Decrease in flesh firmness was negatively correlated to the increase in SSC during maturation as shown by the values of -0.88, -0.92 and -0.94 for all temperature treatments in Experiments 1, 2 and 3, respectively. Similar correlations were attempted with five different strains of 'Red Delicious' apples in two consecutive years (Ingle and D'Souza, 1989). The values for their correlations were lower than for those with kiwifruit in this work and in some strains there was a positive correlation between firmness and SSC, while in others there was a negative correlation. Similarly, there was variation in

correlation between decrease in flesh firmness and increase in SSC among seven melon cultivars (Miccolis and Saltveit, 1991). Decrease in flesh firmness and increase in SSC both occur in many fruit species during maturation, but not necessarily at the same time or rate (Coombe, 1976).

Dry matter is a measure of the total solid material in kiwifruit and values increase to a maximum about the same time as fruit reach maturity, that is, it reflects the change in total carbohydrate concentration (Hopkirk, 1991). Research in Australia and California has investigated use of dry matter as an alternative index for kiwifruit maturity, instead of SSC (Scott *et al.*, 1986). The advantage of this test is that it would be less influenced by the conversion of starch to sugar (Hopkirk, 1991). Increase in dry matter during Experiments 2 and 3 was similar to that found by other researchers. MacRae *et al.* (1989a) recorded a dry matter of 16.5% for fruit from an orchard in Te Puke on 26 March, and this had increased to 20.3% by 15 May. At Kumeu, dry matter from control vines at harvest was 17.6% in 1986 and 16.0% in 1987 (Hopkirk *et al.*, 1989) whereas, in California, dry matter at harvest was 18.5% (Walton and de Jong, 1990a). There are differences between sites and within sites between years in fruit dry matter, possibly reflecting the environmental conditions and management procedures that affect photosynthesis and thus carbohydrate status of fruit.

However, in this study there was a varied relationship between dry matter and total non-structural carbohydrate (starch and sugar concentration). The correlation coefficients were r=0.41, 0.44, 0.76 and 0.77 for the treatments of 15/15, 11/7, 15/11 and 26/20C, respectively. In addition, rate of increase in dry matter was not entirely related to the different temperature treatments and hence, change in SSC. For example, at the end of Experiment 2, dry matter for all treatments was 20.6 ±1.2% where SSC ranged from 9.1 to 15.0%. A further lack of consistency in the relationship between dry matter and SSC was shown when dry matter was 16.0 and 16.5% and SSC was 7.1 and 4.5%, respectively (Hopkirk *et al.*, 1989; MacRae *et al.*, 1989a). Hopkirk *et al.* (1989) found no differences in dry matter content of kiwifruit subjected to different temperature treatments, although there

was a trend for higher dry matter and lower SSC in treatments that were above ambient. It therefore appears that dry matter does not consistently correlate to starch and sugar concentrations or SSC in kiwifruit, and contrasts to the implication that dry matter may be better than SSC as a maturity index for harvesting kiwifruit (Scott *et al.*, 1986; Hopkirk, 1991). Further work is needed to establish whether dry matter would be a suitable index of maturity for kiwifruit.

During maturation there was an increase in SSC in fruit from container-grown kiwifruit vines with a similar pattern to that observed in field-grown fruit (Chapter 3) and, for example, by Okuse and Ryugo (1981), MacRae *et al.* (1989a) and Sawanobori and Shimura (1990). An increase in SSC during maturation was also found in apple (Workman, 1963; Ingle and D'Souza, 1989) and pear (Mann and Singh, 1988). Many studies with fruit crops have shown differences in harvest date among sites and among years. For example, studies have shown variation in mean flowering and mean picking date of apple (Kronenberg, 1988) and maturation of kiwifruit (Harman and Hopkirk, 1982). This may be due to different temperatures experienced by plants between regions or years. A popular belief among kiwifruit growers is that SSC increases at a faster rate with cooler night (minimum) temperatures (Hopkirk *et al.*, 1989; Kelly, 1990). If the effect of temperature on kiwifruit maturation can be quantified and harvest date predicted, it would assist growers, the New Zealand Kiwifruit Marketing Board, packaging and shipping companies, and allied support industries, as at present there is considerable uncertainty regarding time of harvest.

There has been considerable interest over the years on the effect of different minimum (night) temperatures on fruit maturation. Many of these experiments were carried out by covering part of the vine or tree with a chamber and increasing temperature within the chamber relative to ambient. Problems associated with such experiments were discussed in Section 1.4.2. Controlled environments have been used to determine the effect of cool (11C) or warm (22C) night temperatures on container-grown apple trees (Blankenship, 1987). There was no significant difference in SSC between temperature treatments, but

the concentration was nearly always higher at 11 than 22C night temperature. Similarly, there was no effect of different night temperatures on SSC of peach (Batjer and Martin, 1965). However, when limb chambers were placed around cherry trees, maturation was delayed in fruit to a greater extent at warmer than cooler minimum (night) temperatures (Tukey, 1952). Again, SSC was higher in muskmelon grown at a night temperature of 12 compared to 21C, although the results were not statistically different (Welles and Buitelaar, 1988).

Temperature effects on field-grown kiwifruit vines have not been widely studied. However, grower observations over a number of seasons have recorded that fruit from southern (cooler) districts, such as Nelson, reached 6.2% SSC before those from northern (warmer) districts, such as Kerikeri (Hopkirk *et al.*, 1989). Whole field-grown kiwifruit vines were surrounded by relocatable greenhouses and the mean temperature increased by 3C above ambient during the day (maximum) and night (minimum) or during the day only (Hopkirk *et al.*, 1989). By 14 May, the SSC were 6.2, 6.2 and 7.0% for the two warm temperature treatments and the control, respectively. There was a 9 day delay in reaching commercial harvest maturity (6.2% SSC) for fruit from the warmed treatments compared to fruit from control vines. The two warmed treatments gave similar results; Hopkirk *et al.* (1989) thought that this was probably due to the ambient night (minimum) temperature not being low enough in that year to reduce the mean temperature sufficiently in the treatment with the warm days to make it different from the treatment with the warm days and nights.

Temperature affects all aspects of plant metabolism, including photosynthesis, translocation of photosynthate, respiration and carbohydrate metabolism. The effect of temperature on these processes will affect increase in SSC during maturation. The increase in SSC may be influenced by:

i) mean temperature
ii) minimum temperature *per se*
iii) magnitude of the difference between maximum and minimum temperature.

Treatments with the coolest mean temperature resulted in the fastest rate of increase in SSC (Fig. 2.19). Change in SSC occurred in response to mean temperature, irrespective of minimum temperature or magnitude of the difference between maximum and minimum temperature. Presumably the vine integrates the current temperature it receives and thus, over a daily cycle, appears to respond to mean temperature. Minimum temperature *per se* or magnitude of the difference between maximum and minimum temperature were not responsible for changes in SSC for the following reasons:

i) If minimum temperature *per se* had caused the changes in SSC in Experiment 1, then all four treatments with a minimum temperature of 8C should have reached the same SSC in Experiment 2, as should the two treatments with a minimum temperature of 12C. Again, this did not occur, as final SSC was 9.1, 10.3, 13.0 and 15.0% for 26/8, 22/8, 18/8 and 14/8C temperature treatments, respectively.

ii) If the magnitude of the difference between maximum and minimum temperature resulted in the differences in SSC between treatments in Experiment 1, then in Experiment 2 the treatments of 22/12 and 18/8C (10C difference between maximum and minimum) should have had a similar final SSC. This did not occur as final SSC was 9.7 and 13.0% for 22/12 and 18/8C temperature treatments, respectively.

Results from controlled environment work described in this chapter clearly show that it is not the minimum temperature *per se*, but the mean temperature that influences the rise in SSC. The popular misconception of minimum (night) temperature accounting for increase in SSC probably arises because the minimum temperature is an integral part of the mean temperature. Until now, temperature has not been manipulated around kiwifruit to determine such a precise relationship between temperature and SSC during fruit maturation.

An interesting exception to the increase in SSC at low temperatures described previously was the lack of difference in SSC between the fixed temperature treatment and the temperature perturbation treatments in Experiments 4 and 5.

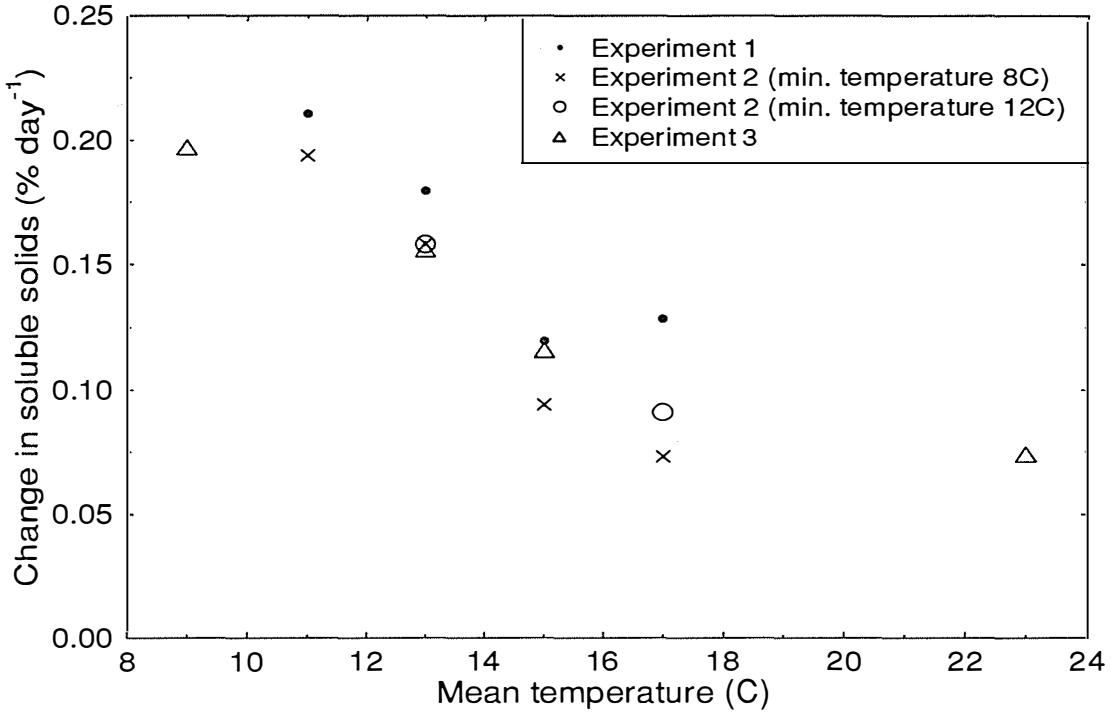


Fig. 2.19 Rate of change in soluble solids concentration (%) of kiwifruit during maturation in controlled environments at different minimum temperatures (Experiment 1) or different maximum and minimum temperatures (Experiments 2 and 3).

There was no effect of either the first or second set of temperature changes from 22/18 to 14/10C for 2, 6 or 13 days, as SSC increased similarly in all treatments in Experiment 4 and in the first temperature perturbation in Experiment 5. However, SSC increased during the second temperature perturbation treatment of 14/10C in Experiment 5. Here, the fruit had presumably reached a developmental stage where it was able to respond to cool temperatures, resulting in an increase in SSC.

The lack of change in SSC when vines were exposed to 14/10C was probably due to the fruit being immature in 1992 and 1993 (Experiments 4 and 5, respectively). In contrast, SSC increased in response to similar periods of cool temperatures in the previous 3 years; these fruit were presumably at a physiological stage of development where the temperature treatment could stimulate hydrolysis of starch to sugar. Seeds in the fruit were still white when first sampled in Experiments 4 and 5 whereas they were brown or black in the other years, even though experiments commenced 11 days earlier in 1990 (Experiment 2) and only 8 days later in 1991 (Experiment 3) than in 1992. The SSC at the beginning of the experiment was 0.5 to 1.0% lower in 1992 and 1993 than in the other years. Even by day 56 in Experiment 4, SSC had increased very little, again demonstrating the immaturity of the fruit, as an increase in SSC had occurred by a similar time in previous experiments. Further evidence for fruit in Experiments 4 and 5 being immature may come from SSC differences at the proximal and distal ends of fruit. As fruit mature, concentration of SSC at the distal end exceeds that at the proximal end, although the physiological significance of this change is unknown (Cotter *et al.*, 1991). Soluble solids concentration at the distal end of the fruit was lower than that at the proximal end throughout Experiments 4 and 5, in contrast to fruit from Experiments 1, 2 and 3 where SSC from the distal end was generally higher. A factor contributing to fruit immaturity in 1992 and 1993 may have been the low temperatures experienced by the vines during the growing season and before transfer to controlled environments. Temperatures from flowering to time of transfer were generally lower in 1992 and 1993 than in the years 1989 to 1991 (Table 2.13).

Low temperatures may have reduced rate of photosynthesis in leaves, rate of translocation of photosynthate from leaves to fruit and conversion of sugar to starch in fruit, hence resulting in the low SSC measured in fruit in 1992 and 1993.

Table 2.13 Mean monthly temperature (C) at Palmerston North (site code E05363) from flowering (November) to harvest (May) in the years corresponding to Experiments 1, 2, 3, 4 and 5, respectively (New Zealand Meteorological Records).

Month	Temperature (C)				
	Year				
	1988/89	1989/90	1990/91	1991/92	1992/93
November	15.4	16.0	14.9	12.3	14.7
December	18.1	15.7	16.4	15.3	15.1
January	19.6	17.6	17.6	17.4	15.7
February	18.0	20.1	17.4	16.7	16.3
March	17.2	17.9	16.7	14.1	14.7
Mean	17.7	17.5	16.6	15.2	15.3

The whole concept of fruit immaturity is interesting, because Experiments 4 and 5 clearly showed that exposing fruit to cool temperatures before a certain physiological stage had no influence on SSC. A similar proposal was made for field-grown fruit by Salinger and Morley-Bunker (1988) who stated that increase in SSC up to 5.0% did not appear to be controlled by temperature. The present study has confirmed that an increase in SSC of kiwifruit cannot be induced by a reduction in temperature before fruit have reached a certain stage of development. Although the mechanism for the change in responsiveness of fruit to temperature is unknown, it is possible that ethylene may be involved and/or that genes coding for SSC are induced and/or expressed at this stage in fruit development. There may be two components of SSC; a 'basal' component and 'maturation' component. Basal SSC is present from fruit set and determined by one set of genes coding for SSC, whereas the maturation component results from

induction of a different group of genes and accounts for increases in SSC during maturation.

In fruit containing starch as a storage carbohydrate, it is typically found that concentration of starch increases during fruit development. During maturation starch is hydrolysed to sugar and there is a concomitant rise in concentration of total sugar. Similar patterns of changes in carbohydrates during maturation of kiwifruit have been recorded by Wright and Heatherbell (1967), Harman (1981), Okuse and Ryugo (1981), Reid *et al.* (1982a), MacRae *et al.* (1989a) and Sawanobori and Shimura (1990). Although kiwifruit are harvested at 6.2% SSC the decrease in starch concentration and increase in sugar concentration observed during maturation continues throughout storage, until fruit are ripe and ready for consumption (MacRae *et al.*, 1989b). Starch concentration at harvest was 53 mg gFW^{-1}, but this decreased to 6 mg gFW^{-1} after 4 weeks at 4C (MacRae *et al.*, 1989b). Other fruit that store starch also show similar changes in carbohydrate during maturation and ripening include apple (Ohmiya and Kakiuchi, 1990), banana (Agravante *et al.*, 1990), pear (Mann and Singh, 1988) and tomato (Dinar and Stevens, 1981). In apple ('Gloster 69'), starch concentration at harvest (19 mg gFW^{-1}) was lower than in kiwifruit, and decreased to 0.7 and 0.1 mg gFW^{-1} by 97 and 200 days of storage at 3.5C, respectively (Knee and Tsantili, 1988). When similar apples were placed at 15C after harvest, starch concentration decreased rapidly from 19 to 4 mg gFW^{-1} in 14 days (Tsantili and Knee, 1991). Starch concentration decreased in tomato (*L. cheesmanii* typicum) from 25 to 0% of dry matter in 18 days when fruit were fully ripe (Garvey and Hewitt, 1991). Banana treated with ethylene ripened in 10 days and starch concentration decreased from 85% of dry matter at the green stage to 9% of dry matter in fully ripe fruit (Marriott *et al.*, 1981). Similarly, starch concentration decreased faster in fruit from kiwifruit vines sprayed with Ethrel (ethylene) compared to fruit from unsprayed vines (Bowen *et al.*, 1988).

Increases in total sugar concentration for each temperature treatment were similar to those seen for SSC as correlation between the two was high. Therefore

SSC measured was largely affected by sugar concentration in the fruit and affected consistently by other components in the juice, such as acids and vitamin C (Harman and Watkins, 1986). Harman (1981) stated that, in kiwifruit, there was a consistent relationship between SSC and sugar concentration, but did not give any data with which to compare results from experiments reported here. Data collected during the latter stages of fruit maturation in pear (from Mann and Singh, 1988) indicate a correlation coefficient between SSC and total sugar concentration of r=0.94, similar to that of r=0.98 obtained for kiwifruit.

Partitioning of total sugars into the component sugars was affected by the temperature treatment. Nevertheless, the increase in the concentrations of glucose, fructose and sucrose in fruit during Experiment 3 were similar to those for field-grown fruit (MacRae *et al.*, 1989a). Glucose and fructose concentrations were similar in each of the temperature treatments, but the (glucose plus fructose)/sucrose ratio was higher in fruit grown at 11/7C than those at 26/20C. It is possible that invertase activity was higher at 11/7 than at 26/20C, resulting in a higher proportion of glucose and fructose. However, there is no evidence of increase in invertase activity at low compared to high temperatures in harvested potato tubers (Morrell and ap Rees, 1986a; Richardson *et al.*, 1990).

The rate of starch hydrolysis and concomitant increase in sugar and SSC during maturation was affected by temperature. At low temperatures the rate of decrease in starch and increase in sugar concentration was faster compared to fruit grown at warmer temperatures. There are very little data available where the effect of temperature on kiwifruit maturation has been studied under controlled conditions. Starch degradation and increase in sugar concentration was slower in fruit matured on field-grown kiwifruit vines that were 3C above ambient during the day, or day and night, than in fruit from control (ambient) vines (Hopkirk *et al.*, 1989). In contrast, much research has been undertaken on harvested potato tubers to investigate the cause of cold sweetening. An analogy will be drawn between potato and kiwifruit accepting that, firstly, there are differences between

the two types of tissues and secondly, that sugars can continue to move into kiwifruit during maturation but cannot in a harvested potato tuber.

Accumulation of reducing sugars in potato tubers stored at low temperatures and their subsequent disappearance when tubers were placed at higher temperatures was recorded by Müller-Thurgau (1882). Since that time, cold sweetening in potato has been shown by Pressey and Shaw (1966), Isherwood (1973), Pollock and ap Rees (1975), Dixon and ap Rees (1980b) and Morrell and ap Rees (1986a). Unfortunately starch concentration was not measured during many of these experiments with potato. Sugar concentration was higher in potato tubers stored at 2, 4 or 5C than those stored at 10, 18 or 10C, respectively (Pressey and Shaw, 1966; Pollock and ap Rees, 1975; Morrell and ap Rees, 1986a). Recalculation of these worker's data indicated that increase in sugar concentration was faster at a storage temperature of 2C than at 4 or 5C. Similarly, the concentration of reducing sugar in potato tubers was greater at 2 than 4C, and both were higher than those stored at 8C (Claassen *et al.*, 1991). There was no change in the concentration of sugar in tubers stored continuously at 8C for 14 weeks (Claassen *et al.*, 1991), 10C for 20 days (Pollock and ap Rees, 1975) or 10C for 10 weeks (Isherwood, 1973). A decrease in sugar concentration was recorded for tubers stored at 18C for 11 weeks (Pressey and Shaw, 1966), perhaps due to an increased rate of respiration at the higher temperature as found in harvested kiwifruit (Wright and Heatherbell, 1967). The faster degradation of starch and synthesis of sugars at low compared to warm temperatures may be explained by examination of control points in the glycolytic and gluconeogenic pathways.

Within fruit from many species there is a marked gradation in carbohydrate concentration along the longitudinal axis. Soluble solids concentration was higher at the distal than proximal end of mature kiwifruit (Hopkirk *et al.*, 1986; Cotter *et al.*, 1991), similar to apple (Harding, 1936), citrus (Ting, 1969) and melon (Scott and MacGillivray, 1940). Very few studies have examined concentrations of starch and sugar in different tissue zones of kiwifruit during maturation, and even

these studies have not necessarily described the tissue from which samples were taken. Starch and sugar concentrations were higher in the core than outer pericarp in the experiments in this thesis, and this difference was also found in field-grown fruit (Hopkirk *et al.*, 1989; MacRae *et al.*, 1989a). It is possible that the dissimilarity between tissue zones can be explained by the different type of cells present in each tissue, as each type of cell has a different function. There are two sizes of cells in the outer pericarp (Hallett *et al.*, 1992). Patterson *et al.* (1991) reported that small cells were packed with starch grains while large cells were devoid of starch. Core tissue is composed of small, irregular shaped cells (Hallett *et al.*, 1992; Gould *et al.*, 1992). If the small cells in the core were the starch containing type, this may account for the higher concentration of starch in the core compared to the outer pericarp. This is confirmed by starch grain counts where the number of starch grains in the outer pericarp and core of fruit with a firmness of 75.5 N were shown to be 3.8 $x10^5$ and 1.28 $x10^6$ grains mm^{-3} tissue, respectively (Wegrzyn and MacRae, 1991). By the time fruit were near eating ripeness (14.7 N) there were 7.0 $x10^4$ and 4.1 $x10^5$ starch grains mm^{-3} tissue in the outer pericarp and core, respectively. Changes in the number of starch grains in ripening kiwifruit correlated well with loss of starch measured by chemical methods (Wegrzyn and MacRae, 1991).

Total carbohydrate (starch plus sugar concentration) in the outer pericarp at the beginning of Experiment 2 was 49 mg gFW^{-1} and in Experiment 3 was 81 mg gFW^{-1}, but by the end of the experiments was 96 and 93 mg gFW^{-1}, respectively. Vines were placed in the treatments 19 days later in Experiment 3 than in Experiment 2, thus allowing extra growth and accumulation of carbohydrate before the commencement of the experiment. A similar maximum concentration of total carbohydrate was obtained in both experiments, perhaps indicating a physiological maximum concentration. There was no difference in total carbohydrate between the temperature treatments at either the beginning or end of the experiments. This indicates that the change in starch and sugar concentration was due to an alteration in partitioning of carbohydrate, perhaps by

induction of temperature-dependent enzymes and not a change in the concentration of carbohydrate *per se*.

There are few data available for changes in carbohydrate concentrations during temperature perturbation treatments with which to compare results from studies in this thesis. The substantial fluctuations in SSC and sugar concentration in the 11/7C temperature perturbation treatment make interpretation of the data difficult. However, the majority of data points (after transfer to 20/13C) fell below the 95% confidence limit of the 11/7C fixed temperature treatment, indicating increase in SSC was lower at the warmer temperature. The temperature change was imposed near to the maximum SSC for fruit on vines in both the fixed temperature treatment of 11/7C and temperature perturbation treatment, and this may have affected the response of fruit in this treatment. With hindsight, it would have been preferable to have brought forward the date of the temperature change from 11/7 to 20/13C. Decrease in SSC after the temperature changed from 11/7 to 20/13C was similar to the decrease in sugar concentration in potato tubers when transferred from either 2 or 4C to 10 or 18C, respectively (Pressey and Shaw, 1966; Isherwood, 1973) and could have been be due to increased respiration by fruit at the higher temperature. The decrease in SSC occurred within two days of transfer to 20/13C and without the longer delay measured in the other temperature perturbation treatment. Following reversion of the temperature to 11/7C SSC increased, as would be expected from fruit on vines exposed to cool temperatures.

Final SSC was only 14%, similar to vines in the fixed temperature treatment of 11/7C. The plateau at 14% SSC in the 11/7C treatment may be due to the leaves on the vine becoming senescent and abscising (Plate 2.4) and therefore not operating at their full photosynthetic potential. The photosynthetic capacity of kiwifruit leaves was highest in spring (13 μmol m^{-2} s^{-1}) and declined during the growing season to 6 μmol m^{-2} s^{-1} in autumn (Greer and Laing, 1992). Under these conditions a severely reduced amount of photosynthate would be entering the fruit and increase in carbohydrate concentration from new photosynthates would

Plate 2.4 Effect of temperature on vines in the fixed temperature treatments in Experiment 3. Left photograph: 11/7C treatment. Right photograph: 26/20C treatment.

be limited. Also, at this stage, virtually all of the starch had been hydrolysed to sugar so that there would have been little further hydrolysis.

In the 26/20C temperature perturbation treatment, when the temperature was changed from 26/20 to 15/11C the concentration of starch decreased and that of sugar increased, similar to the rise in SSC. In potato tubers, when the temperature was changed from 10 to 2C, increase in concentration of total sugar was thought to be due to the conversion of starch to sugar, after allowance was made for respiration (Isherwood, 1973). After the temperature change from 26/20 to 15/11C, the 5 day lag phase observed in SSC was also reflected in the change in sugar concentration. Similarly, in potato tubers transferred from 10 to 2C there was no change in total sugar concentration for about 4 days after the transfer (Isherwood, 1973). Pressey and Shaw (1966) also undertook transfer experiments with potato tubers between different temperature treatments, but their measurements of change in sugar concentration were taken at 14 day intervals, far too long to detect short term changes. The time delay seems surprising in the context of rapidity of changes in enzymes or sugar substrates that can take place. In kiwifruit, metabolite and enzyme concentrations may change quickly, but presumably it would take time for the results of these changes to be incorporated into SSC, starch and sugar concentrations at a sufficient level to be detected by the methods used. Temperature also affects the rate of respiration. In kiwifruit, respiration was lowest at low temperatures and under these conditions sugars could accumulate instead of being respired. Respiration decreased in kiwifruit when the temperature was changed from 26/20 to 15/11C, but this measurement was taken 6 days after the temperature change, once SSC had begun to increase. It would be interesting to study the rate of respiration more closely in the period immediately following the temperature change.

Respiration can be separated into growth and maintenance components (Penning de Vries, 1975). Growth respiration is the energy source for the synthesis of new biomass while maintenance respiration supplies energy for maintenance of

current biomass. Both Jones (1981) and Walton and de Jong (1990b) recorded a decrease in respiration during the growing season in apple and kiwifruit, respectively. Such a decrease was especially noticeable in the 11/7C temperature treatment. In this treatment, as described earlier, vines were beginning to senesce and the leaves abscise (Plate 2.4). A reduced quantity of photosynthate would therefore be available for the fruit; as a consequence there would be little fruit growth or other active processes, hence the reduction in respiration. There was no decline in respiration with maturation in the 26/20C fixed temperature treatment, presumably due to the ideal growing conditions in this treatment (Plate 2.4).

The higher respiration rate of fruit in the 26/20 than 11/7C fixed temperature treatment was similar to the differences in respiration rate with temperature found in kiwifruit by Walton and de Jong (1990b) and by Jones (1981) in apple. There was no obvious explanation for the lower respiration rates in the 15/15C compared to 15/11C temperature treatment as these fruit appeared to behave in accordance with the temperature treatments in all other respects.

In the 11/7 to 20/13C temperature perturbation treatment there was a decrease in respiration once the temperature reverted back to 11/7C as would be expected in response to the cooler temperature. In the temperature perturbation treatment when the temperature changed from 26/20 to 15/11C, respiration decreased, probably due to metabolic processes slowing at cooler temperatures. Respiration during the period at 15/11C was lower on day 43 and higher on day 50 than for fruit on vines grown in the 15/11C fixed temperature treatment. Although this may indicate over-compensatory swings in respiration, respiration was not measured frequently enough to determine if this was true.

From these experiments conducted in controlled environments, it can be concluded that kiwifruit maturation was affected by temperature. Increase in SSC, total sugar concentration and decrease in starch concentration was faster at cooler than warmer temperatures, irrespective of the minimum temperature *per*

se or magnitude of the difference between maximum and minimum temperature. Changes other than carbohydrates occur during maturation. Flesh firmness decreased during the experiments and tended to be fastest at the coolest temperature, but the temperature effect was not as clear as with carbohydrates. Fruit respiration was measured and found to be related to temperature at the time of measurement. Respiration was highest in the warmest temperature treatments and lowest in the cooler temperature treatments. The precise effect of temperature on kiwifruit maturation, in particular SSC, was recorded under controlled conditions. Such data should allow development of a mathematical model (Chapter 5), the temperature function of which may be applicable to field data (Chapter 3), and ultimately used to assist harvest date prediction at orchards in different geographical locations around the country.

CHAPTER 3

MATURATION OF KIWIFRUIT GROWN AT ORCHARDS IN FOUR CONTRASTING TEMPERATURE ENVIRONMENTS

3.1 Introduction

Studies with many crops have shown differences in rate of maturation and date of harvest, both among sites and at the same site among years. For example, there was a 30 day difference in the date of ripening of apricot between two sites 22 km apart (Lilleland, 1936). Mean flowering date and mean picking date varied in apple, both among sites and between years (Kronenberg, 1988). In British Columbia, kiwifruit flowered several weeks later than in California, but fruit were picked at similar times (Kempler *et al.*, 1992). It is possible that the time of flowering and harvest may be affected by temperature.

The maturity index used to determine harvest dates for kiwifruit in New Zealand is based on SSC; fruit can be harvested for export when its SSC exceeds a minimum of 6.2% (Beever and Hopkirk, 1990). Soluble solids concentration was positively correlated to total sugar concentration (Harman, 1981; Chapter 2). Increase in sugar concentration during maturation of kiwifruit is due to the combination of inflow of current assimilate and hydrolysis of starch (MacRae *et al.*, 1989a), a process typical of other fruit containing starch (Tucker and Grierson, 1987).

Soluble solids concentration at harvest has proved to be a valuable indicator of fruit maturity over the 12 years since it was introduced as a standard to ensure that immature fruit were not harvested for export (Hopkirk, 1992). However, studies have shown that fruit harvested at 6.2% SSC are not ideal for long term storage and frequently do not have optimum eating quality,

whereas fruit harvested at higher SSC store for a longer time and have higher eating quality (Harman, 1981; MacRae *et al.*, 1989a; Mitchell *et al.*, 1992). The SSC at harvest was reported to be of little use for predicting quality of kiwifruit post-storage (Scott *et al.*, 1986), probably because rate of softening rather than eating quality of the fruit was the major consideration when the index was developed (Harman, 1981; Hopkirk, 1991).

Use of dry matter concentration has been investigated as an alternative measure of kiwifruit maturity in Australia, California and New Zealand (Scott *et al.*, 1986; Hopkirk, 1991; Mitchell *et al.*, 1992). The advantage of this test is that it is not influenced by conversion of starch to sugar, as the change in total solid material is closely related to change in total carbohydrate concentration (Hopkirk, 1991).

The effect of temperature on kiwifruit maturation was discussed in detail in Chapters 1 and 2. Maturation is accelerated by warm temperatures in fruit not containing appreciable concentrations of starch, such as stonefruit and grapes (Lilleland, 1936; Tukey, 1952, 1958; Batjer and Martin, 1965). However, in fruit such as pear and kiwifruit which contain starch, maturation is enhanced by cool temperatures (Wang *et al.*, 1971; Hopkirk *et al.*, 1989). Controlled environment studies have further defined the responses of kiwifruit maturation to temperature, especially in relation to changes in SSC, starch and sugar concentrations, flesh firmness and dry matter concentration (Chapter 2). These studies showed that once fruit had reached a certain stage of maturity (6.0% SSC in fruit from container-grown vines), the rate of maturation was then determined by temperatures to which fruit were exposed. As mean temperature decreased, rate of increase in SSC and total sugar concentration and decrease in starch concentration became faster.

Within New Zealand there are differences in timing of commercial harvest among seasons (Harman, 1981), among regions (Hopkirk, 1986; MacRae *et al.*, 1989a), within regions (Snelgar *et al.*, in prep.) and within vines (Hopkirk

et al., 1986; Smith *et al.*, 1992). Differences have also been recorded between regions in Japan (Sawanobori and Shimura, 1990), California (Cristoto *et al.*, 1984) and British Columbia (Kempler *et al.*, 1992). These differences in time taken to reach 6.2% SSC may be due, at least in part, to temperature. Few studies have accurately recorded temperature when comparing fruit maturation between different orchards or different years. Based on results from controlled environment studies, it would be expected that rate of increase in SSC and total sugar concentration and decrease in starch concentration would be fastest at the coolest orchards. The aim of this study was to evaluate the effect of temperature on maturation of field-grown kiwifruit in four contrasting temperature environments.

3.2 Materials and methods

3.2.1 Description of orchard sites

At each site, kiwifruit vines consisted of 'Hayward' scions grafted onto 'Bruno' rootstocks, trained on T-bars with rows orientated north to south. Vines were in full production and received normal commercial management, such as pruning, spray programmes and cultural treatments.

The HortResearch orchard at Kerikeri (35° 10'S, 173° 57'E) was situated on an Okaihau gravelly loam (Orthoxic Palehumult). The 6 year old kiwifruit vines were spaced 4.5 m apart within a row and 5 m between rows.

The HortResearch orchard at Te Puke (37° 49'S, 176° 19'E) was situated on a composite yellow brown pumice soil over yellow brown loam (Oropi sand on Kaharoa ash) (Typic Hapludand). The 15 year old kiwifruit vines were spaced 4.5 m apart within a row and 5.5 m between rows.

The Fruit Crops Unit orchard, Massey University at Palmerston North (40° 23'S, 175° 37'E) was situated on a Manawatu silty and sandy loam (Dystric Fluventic Eutrochrept). The 7 year old kiwifruit vines were spaced 4.5 m apart within a row and 5 m between rows.

The commercial grower's property at Riwaka (44° 06'S, 172° 58'E) was situated on a Riwaka silty and sandy loam, overlaying gravel (Dystric Eutrochrept). The 13 year old kiwifruit vines were spaced 5 m apart within a row and 4 m between rows.

3.2.2 Measurements

Fruit were harvested weekly (from 26 February 1990) from six adjacent vines at each orchard on a predetermined schedule (Table 3.1). Each week, six

similar sized fruit were harvested from each vine (36 fruit in total), packed into a polystyrene box with ice pads (wrapped in paper to prevent fruit from freezing) and sealed before sending by courier to Palmerston North. Fruit collected from the orchard in Palmerston North were stored similarly, but in the laboratory, for 24 hours. Samples that did not arrive within 24 hours of picking were discarded. To determine whether 24 hours in transit affected physical characteristics and chemical composition of fruit sent from each site, each week an additional 36 fruit were harvested from vines in Palmerston North and measured within 2 hours of picking. Furthermore, four additional harvests were taken at Kerikeri, Te Puke and Riwaka during the experiment and SSC was measured at the site within 2 hours of picking to allow comparison with the post-transit values measured at Palmerston North.

Table 3.1 Date of measuring kiwifruit sent to Palmerston North from different orchard locations.

Week of experiment	Orchard site			
	Kerikeri	Te Puke	Palmerston North	Riwaka
	Date of measurement in Palmerston North			
1	27/2	28/2	3/3	1/3
2	6/3	7/3	10/3	8/3
3	13/3	14/3	17/3	15/3
4	20/3	21/3	24/3	22/3
5	27/3	28/3	31/3	29/3
6	3/4	4/4	7/4	5/4
7	10/4	11/4	14/4	12/4
8	19/4	18/4	21/4	18/4
9	-	27/4	28/4	27/4
10	1/5	4/5	5/5	4/5
11	10/5	9/5	12/5	11/5
12	15/5	16/5	19/5	18/5
13	22/5	23/5	26/5	22/5
14	29/5	30/5	30/5	-

At Palmerston North, fruit from each harvest were measured to determine SSC, flesh firmness, dry matter concentration and fruit growth as described in Section 2.2.1. Samples from outer pericarp and core tissue were separately stored for later analysis of starch and total sugar concentration in each tissue following the method outlined in Section 2.2.1, except that fruit were bulked into two replicates, each of three fruit. Subsamples of sugar extracts from fruit harvested at Te Puke and Riwaka were analysed to determine component sugars using an HPLC (Section 2.3.1). Extracts for analysis were selected from the beginning and end of the experiment, and strategic points in between.

Temperatures were recorded at each orchard as 30 minute averages using thermistor probes coupled to Campbell Scientific CR21 data loggers (Campbell Scientific, Logan, Utah); the loggers were also programmed to record daily maximum and minimum temperatures. Data were recorded onto a cassette tape sent each month to Palmerston North for transcription. Thermistor probes supplied to match the loggers were calibrated in an oil bath using thermometers calibrated against New Zealand standard thermometers. Air temperature measurements were taken above and below the kiwifruit canopy (at 2.9 and 1.3 m above the ground, respectively) within a louvred screen (Henshall and Snelgar, 1989). Soil temperatures were measured at 200 mm below the soil surface. Temperature records for each orchard are given in Appendix 4.

At both Kerikeri and Te Puke there were operational problems with the data loggers resulting in loss of some data. For these sites, meteorological data were obtained from the closest NZ Meteorological Station at HortResearch, Kerikeri (A52391) and HortResearch, Te Puke (B76835) for February to May 1990. Maximum and minimum temperatures from these stations were regressed against available data from the logger over the same period. The resultant equation was used to adjust the Meteorological Station temperature data for Kerikeri and Te Puke from February to May. There can be a difference between temperature records from data loggers and standard

meteorological stations. Daily maximum and minimum temperatures are measured in the 24 hour period midnight to midnight with a data logger, but are recorded over the 09.00 to 09.00 period at meteorological stations. A difference in temperature measured with a data logger compared to the meteorological station will occur when minimum temperature does not occur between midnight and 09.00, as happens in approximately 20% of cases (A. Hall, HortResearch, Palmerston North, pers. comm.). This difference has not been taken into account in this work.

3.2.3 Statistical analyses

A linear regression was used to fit flesh firmness data from successive harvests. A t-test was used to determine the difference in soluble solids, starch and sugar concentration of fruit measured within 2 hours of harvest and those transported to Palmerston North or those stored for 24 hours in Palmerston North. Soluble solids concentration data were fitted with a logistic curve where upper asymptote is 16% SSC (Section 2.2.1). The least significant difference (LSD) test was used to separate differences in starch and total sugar concentrations from fruit harvested at the four orchards.

3.3 Results

The mean monthly temperatures for the four orchards showed that Riwaka was the coldest orchard, Kerikeri the warmest and that Te Puke and Palmerston North were similar during the period of study in 1990 (Table 3.2). Decrease in mean monthly temperature from February to May was 6.2, 7.6, 8.9 and 9.1C at Kerikeri, Te Puke, Palmerston North and Riwaka, respectively.

Table 3.2 Mean temperatures (C) (February to May 1990) from orchards at four different locations.

	Mean temperature (C)				
Orchard location	February	March	April	May	Mean
Kerikeri	20.0	19.2	16.6	13.8	17.4
Te Puke	19.1	18.0	14.0	11.5	15.7
Palmerston North	20.5	18.3	14.2	11.6	16.2
Riwaka	18.6	16.3	12.5	9.5	14.2
Mean	19.6	18.0	14.3	11.6	

Fruit increased in size during the study, with the increase in fruit volume being 0.47 mm^3 day^{-1} at Kerikeri compared to 0.29 and 0.36 mm^3 day^{-1} at Riwaka and Palmerston North, respectively (Table 3.3). Fruit volume data at the end of the experiment were not collected at Te Puke. Mean fruit weight at the beginning and end of the study was 69, 72, 76 and 85 g and 103, 101, 109 and 129 g for fruit harvested from Palmerston North, Riwaka, Te Puke and Kerikeri, respectively. This represents an increase of 0.32, 0.36, 0.37 and 0.48 g day^{-1} for fruit from Riwaka, Te Puke, Palmerston North and Kerikeri, respectively.

Table 3.3 Fruit volume (mm^3) at the beginning and end of the experiment for kiwifruit harvested from orchards at different locations.

Orchard location	Fruit volume (mm^3)	
	Time of measurement (days)	
	0	92
Kerikeri	90 (1.7)[1]	133 (2.4)
Te Puke	86 (1.5)	118[2]
Palmerston North	49 (1.4)	82 (2.2)
Riwaka	70 (1.3)	97 (2.0)[3]

[1] ±SE (standard error)
[2] interpolated from fresh weight
[3] day 84

Flesh firmness decreased during the experiment as fruit softened with increasing maturity (Fig. 3.1). Initial firmness was 110.0 N and the estimated rate of change in firmness was -0.53, -0.53, -0.56, and -0.62 N day^{-1} for fruit harvested from Te Puke, Palmerston North, Riwaka and Kerikeri, respectively. Fruit harvested in Kerikeri softened faster than fruit harvested from other locations ($P \leq 0.05$). There was a negative correlation ($r=-0.82$) between flesh firmness and SSC for data pooled across all locations.

Dry matter of fruit harvested from different locations increased from the first measurement at the end of February to the final measurement three months later (Table 3.4). Dry matter of fruit from Kerikeri was higher than that from other orchards at both the beginning and end of the experiment. The rate of increase in dry matter was 2.5, 2.8, 3.0 and 3.2% day^{-1} ($x10^{-2}$) for fruit harvested from Palmerston North, Te Puke, Kerikeri and Riwaka, respectively.

Tests were undertaken to check whether SSC, starch concentration and total sugar concentration were affected by measuring fruit 24 hours after harvest compared to those measured within 2 hours of harvest. Soluble solids concentration was different in only three of the 14 harvests among fruit

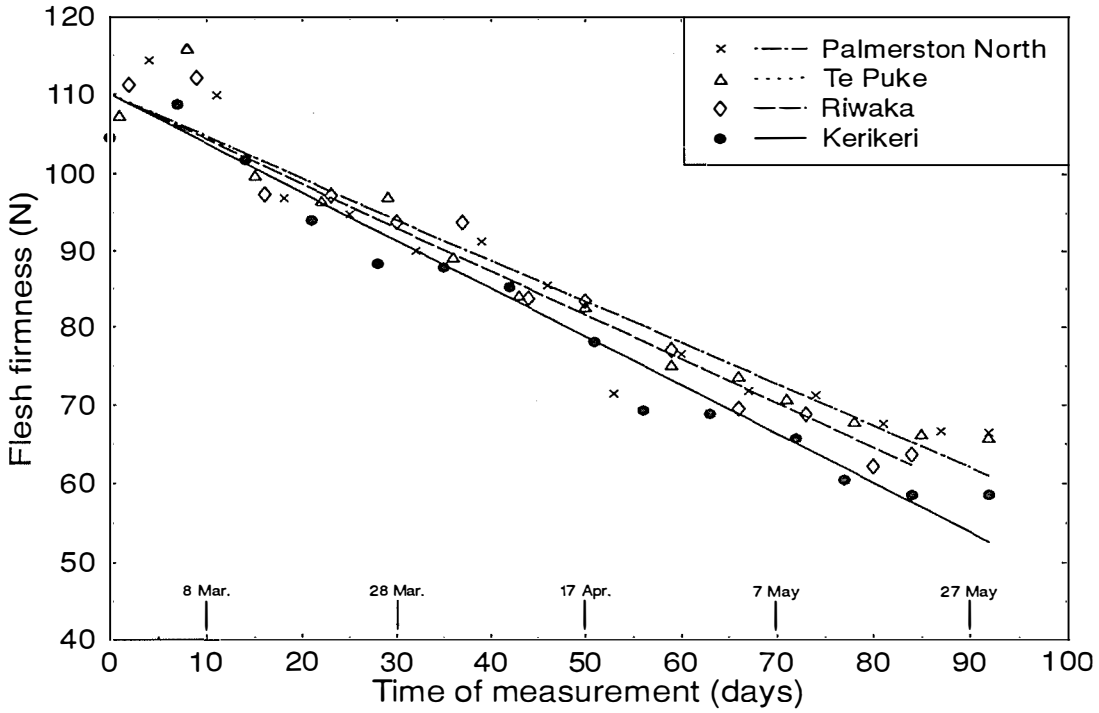


Fig. 3.1 Flesh firmness (N) of kiwifruit grown at four different orchard locations. Firmness of fruit harvested from Palmerston North and Te Puke were similar. Regression coefficients in A.3.7.

Table 3.4 Dry matter (%) at the beginning and end of the experiment for kiwifruit harvested from orchards at different locations.

Orchard location	Dry matter (%)	
	Time of measurement (days)	
	0	92
Kerikeri	13.2 (0.3)[1]	16.0 (0.3)
Te Puke	11.6 (0.2)	14.2 (0.6)
Palmerston North	12.1 (0.3)	14.4 (0.3)
Riwaka	11.7 (0.2)	14.6 (0.3)[2]

[1] ±SE (standard error)
[2] day 84

measured 2 hours after harvest in Palmerston North compared to those measured 24 hours later (Table 3.5). The difference in SSC between fruit measured 2 and 24 hours after harvest was negative on five occasions and positive on nine occasions; these trends however, did not appear to be related to stage of maturity. Similarly, there was reasonable agreement in SSC values between the two measurement times for fruit harvested from Kerikeri and Riwaka (Table 3.6). In contrast, differences in SSC consistently occurred in fruit measured at 2 and 24 hours from Te Puke (Table 3.6). The difference in SSC between the two measurement times was always less than 0.6% for fruit measured at Palmerston North (Table 3.5) and Kerikeri, Te Puke and Riwaka (Table 3.6).

Overall, there were no consistent differences in starch and total sugar concentrations in outer pericarp or core tissue between fruit measured 2 hours after harvest in Palmerston North and those stored and measured 24 hours later (Table 3.7). The differences in starch and total sugar concentrations between the two measurement times were less than 19 and 11 mg gFW^{-1}, respectively (Table 3.7).

Table 3.5 Difference in soluble solids concentration (%) between kiwifruit measured within 2 hours of harvest and those stored for 24 hours. Fruit were harvested in Palmerston North.

Time of measurement (days)	Difference in soluble solids concentration (%)	P≤0.01
0	-0.59 (0.16)[1]	-[2]
7	-0.57 (0.08)	*
14	0.00 (0.09)	-
21	0.02 (0.03)	-
28	0.15 (0.04)	*
35	-0.01 (0.07)	-
42	0.00 (0.03)	-
49	0.19 (0.08)	-
56	0.00 (0.10)	-
63	-0.03 (0.08)	-
70	0.46 (0.10)	*
77	0.21 (0.13)	-
84	-0.34 (0.16)	-
92	0.00 (0.21)	-

[1] ±SE (standard error)
[2] * significantly different at P≤0.01 determined by t-test.

The method used to transport fruit from the different locations to Palmerston North before measurement was therefore considered acceptable as fruit carbohydrate metabolism was not affected. In the experiments described here, fruit were transported in chilled boxes which would help to reduce the potential increase in SSC during transit. Harman (1981) found the increase in SSC in detached fruit after 24 hours at room temperature was faster than in similar fruit attached to the vine. Differences in SSC, starch or total sugar concentration between the two measurement times were not significant for the majority of measurements, and those that were significant did not appear to

be related to any particular stage of maturity. In addition, even though all the differences in SSC measurements at Te Puke were significant, they were small, ranging from 0.2 to 0.5%.

Table 3.6 Difference in soluble solids concentration (%) between kiwifruit measured within 2 hours of harvest at Kerikeri, Te Puke and Riwaka and those transported to Palmerston North within 24 hours.

Orchard location	Time of measurement (days)	Difference in soluble solids concentration (%)	P≤0.01
Kerikeri	0	0.12 (0.05)[1]	-[2]
	21	0.22 (0.05)	*
	49	0.22 (0.09)	-
	91	0.09 (0.24)	-
Te Puke	0	-0.15 (0.02)	*
	22	0.27 (0.03)	*
	48	0.20 (0.04)	*
	92	0.51 (0.17)	*
Riwaka	0	-0.20 (0.03)	*
	23	0.28 (0.04)	*
	48	0.15 (0.08)	-
	84	-0.08 (0.24)	-

[1] ±SE (standard error)
[2] * significantly different at P≤0.01 determined by t-test.

Table 3.7 Difference in starch and total sugar concentration (mg gFW^{-1}) between kiwifruit measured within 2 hours of harvest and those stored for 24 hours. Fruit were harvested in Palmerston North.

Tissue type	Time of measurement (days)	Difference in concentration (mg gFW^{-1})			
		Starch	P≤0.01	Sugar	P≤0.01
Outer pericarp	0	-6.33 (2.6)[1]	-[2]	-3.91 (0.9)	-
	28	-8.58 (2.7)	*	-1.06 (0.4)	-
	42	18.14 (2.8)	*	0.77 (0.5)	-
	56	-9.40 (1.6)	*	0.57 (0.9)	-
	70	-3.15 (1.2)	-	5.87 (1.3)	*
	92	1.40 (1.4)	-	-4.39 (1.7)	-
Core	0	-13.33 (4.0)	-	-1.83 (0.7)	-
	28	-6.37 (6.8)	-	0.09 (0.7)	-
	42	-3.07 (4.6)	-	0.99 (0.5)	-
	56	1.86 (3.2)	-	0.55 (1.2)	-
	70	6.29 (5.0)	-	10.69 (1.8)	*
	92	-2.11 (2.0)	-	-5.94 (2.7)	-

[1] ±SE (standard error)
[2] * significantly different at P≤0.01 determined by t-test.

Soluble solids concentration remained below 5% at all orchards until early to mid April, depending on the orchard, and then increased rapidly (Fig. 3.2). Rate of SSC increase was faster, and consequently earlier, in fruit harvested at Riwaka compared to fruit from Kerikeri, Palmerston North and Te Puke (Table 3.8). This difference was exemplified by the number of days projected to reach the inflection point (Fig 2.3) which was 85, 103, 104 and 109 days for fruit harvested at Riwaka, Kerikeri, Palmerston North and Te Puke respectively (Table 3.8). In each fruit, SSC was measured from both the distal and proximal ends. Soluble solids concentration from the distal end of fruit was greater than that at the proximal end after the first harvest at Kerikeri, fourth harvest at Palmerston North and fifth harvest at Te Puke and Riwaka (Appendix 2).

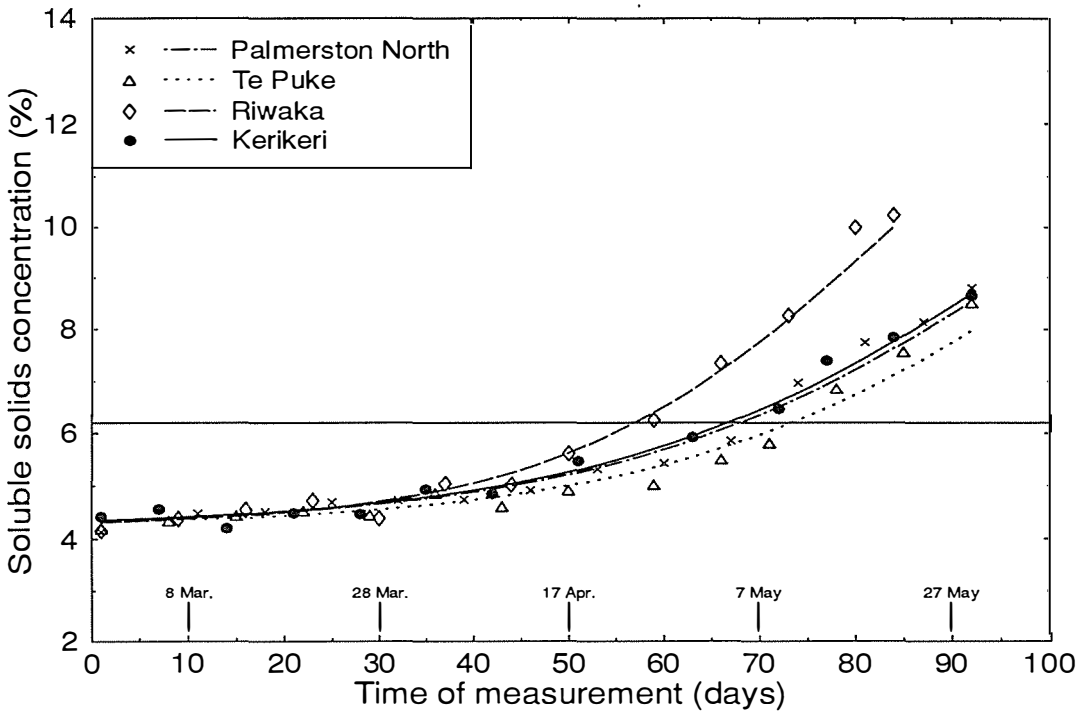


Fig. 3.2 Soluble solids concentration (%) of kiwifruit grown at four different orchard locations. Regression coefficients in A.3.8.

Table 3.8 Comparison of the maximum rate of change in soluble solids concentration (slope) and number of days to the inflection point of kiwifruit harvested from orchards at different locations. Values from fitted logistic curves where a maximum soluble solids concentration of 16% was assumed.

Orchard location	Slope (% day^{-1})	Time to inflection point (days)
Kerikeri	0.044 (0.004)[1]	103
Te Puke	0.045 (0.003)	109
Palmerston North	0.044 (0.002)	104
Riwaka	0.058 (0.004)	85

[1] ±SE (standard error)

Concentration of starch was higher in core tissue than in outer pericarp tissue, and this difference was consistent in fruit from all locations (Fig. 3.3). During this study, starch concentration in outer pericarp tissue initially increased until it reached an apparent plateau where starch concentration remained the same for 20 to 30 days before declining. Similarly, starch concentration in core tissue increased by approximately 20 mg gFW^{-1} more than in outer pericarp tissue, then reached a plateau and finally decreased in the last 20 to 30 days of the study. Decrease in starch concentration was more rapid in fruit from Riwaka than in those from Kerikeri. By the end of the study, starch concentration was lowest from fruit harvested at Riwaka and highest in fruit from Kerikeri in both outer pericarp and core tissues; concentrations from Te Puke and Palmerston North were between those measured at Kerikeri and Riwaka. The increase in total sugar concentration began before the decrease in starch concentration. Total sugar concentrations from outer pericarp and core tissue were similar at the beginning of the study, but increased to a higher final concentration in core than outer pericarp tissue by the end of the study. Increase in total sugar concentration was faster at Riwaka than Kerikeri, Palmerston North and Te Puke, respectively (Fig. 3.3). Correlation coefficient (r) for the relationship between total sugar concentration and SSC was 0.99 when data were pooled for all orchards and was 0.98, 0.99, 0.99 and 1.00 for Te Puke, Kerikeri, Palmerston North and Riwaka, respectively (Fig. 3.4).

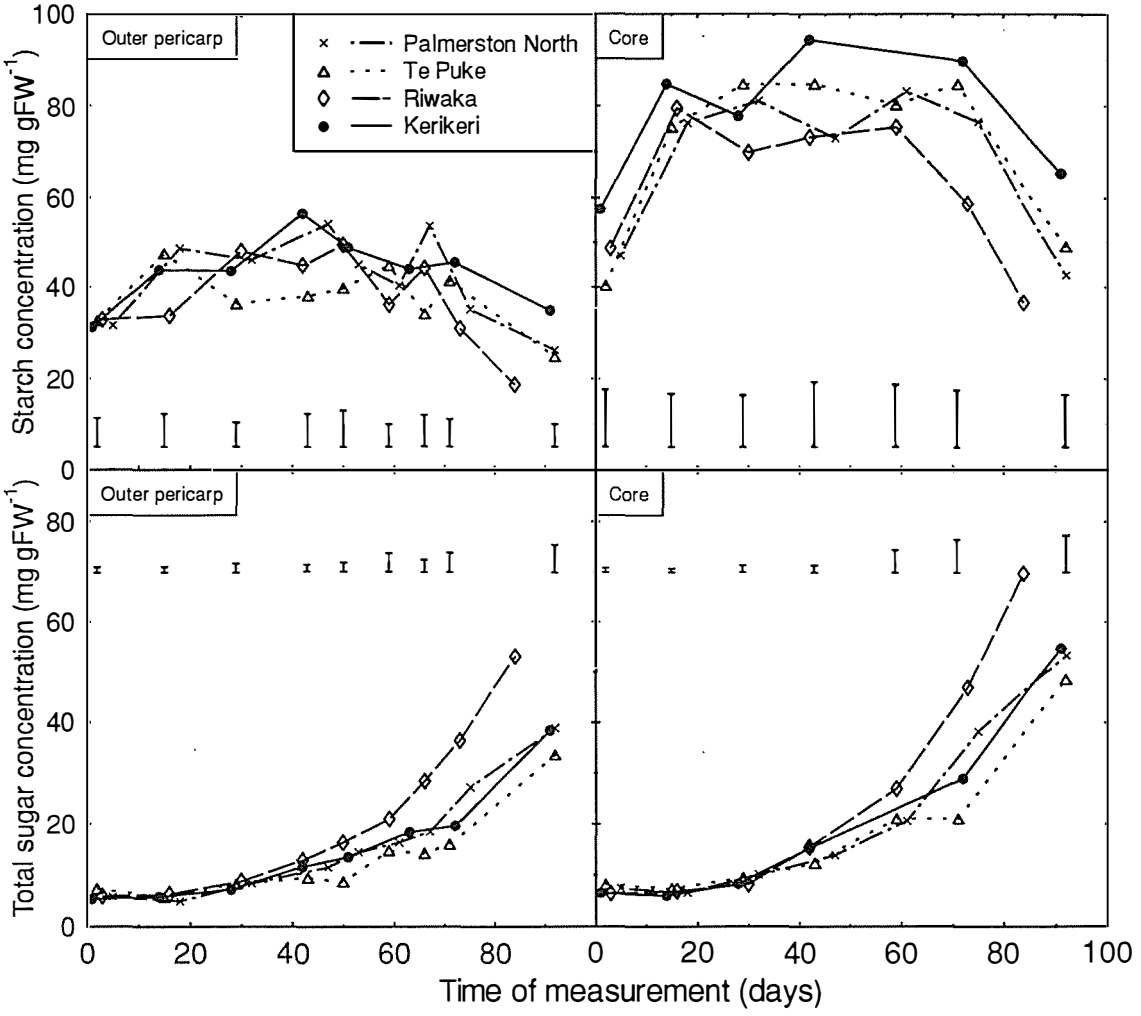


Fig. 3.3 Starch and total sugar concentrations (mg gFW^{-1}) of outer pericarp and core tissue of kiwifruit grown at four different orchard locations. Bars = LSD at $P \leq 0.05$. Day 0 = 27 February.

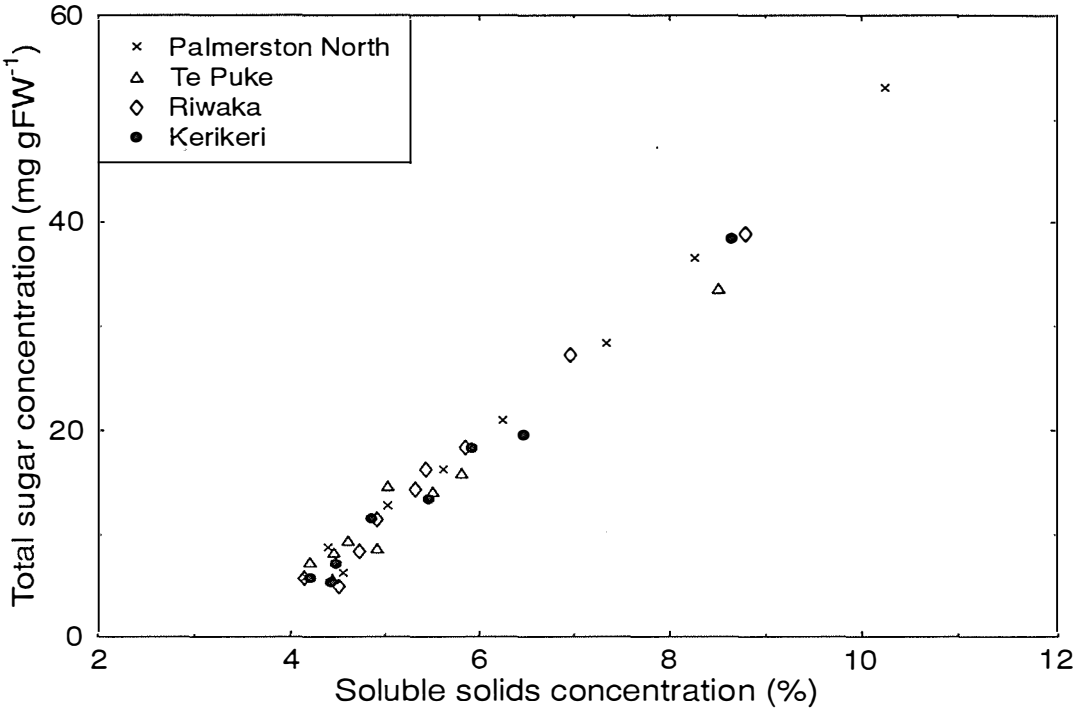


Fig. 3.4 Correlation of total sugar concentration (mg gFW^{-1}) and soluble solids concentration (%) of kiwifruit grown at four different orchard locations. Y = - 25.6 + 7.4% SSC (1.0 standard deviation) (R^2 = 0.98).

Glucose and fructose were the dominant sugars present in kiwifruit, and sucrose generally comprised less than 10% of total sugars. The concentrations of glucose, fructose and sucrose increased from February to May at Te Puke and Riwaka (Table 3.9). In general, the concentration of each sugar was higher in core tissue than in outer pericarp tissue throughout the study. Concentrations of glucose and fructose were similar at both Te Puke and Riwaka and in outer pericarp and core tissues. The (glucose plus fructose)/sucrose ratio in fruit from Te Puke was generally equal to or higher than in fruit from Riwaka in both outer pericarp and core tissues. However, within each tissue type there were no obvious trends in the ratio as the study progressed.

Table 3.9 Concentrations of glucose, fructose and sucrose (mg gFW^{-1}) and (glucose plus fructose)/sucrose ratio in fruit harvested from orchards at Te Puke and Riwaka.

Orchard location	Tissue type	Time of measurement (days)	Sugar concentration (mg gFW^{-1})			
			Glucose	Fructose	Sucrose	$\frac{G+F}{S}$[1]
Te Puke	Outer pericarp	0	3.9 (1.2)[2]	1.1 (0.3)	0.2 (0.1)	25
		42	2.3 (0.8)	2.0 (0.6)	0.0 (0.0)	0
		56	7.6 (1.7)	7.8 (1.1)	0.5 (0.2)	31
		70	8.8 (1.2)	9.0 (0.8)	1.0 (0.2)	18
		92	9.6 (1.7)	9.8 (0.9)	0.9 (0.4)	22
	Core	0	2.3 (0.8)	2.0 (0.6)	0.0 (0.0)	0
		42	5.1 (0.0)	4.4 (0.0)	0.2 (0.0)	48
		56	8.4 (0.9)	10.0 (0.2)	0.1 (0.1)	184
		70	10.6 (2.3)	10.1 (0.8)	1.0 (0.2)	21
		92	12.6 (1.0)	12.6 (1.9)	1.4 (0.4)	18
Riwaka	Outer pericarp	0	2.4 (1.1)	0.9 (0.2)	0.1 (0.0)	33
		42	5.4 (2.0)	6.1 (1.6)	1.2 (0.4)	10
		56	9.4 (1.0)	9.2 (0.5)	0.6 (0.1)	31
		70	15.6 (3.2)	12.7 (2.2)	3.0 (1.0)	9
		84	12.8 (2.0)	13.4 (2.2)	2.2 (0.4)	12
	Core	0	1.5 (1.1)	1.4 (0.7)	0.1 (0.0)	29
		42	5.7 (2.4)	7.1 (2.5)	0.3 (0.0)	43
		56	10.8 (1.5)	11.3 (1.4)	1.1 (0.5)	20
		70	20.1 (4.2)	16.4 (4.8)	5.3 (2.2)	7
		84	14.0 (2.6)	14.9 (0.0)	0.7 (0.6)	41

[1] (glucose plus fructose)/sucrose ratio
[2] ±SE (standard error)

3.4 Discussion

Temperatures in the main kiwifruit producing regions of New Zealand during February to May 1990 were warmer than the long term average (cf. Tables 2.1 and 3.2) and coincided with delayed harvest of kiwifruit throughout the country (Kelly, 1990). This is consistent with results from controlled environment experiments (Chapter 2) where rate of SSC increase in fruit was slowest when vines were grown at the warmest temperatures and fastest in fruit from vines grown at the coolest temperatures. An increase in SSC and decrease in starch concentration, which is characteristic of the maturation process in kiwifruit, usually commences about the beginning of April for field-grown fruit. Temperatures in the field during February and March are unlikely to influence the time at which fruit reach the minimum acceptable commercial harvest maturity of 6.2% SSC (Salinger and Morley-Bunker, 1988); fruit have not reached a sufficient stage of physiological development at which cool temperatures could stimulate hydrolysis of starch to sugar. This study is the first to accurately record temperatures and to measure compositional changes in kiwifruit during maturation at a range of contrasting locations in New Zealand. Examples of the effect of temperature on increase in SSC will be discussed generally in this chapter, but interpreted in more detail in Chapter 5.

Increases in fruit growth over the whole study were similar for fruit harvested at Riwaka, Te Puke and Palmerston North, but the greatest increase was at Kerikeri. Fruit from vines grown at Kerikeri were heavier and had higher dry matter than fruit from the other locations. This may have been due to the light crop load at Kerikeri in the 1990 season, which may have been a consequence of a lack of winter chilling and/or hard winter pruning in 1989 (A. Richardson, HortResearch, Kerikeri, pers. comm.). Low winter chilling results in poor budbreak and consequently low numbers of fruit and flowers (Warrington and Stanley, 1986) and would have resulted in a light crop load. Studies which manipulated crop load showed that vines with a light crop load have heavier fruit than those with a heavy crop load (Antognozzi *et al.*, 1992;

Cooper and Marshall, 1992; Costa *et al.*, 1992a). Physiologically, vines with a light crop load would have a greater quantity of photosynthate available per fruit than vines with a heavy crop load, resulting in heavier fruit on light cropping vines.

Flesh firmness decreased in fruit harvested from four different orchard locations from the beginning of March to the end of May, in a similar way to kiwifruit measured by MacRae *et al.* (1989a), Beever and Hopkirk (1990) and for container-grown vines (Chapter 2). However, there were differences in the rate of decrease in firmness between orchard locations, with firmness decreasing faster in fruit from Kerikeri than in those from Riwaka. In contrast, there were no differences in flesh firmness between fruit harvested from two different orchard locations in Japan (Sawanobori and Shimura, 1990).

A decrease in flesh firmness during maturation has been found in many other species of fruit, such as apple (Workman, 1963), pear (Mann and Singh, 1988) and peach (Maness *et al.*, 1992). However, differences in the rate and extent of softening have been found among species and between years. Flesh firmness in peach at physiological maturity ranged from 4 to 8 N depending on cultivar, but decreased to 0.5 to 5 N when fully mature (Maness *et al.*, 1992), similar to firmness at eating ripeness in kiwifruit (Beever and Hopkirk, 1990). In contrast, immature guava were very firm (>295 N), but firmness decreased rapidly during a 14 day period to result in fruit that were 98 N at eating ripeness (Yusof and Mohamed, 1987). Changes in firmness result from the action of cell wall degrading enzymes such as polygalacturonase, pectinmethylesterase and cellulase; activities of these enzymes can be low or absent in unripe fruit, but increase during maturation and ripening (Rhodes, 1980). It is possible that the higher firmness in guava than kiwifruit or peach at eating ripeness may be due to a lower concentration, and/or decreased activity of cell wall degrading enzymes in guava.

Dry matter of fruit harvested from Palmerston North, Te Puke, Kerikeri and Riwaka increased by 2.3, 2.6, 2.8 and 2.9%, respectively during the period of study from March to May. MacRae *et al.* (1989a) measured a 3.8% increase in dry matter from fruit harvested at Te Puke during a similar period. At Kumeu, the dry matter from control vines at harvest was 17.6% in 1986 and 16.0% in 1987 (Hopkirk *et al.*, 1989), indicating variability between years as found with SSC (Harman, 1981). Dry matter at harvest ranged from 16.0 to 20.3% in different countries or in different regions of New Zealand (Hopkirk *et al.*, 1989; MacRae *et al.*, 1989a; Walton and de Jong, 1990a). These values were higher than the dry matter measured in the present study, perhaps due to different vine management or temperatures in the different seasons affecting rate of photosynthesis, translocation of assimilate and subsequent conversion to starch in fruit.

Soluble solids concentration remained below 4.5% at all orchards until the beginning of April, but increased after this time. Similar patterns of increase in SSC were found in maturing kiwifruit in other orchards within New Zealand (Reid *et al.*, 1982a; Beever and Hopkirk, 1990), in the USA (Okuse and Ryugo, 1981) and in Japan (Sawanobori and Shimura, 1990). For example, SSC was 4.4 and 4.5% at Kumeu and Te Puke, respectively on 25 March and exceeded 5% SSC at both sites after 18 April 1985 (MacRae *et al.*, 1989a). At Kumeu in 1987, SSC was 4.5% on 12 March and remained at that concentration until 2 April when it increased to reach 5% two weeks later (Hopkirk *et al.*, 1989). Results obtained from fruit grown in Japan showed that SSC remained at 4.5% from 6 to 18 weeks after full bloom (roughly equivalent to 12 April) and increased thereafter (Sawanobori and Shimura, 1990).

Fruit has been shown to reach commercial harvest maturity, that is exceeding a minimum of 6.2% SSC, at different times depending on orchard location and season (Beever and Hopkirk, 1990). Differences in the timing of harvest may be due, in part, to temperature. Although maturation of fruit at different orchards was studied in this work in only one season, orchards were selected

according to their geographical location to give as wide a range of temperatures as possible. Fruit measured in Riwaka reached 6.2% SSC 10, 11 and 16 days before fruit from Kerikeri, Palmerston North and Te Puke, respectively. However, fruit from the Nelson region reached 6.2% SSC less than one week earlier than fruit from Te Puke orchards in each of the 1981 to 1984 seasons (Hopkirk, 1986) and in 1985 there was no difference in the timing of fruit reaching 6.2% SSC when harvested from Te Puke and Kumeu (MacRae *et al.*, 1989a). Soluble solids concentration of kiwifruit harvested from two orchards in Japan increased similarly in one year, but there was a 14 day difference in the following year (Sawanobori and Shimura, 1990). Low temperature effects an increase in SSC once SSC is greater than 5% (Salinger and Morley-Bunker, 1988). To understand the reasons for the different harvest times in each of the regions and seasons, detailed temperature records from early March through May need to be known for each of the years concerned.

Results from controlled environment studies (Chapter 2) clearly showed that SSC increased fastest in treatments with coolest mean temperatures. In the field, the fastest increase in SSC occurred at Riwaka, the coldest site compared to the other orchards, which confirmed results from controlled environment studies. If SSC increased solely according to the current temperature in April and May, then the increase in SSC for fruit from Te Puke and Palmerston North should have been similar, and intermediate to those for Riwaka and Kerikeri; this did not occur. However, comparison of SSC change with mean monthly temperatures is a very crude approximation, as the frequency of time spent at each temperature is not taken into account. In addition, chemical reactions involved in converting starch to sugar are likely to be both temperature and time dependent. In harvested potato tubers, changes in starch and sugar concentrations occurred several days after a temperature perturbation (Pressey and Shaw, 1966; Isherwood, 1973), similar to changes in kiwifruit (Chapter 2). A more detailed analysis of the relationship of SSC with temperature from these orchards has been undertaken in Chapter 5 using the

discrete-rate analysis where the frequency of time spent at each temperature is taken into account (McNaughton *et al.*, 1985).

During maturation many independent chemical changes are taking place in the fruit, each of which have their own timing and control which may be interrelated, but the events need not be coincident (Coombe, 1976). For example, SSC in two strains of red 'd' Anjou pear did not change while flesh firmness decreased during ripening and storage (Chen *et al.*, 1993). In contrast, there was variation in correlation between decrease in flesh firmness and increase in SSC among seven cultivars of melon (Miccolis and Saltveit, 1991). MacRae *et al.* (1989a) found that SSC increased at a similar rate in fruit harvested from Te Puke and Kumeu, but that flesh firmness decreased faster in fruit from Kumeu compared to those from Te Puke. In this study, the rate of decrease in flesh firmness at Kerikeri and Riwaka was not related to the rate of increase in SSC, as firmness decreased fastest at Kerikeri while SSC increased fastest at Riwaka.

Likewise, changes in flesh firmness are not related as closely to temperature as are changes in SSC. No difference in flesh firmness was detected between the control and warmed treatments when kiwifruit were grown in relocatable greenhouses, while there were differences in SSC of up to 1.4% between treatments by the end of May (Hopkirk *et al.*, 1989). In contrast, when field-grown vines in British Columbia were either covered with a plastic greenhouse or left uncovered all season (control), flesh firmness was 10 to 20 N higher for fruit in control than in fruit from covered vines during maturation, while fruit from control vines reached 6.2% SSC 2 days after those in the covered treatment (Kempler *et al.*, 1992). The trend for the greatest decrease in firmness to occur in the treatments with the coolest mean temperature (Chapter 2) was not evident in the current study where the fastest decrease occurred at Kerikeri (warmest site), and the firmness at the coldest site (Riwaka) decreased more slowly.

Changes in starch and sugar concentrations during fruit maturation in field-grown vines measured in this study were similar to those for other field-grown vines (Wright and Heatherbell, 1967; Harman, 1981; Okuse and Ryugo, 1981; Reid *et al.*, 1982a; MacRae *et al.*, 1989a; Sawanobori and Shimura, 1990) and for container-grown vines (Chapter 2). Examples of other fruit containing starch which show similar patterns of carbohydrate interconversions during maturation and ripening are apple (Ohmiya and Kakiuchi, 1990), banana (Agravante *et al.*, 1990), pear (Mann and Singh, 1988) and tomato (Dinar and Stevens, 1981). However, the rates of these interconversions are different between the different species. For example, starch concentration decreased in tomato (*L. cheesmanii* typicum) from 24 to 0% dry matter in 18 days when fruit were fully ripe (Garvey and Hewitt, 1991). In 'Gloster 69' apple placed at 15C after harvest, starch concentration decreased from 19 to 4 mg gFW^{-1} in 14 days (Tsantili and Knee, 1991). Starch concentration in kiwifruit at harvest was 53 mg gFW^{-1}, but this decreased to 6 mg gFW^{-1} after 4 weeks at 4C (MacRae *et al.*, 1989b).

The most noticeable difference between orchards was that concentration of starch decreased and sugar increased to a greater extent in both outer pericarp and core tissue in fruit harvested from Riwaka compared with those from other orchards. The cooler temperatures in April and May at Riwaka compared to Kerikeri, Te Puke and Palmerston North probably accounted for the rapid increase in SSC and total sugar concentration and the decrease in starch concentration in fruit harvested from Riwaka. Similar changes occurred in container-grown vines in controlled environments, where the decrease in starch concentration and increase in total sugar and SSC was faster at the coolest temperatures (Chapter 2). The faster hydrolysis of starch and synthesis of sugars at low compared to high temperatures may be due to the effect of temperature on control points in the glycolytic and gluconeogenic pathways (Chapter 6). The high correlation between SSC and total sugar concentration from field-grown fruit was similar to that in container-grown vines (Chapter 2), confirming that the processes of maturation were similar between the two

populations of vines, even though SSC was higher in container-grown vines. Higher concentrations of sugar and starch were found in the core than the outer pericarp in fruit from field-grown vines (MacRae *et al.*, 1989a) and container-grown vines (Chapter 2). The dissimilarity between the tissue zones may be due to the different types of cells present in each tissue (Section 2.3.2).

The increases in concentrations of glucose, fructose and sucrose in fruit harvested from Te Puke and Riwaka were similar to those for other field-grown fruit (MacRae *et al.*, 1989a) and for fruit from container-grown vines. A high (glucose plus fructose)/sucrose ratio can indicate high invertase activity, as both acid and alkali invertase catalyse the reduction of sucrose to glucose and fructose (Fig. 1.1). The lower (glucose plus fructose)/sucrose ratio in the core than outer pericarp (MacRae *et al.*, 1989a) was also shown for fruit harvested at Te Puke and Riwaka at most sampling times. It is possible that invertase activity may be reduced at cooler temperatures, resulting in a low (glucose plus fructose)/sucrose ratio. A low ratio was found at Riwaka, where temperatures were cooler than at Te Puke, and in container-grown vines grown at 11/7C compared with 26/20C (Chapter 2).

The mean total (non-structural) carbohydrate concentration (starch plus sugar concentration) in the outer pericarp of fruit from all orchards was similar at the beginning of the study (39 ±1.6 mg gFW^{-1}) but by the end had increased to 67 ±6.9 mg gFW^{-1}. Fruit from Riwaka and Kerikeri contained a similar concentration of total carbohydrate at the end of the study, the proportion of starch was 25 and 48% of total carbohydrate. Degradation of starch was faster in fruit harvested from Riwaka than Kerikeri, similar to that in fruit from container-grown vines grown at cooler compared to warmer temperatures (Chapter 2).

Total carbohydrate concentration in fruit at harvest may be important in determining the length of time fruit can be stored and still produce a fruit of

acceptable eating quality. Concentration of sugar can increase in fruit on the vine by a combination of starch hydrolysis and direct inflow of assimilate. Once fruit have been harvested there can be no further inflow of carbohydrate, and repartitioning of the existing carbohydrate will determine final SSC at eating ripeness. Therefore, starch concentration at harvest is an important contributor to final SSC at eating ripeness. The important contribution of starch concentration to the final SSC attained in the fruit was shown when fruit were grown in the controlled environment treatment of 11/7C (Section 2.3). Soluble solids and total sugar concentrations increased rapidly with a concurrent decrease in starch concentration, resulting in a relatively low final SSC (14%). In that treatment starch concentration in fruit decreased to a low level and there were no further increases in sugar concentration as leaves had senesced, hence no further photosynthates were being produced and imported into the fruit. Unfortunately, fruit from the study were not able to be stored and reanalysed after storage. It would have been interesting to determine whether quality of fruit post-storage was affected by the starch, the sugar or the total carbohydrate concentration in the fruit at harvest.

There are few experiments on kiwifruit reported in the literature which have measured the concentration of starch and sugar both before and after storage. Studies of fruit harvested from Nelson and Te Puke did not measure the starch and sugar concentrations (Hopkirk, 1986). Other experiments have either treated fruit of the same initial carbohydrate concentration (Matsumoto *et al.*, 1983) or sufficient data was not available to give meaningful interpretation (MacRae *et al.*, 1989a, 1989b). If starch concentration in fruit at harvest was of overriding importance in determining SSC at eating ripeness (after storage) then, taking the extremes, fruit from Riwaka would be less acceptable in terms of SSC than fruit from Kerikeri. However, if total carbohydrate concentration at harvest was the critical factor determining SSC at eating ripeness, then fruit from Te Puke would be the least acceptable and those from Palmerston North the most acceptable; total carbohydrate concentrations were 57 and 71 mg gFW^{-1} for fruit harvested at 6.2% SSC from Te Puke and Palmerston North,

respectively. Further conclusions and recommendations cannot be drawn from these studies as SSC was not determined after storage.

Dry matter of kiwifruit is being considered for use as an alternative or better maturity index than SSC, because it accounts for combined starch and sugar concentration in the fruit (Scott *et al.*, 1986; Hopkirk, 1991). Although Hopkirk (1991) indicated that dry matter reflected the change in total (non-structural) carbohydrate concentration, contrary results were obtained from the four orchards in this study and from container-grown vines (Section 2.4). The correlation coefficients (r) for the relationship between dry matter and total carbohydrate concentration were 0.60, 0.74, 0.88 and 0.91 for fruit harvested from Te Puke, Riwaka, Kerikeri and Palmerston North, respectively. This shows that the change in dry matter is variable among orchards and not reflected solely by total carbohydrate concentration. The reason for the different values obtained for correlation of dry matter and total carbohydrate concentration is unclear. However, Young *et al.* (1993) found that components of soluble solids other than sugars, such as pectin, soluble starch, amino acids, lipids and minerals affected accumulation of fruit dry matter in tomato. Further tests will be required before dry matter can be used satisfactorily as a maturity index for the harvest of kiwifruit, or a different test for total carbohydrate concentration needs to be found. Any new maturity index needs to be suitable for fruit harvested from all orchard locations.

Differences in rate of kiwifruit maturation were found among orchard sites at Kerikeri, Te Puke, Palmerston North and Riwaka, and some of these differences could be attributed to the effect of temperature. Decrease in flesh firmness was not influenced as much by temperature as were SSC, sugar and starch concentrations. The cooler temperatures at Riwaka resulted in faster increases in SSC, total sugar concentration and decrease in starch concentration than that occurring at the other orchards. It is possible that carbohydrate status (starch and/or total sugar concentration) of fruit before storage may affect the quality of fruit after storage with fruit having low starch and/or total sugar concentration being of lower quality at eating ripeness. Such information may influence storage and marketing strategies.

CHAPTER 4

MANIPULATION OF CARBOHYDRATE CONCENTRATIONS IN KIWIFRUIT

4.1 Introduction

Fruit growth depends on the ability of fruit to compete with concurrent vegetative growth for a supply of assimilates from leaves. Fruit yield depends on the balance between source strength (supply of assimilates from leaves) and sink strength (governed by fruit number, fruit size and their competitive ability) (Wareing and Patrick, 1975).

Numerous studies using $^{14}CO_2$ have shown that assimilates flow from the source (leaves) to the closest sink (fruit) along a gradient where some sinks have a higher priority than others. Such studies have been undertaken in, for example, apple (Hansen, 1969), kiwifruit (Lai *et al.*, 1989) and muskmelon (Hughes *et al.*, 1983). Studies on tomato showed preferential movement of carbon into proximal fruits on a truss compared to distal fruits (Ho *et al.*, 1983). Several leaves may be involved in assimilate flow into a fruit, but the direction of flow to the sink can be very specific, such as in citrus. Here, the fruit quarter in direct vertical alignment with the labelled source leaf received the majority of translocated ^{14}C and conversely, the lowest level of radioactivity was found in the fruit quarter opposite that aligned with the source leaf; leaves other than the source leaf contributed to filling the other fruit quarters (Koch, 1984).

In kiwifruit, the principal source leaves which supplied fruit were those which subtended the fruit. The source leaves supplied as much as 62% of their total ^{14}C-assimilate exclusively to their own fruit (Lai, 1987). Smaller amounts of ^{14}C-assimilate also came from some distal leaves via vascular connections which linked the node (n) with the fruit, these connections were with the n, n+5 and n+8 nodes. However, when the shoot was pruned an increased amount (78% of ^{14}C-

assimilate) came from the subtending leaf plus assimilate from every distal leaf (Lai, 1987). Lai (1987) concluded that new vascular connections may be developed after pruning in response to the need for redirecting assimilates to sink demands.

The majority of photosynthates produced by leaves are translocated out of the leaf to other parts of the plant. The movement of assimilates from leaves to fruit is usually regarded as being hormonally directed, since growth centres are regions of high hormonal concentrations (Davison, 1990). Whilst carbohydrate can be transported to kiwifruit over 2 m from the nearest leaf (Snelgar *et al.*, 1986), the direction of flow of photosynthate from the source was normally to the adjacent sink (Buwalda and Smith, 1990). Similarly, removal of fruit from selected branches of apple trees showed that leaves on non-bearing branches support fruit growth on neighbouring branches (Hansen and Christensen, 1974).

The flow of photosynthates to fruit can be altered by girdling. Girdling of the trunk or a branch of a fruit tree is a technique which has been used to increase fruit set and fruit size in apple (Priestley, 1976a, 1976b), grape (Coombe, 1959) and yield, by 60%, in kiwifruit (Davison, 1980). In this chapter, girdling refers to the removal of a ring of bark, thus breaking the phloem connections with the remaining parts of the vine, but leaving the xylem intact (see photograph in Davison, 1980). Girdling a fruiting lateral on a kiwifruit vine isolates the lateral from other parts of the vine. Carbohydrate supply is restricted by the number of leaves present on girdled laterals, but in ungirdled laterals carbohydrate can be imported from outside the lateral. Consequently, fruit growth is totally dependent on the supply of photosynthate from leaves within that lateral (Lai *et al.*, 1989). Fruit size was reduced on a girdled lateral in the 1:1 compared with the 2:1 leaf:fruit ratio treatment but was not affected by the leaf:fruit ratio treatment on an ungirdled lateral (Lai *et al.*, 1989). Fruit size was progressively reduced as the leaf:fruit ratio on girdled kiwifruit laterals ranged from 5:1 to 1:1 (Snelgar *et al.*, 1986; Woolley *et al.*, 1992). The minimum leaf:fruit ratio required to support maximum fruit growth in the kiwifruit lateral was 2:1 (Lai *et al.*, 1989).

Many changes occur in fruit during maturation, including increases in SSC, total sugar concentration, dry matter concentration and fresh weight, and decreases in starch concentration and flesh firmness (Beever and Hopkirk, 1990). Altering the leaf:fruit ratio on girdled laterals in addition to influencing fruit weight (Snelgar *et al.*, 1986; Lai *et al.*, 1989; Woolley *et al.*, 1992) also increased SSC (Snelgar and Thorp, 1988) where both were higher on laterals with a higher leaf:fruit ratio. Girdling studies have tended to measure mainly fruit weight but not other changes in the fruit that occur during maturation, such as carbohydrate, flesh firmness and fruit respiration.

In the present study, girdling of kiwifruit laterals was used as a tool to manipulate the carbohydrate concentration in fruit to determine whether different carbohydrate concentrations affected rate of fruit maturation.

4.2 Materials and methods

Six 'Hayward' kiwifruit vines were chosen in the same orchard block at Palmerston North as described in Section 3.1.1. On 14 January 1991, 30 laterals (Fig. 4.1) on each vine were selected, girdled and pruned to give two different leaf:fruit ratio treatments (1 leaf:1 fruit or 5 leaves:1 fruit). Care was taken to ensure that fruit selected were approximately the same size in both treatments. Girdling involved removal of a 10 mm portion of bark on the lateral closest to the vine leader. The appropriate number of fruit and leaves were then removed from the laterals by pruning to give the required leaf:fruit ratio. Throughout the experiment the girdled region was recut when necessary to prevent reconnection of the phloem.

Two fruit from each treatment were harvested from each of the 6 vines on 5 occasions (21 March (day 0), 11 April, 26 April, 6 May and 15 May). Measurements taken on each fruit were: SSC, flesh firmness, dry matter and fresh weight (Section 2.2.1). These same two fruit in each treatment were used to take samples of outer pericarp and core tissue, where samples from the same vine were bulked and stored for subsequent analysis of starch and total sugar concentrations (Section 2.2.1).

An additional 6 fruit per treatment (1 per vine) were harvested on each occasion for measurement of respiration. These fruit were held in a constant temperature room at 20C, and measured at least three times during 10 days at 20C. Each fruit was placed in an airtight 500 ml jar which was open between measurements, but sealed before a measurement with a rubber bung fitting tightly into a hole made in the lid. The jars were flushed with CO_2-free air then sealed for 90 minutes, the time necessary for accumulation of a detectable concentration of CO_2 for fruit from both treatments. After this time a 1 ml sample of gas was taken from each jar, through the rubber bung, using a disposable 1 ml syringe (Monoject Tuberculin) fitted with a hypodermic needle (15.9 mm 25 gauge) and

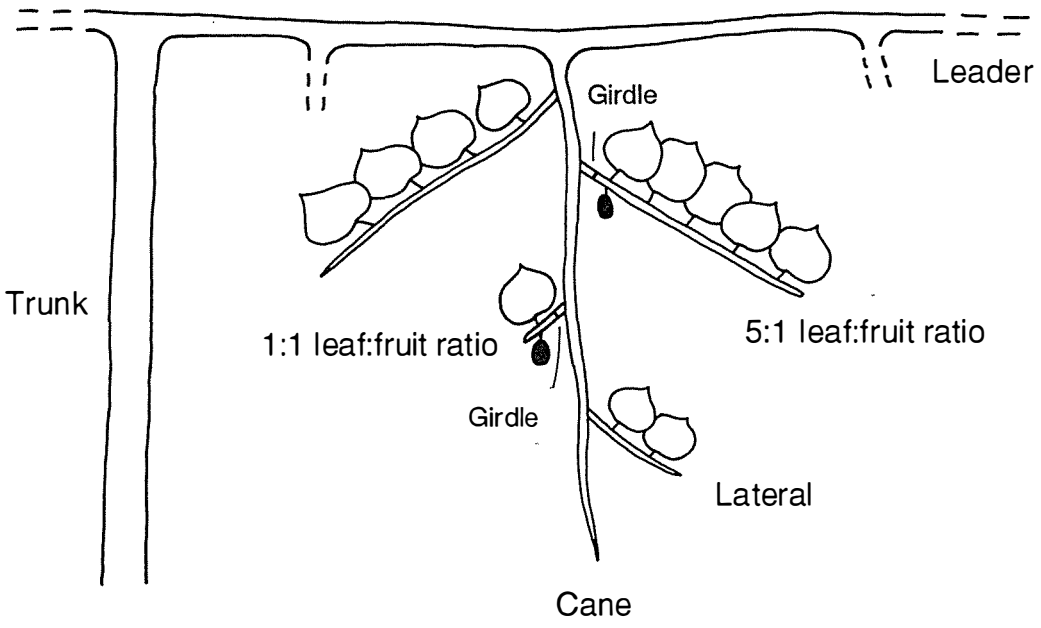


Fig. 4.1 Schematic diagram of the nomenclature of kiwifruit vine structure (based on Sale and Lyford, 1990) showing 1:1 and 5:1 leaf:fruit ratios and positions of girdles.

injected into a gas chromatograph. After measurement, jars were unsealed until the next measurement.

Carbon dioxide was measured using a thermal conductivity detector at 60C and 80 mA current in a Shimadzu GC 8A gas chromatograph with an Alltech CTR I column (Alltech catalogue no. 8700) at 30C and hydrogen at a flow rate of 1800 ml sec^{-1} as a carrier gas. The Alltech CTR I column is a dual column, the outer column (1.83 m x 6.35 mm) packed with an activated molecular sieve and the inner column (1.83 m x 3.18 mm) packed with a porapak mixture.

4.2.1 Statistical analyses

A linear regression was used to fit flesh firmness data. An exponential equation was fitted to SSC data. The least significant difference (LSD) test was used to separate the differences in starch and total sugar concentrations between the leaf:fruit ratio treatments.

4.3 Results

Leaf:fruit ratio treatments were applied in January to fruit of approximately the same size. The treatments had a major effect on fruit growth by the first harvest in March, as fruit in the 5:1 leaf:fruit ratio treatment were 39 g heavier than those in the 1:1 leaf:fruit ratio treatment (Table 4.1). Fruit in the 5:1 leaf:fruit ratio treatment were consistently over 35 g heavier than those in the 1:1 leaf:fruit ratio treatment at each harvest. The increases in size during this study were 0.16 and 0.27 g day^{-1} for fruit harvested from the 1:1 and 5:1 leaf:fruit ratio treatments, respectively.

Table 4.1 Fruit weight (g) at each harvest for kiwifruit grown at two different leaf:fruit ratios.

Leaf:fruit ratio treatment	Fruit weight (g)				
	Time of measurement (days)				
	0[1]	21	36	46	55
1:1	47.6 (4.0)[2]	59.3 (4.4)	55.5 (4.2)	65.6 (7.1)	57.2 (3.8)
5:1	86.7 (4.9)	94.5 (4.7)	97.3 (7.4)	108.5 (5.3)	101.9 (6.6)

[1] Day 0 = 21 March
[2] ±SE (standard error)

Flesh firmness decreased throughout the study in both treatments ($P \leq 0.05$) (Fig. 4.2). Initial firmness was 100.4 N and estimated rate of change in firmness was -0.55 and -0.62 N day^{-1} for fruit harvested from the 1:1 and 5:1 leaf:fruit ratio treatments, respectively. The interaction between leaf:fruit ratio and time was not significant.

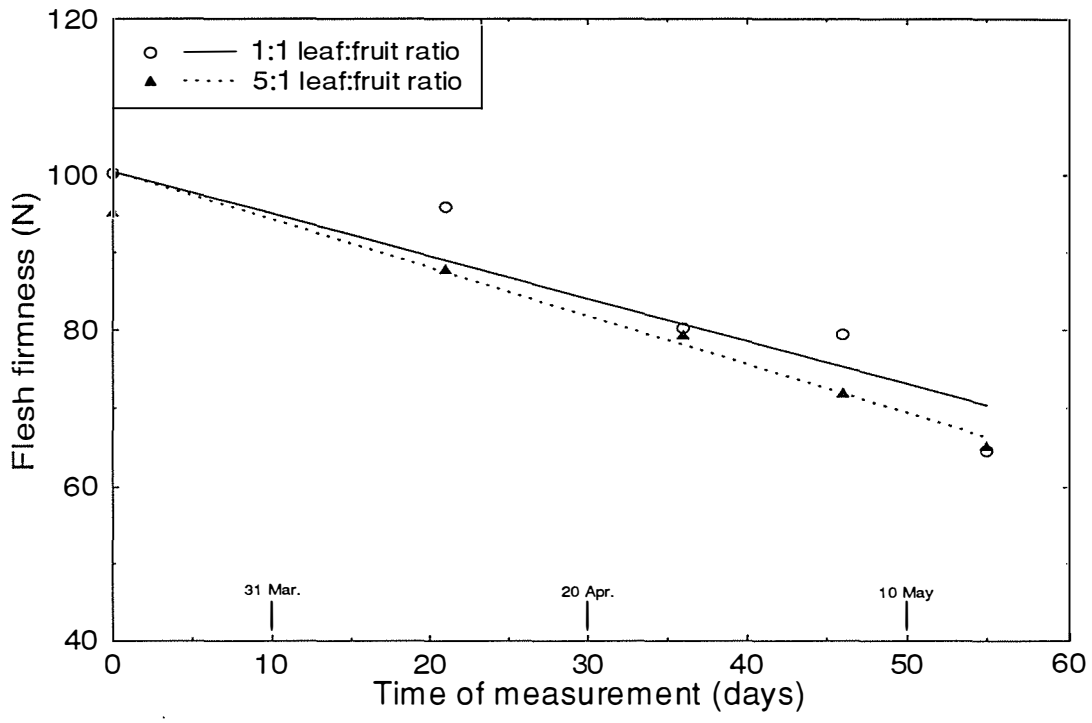


Fig. 4.2 Flesh firmness (N) of kiwifruit grown at two different leaf:fruit ratios. Regression coefficients in A.3.9.

At the first harvest, dry matter was over 2% higher in the 5:1 than in the 1:1 leaf:fruit ratio treatment. In subsequent harvests, dry matter of fruit from the 5:1 leaf:fruit ratio treatment was 1.2 to 3.7 g higher than in the 1:1 leaf:fruit ratio treatment (Table 4.2). Rates of increase in dry matter during the study were 2.0 and 3.8% day^{-1} ($x10^{-2}$) for fruit harvested from the 1:1 and 5:1 leaf:fruit ratio treatments, respectively.

Table 4.2 Dry matter (%) at each harvest for kiwifruit grown at two different leaf:fruit ratios.

Leaf:fruit ratio treatment	Dry matter (%)				
	Time of measurement (days)				
	0[1]	21	36	46	55
1:1	10.8 (0.6)[2]	12.6 (0.9)	11.7 (1.1)	11.6 (0.6)	11.9 (1.2)
5:1	12.9 (0.5)	13.8 (0.4)	14.2 (0.5)	15.3 (0.2)	15.0 (0.5)

[1] Day 0 = 21 March
[2] ±SE (standard error)

Initial SSC was similar for fruit from the 1:1 and 5:1 leaf:fruit ratio treatments ($P \leq 0.05$) (Fig. 4.3). However, SSC increased faster in the 5:1 than the 1:1 leaf:fruit ratio treatment ($P \leq 0.05$). Final SSC was 4.9 and 7.9% in the 1:1 and 5:1 leaf:fruit ratio treatments, respectively.

Starch concentration tended to be higher in the 5:1 than the 1:1 leaf:fruit ratio treatments and higher in the core compared to the outer pericarp tissue, although these differences were generally not significant ($P \leq 0.05$) (Fig. 4.4). Concentration of total sugar tended to be higher in the 5:1 than 1:1 leaf:fruit ratio treatment in outer pericarp at all harvests. In core tissue total sugar concentration was similar in both treatments for the first three harvests and subsequently increased faster in the 5:1 than 1:1 leaf:fruit ratio treatment. Sugar concentration in the core was

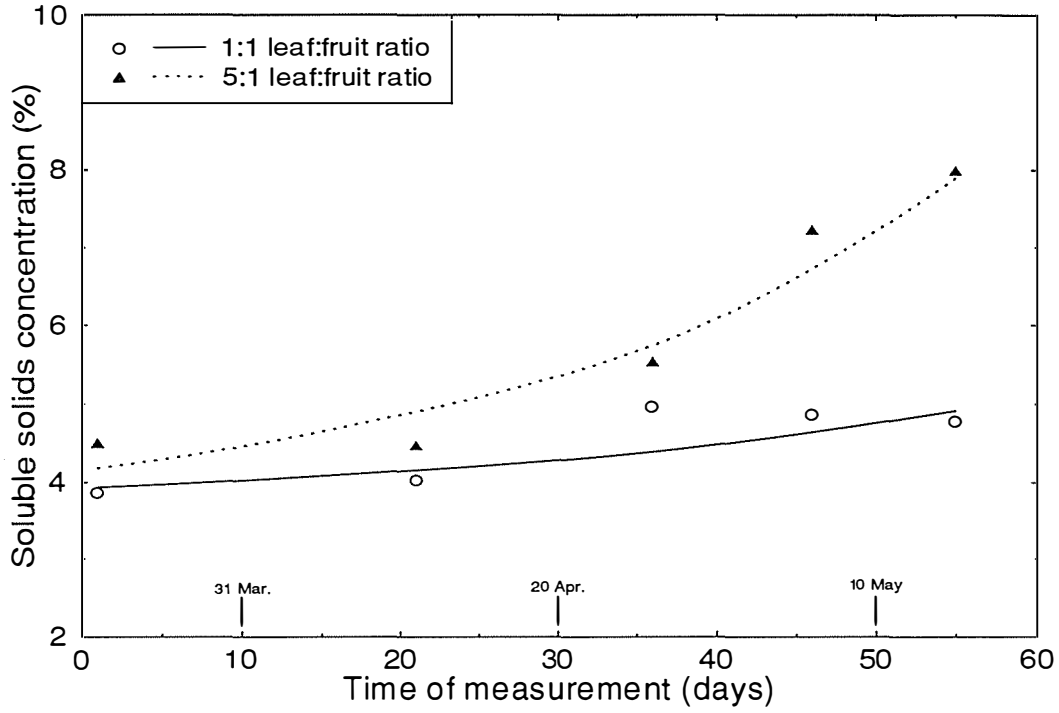


Fig. 4.3 Soluble solids concentration (%) of kiwifruit grown at two different leaf:fruit ratios. Regression coefficients in A.3.10.

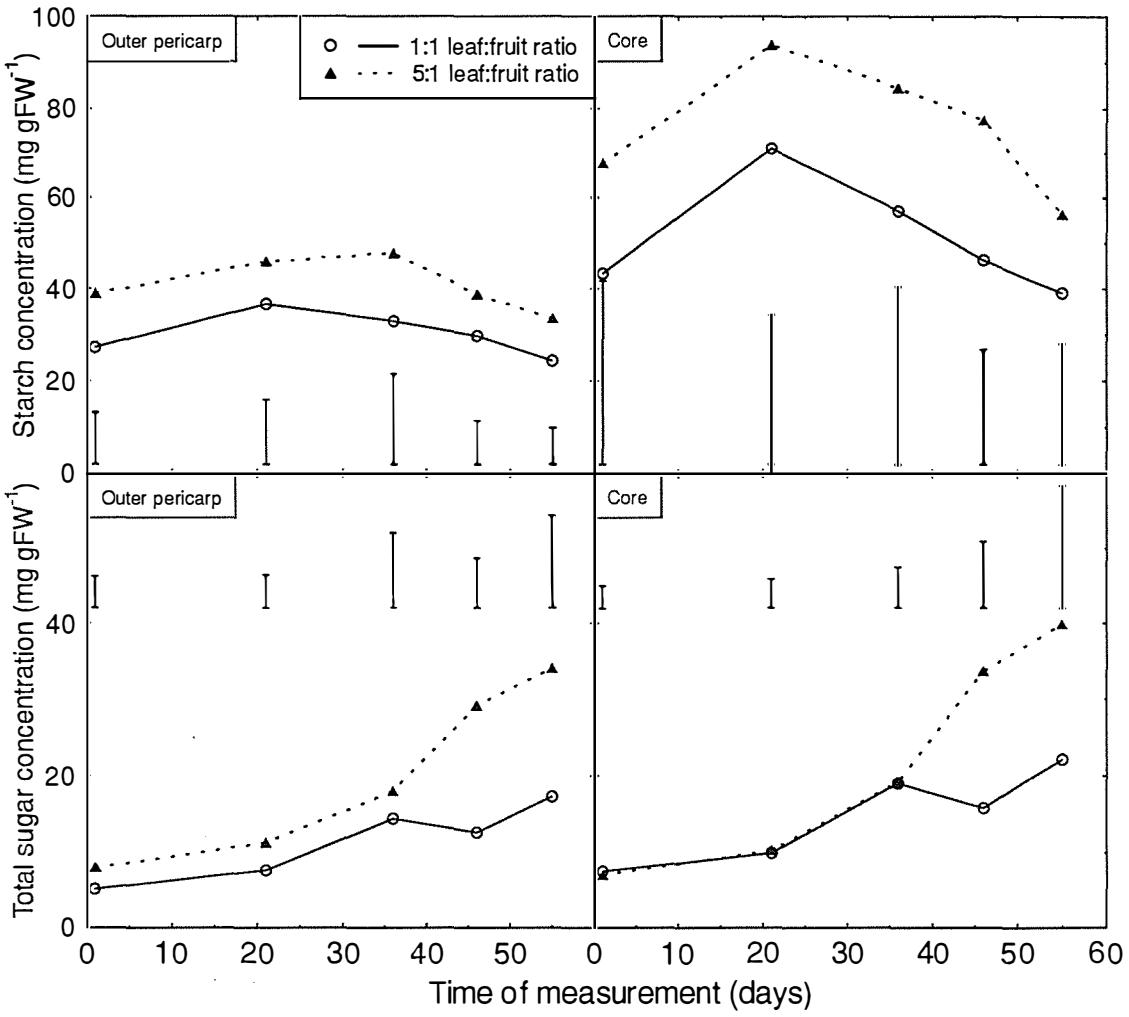


Fig. 4.4 Starch and total sugar concentrations of outer pericarp and core tissue of kiwifruit grown at two different leaf:fruit ratios. Bars = LSD at P $\leq$0.05. Day 0 = 21 March.

generally greater than or equal to that in the outer pericarp at all harvests. The decrease in starch concentration after the second or third harvest corresponded to an increase in total sugar concentration. The difference in sugar concentration between treatments was significant for both the outer pericarp and core tissue at the harvests on 6 and 15 May (days 46 and 55) ($P \leq 0.05$).

Respiration rates were very variable, with no obvious trends when measured at intervals after any of the five harvests. Therefore, only the mean respiration rate from each treatment at each harvest has been given (Table 4.3). Mean rates of respiration were generally higher in the 5:1 than 1:1 leaf:fruit ratio treatments throughout the study.

Table 4.3 Mean respiration (nmol CO_2 kg^{-1} s^{-1}) at each harvest for kiwifruit grown at two different leaf:fruit ratios.

Leaf:fruit ratio treatment	Mean respiration[1] (nmol CO_2 kg^{-1} s^{-1})					
	Time of measurement (days)					
	0[2]	21	36	46	55	Mean
1:1	326.0 (58.2)[3]	283.9 (25.0)	330.3 (22.0)	297.6 (20.6)	191.5 (17.7)	286
5:1	368.3 (41.3)	255.8 (4.5)	353.4 (19.5)	432.4 (18.4)	253.5 (18.5)	333

[1] Mean of 3 measurements over 10 days at 20C
[2] Day 0 = 21 March
[3] ±SE (standard error)

4.4 Discussion

In previous studies, the weight of kiwifruit on girdled laterals increased with an increase in leaf area (Snelgar *et al.*, 1986; Snelgar and Thorp, 1988; Lai *et al.*, 1989; Woolley *et al.*, 1992). Kiwifruit grown on girdled laterals with a 5:1 leaf:fruit ratio were 62 g (Woolley *et al.*, 1992) and 52 g (Snelgar *et al.*, 1986) heavier than those from the 1:1 leaf:fruit ratio treatments. These differences are similar to the 45 g difference at the end of the experiment reported here. In other crops, for example tomato, an increase in leaf area resulted in a 40% increase in the volume of fruit (Gustafson and Stoldt, 1936).

During maturation many changes are taking place in the fruit, such as decreases in flesh firmness and starch concentration and increases in dry matter, soluble solids and total sugar concentrations. There were differences in some of the indicators of fruit maturity between the two leaf:fruit ratio treatments. Flesh firmness decreased in this study during the period from end March to mid May; which is similar to the decrease in firmness measured in other field-grown vines (MacRae *et al.*, 1989a; Fig. 3.1).

At the end of this study, dry matter in the 5:1 leaf:fruit ratio treatment was higher than that in the 1:1 treatment, similar to the results found in other kiwifruit studies (Snelgar and Thorp, 1988; Woolley *et al.*, 1992). Dry matter is a measure of the total weight of solid material in kiwifruit and is related to total carbohydrate concentration in the fruit (Hopkirk, 1991). Here, total carbohydrate concentration is comprised of both starch and sugar concentrations present in the fruit and both were higher in fruit in the 5:1 than in the 1:1 leaf:fruit ratio treatment. At the end of this study, concentrations of total carbohydrate were 42 and 68 mg gFW^{-1} in the outer pericarp and 61 and 96 mg gFW^{-1} in core tissue, for the 1:1 and 5:1 leaf:fruit ratio treatments, respectively. Total carbohydrate concentration and dry matter was lower in fruit from the 1:1 compared to the 5:1 leaf:fruit ratio treatment. Hence, dry matter of fruit may be affected by the different concentrations of carbohydrate available from the two leaf:fruit ratio treatments.

A girdled lateral is isolated from other parts of the vine so that fruit growth is totally dependent on the supply of photosynthate from leaves within that lateral. Girdled laterals with a greater number of leaves would potentially have higher production of photosynthates and therefore higher carbohydrate concentration available for fruit growth and development than that from ungirdled laterals. Production of photosynthates from a leaf:fruit ratio treatment of 2:1 was sufficient to produce 'export' sized fruit, but a ratio of 1:1 was not sufficient (Lai *et al.*, 1989). The 5:1 leaf:fruit ratio treatment in this study should, therefore produce ample carbohydrate for normal fruit growth. Kiwifruit in the 5:1 leaf:fruit ratio treatment were heavier and the dry matter, SSC, starch and total sugar concentrations were higher than for fruit in the 1:1 leaf:fruit ratio treatment.

The pattern of increase in SSC during maturation for fruit from the 5:1 leaf:fruit ratio treatment in this study was typical of that occurring in fruit of field-grown vines, where SSC increased rapidly once a minimum SSC of 5 to 5.5% had been exceeded (Reid *et al.*, 1982a; MacRae *et al.*, 1989a; Beever and Hopkirk, 1990; Fig. 3.2). Soluble solids concentration was higher and the rate of increase faster in fruit from the 5:1 than fruit from the 1:1 leaf:fruit ratio treatment. In other studies, fruit on girdled laterals had a high SSC which was associated with a high leaf:fruit ratio (Snelgar and Thorp, 1988). Again, this shows that the 5:1 leaf:fruit ratio treatment provided sufficient assimilates for fruit to be similar to those from ungirdled laterals, where assimilate can be transported considerable distances around the vine (Snelgar *et al.*, 1986). However, in the 1:1 leaf:fruit ratio treatment, SSC did not reach 5% even by the end of the study indicating that the quantity of assimilate was limiting for fruit growth and development. Hence, accumulation of carbohydrates would be prevented and consequently SSC would not increase to the same extent as in fruit from the 5:1 leaf:fruit ratio treatment.

Previous studies where different leaf:fruit ratio treatments have been imposed on kiwifruit vines have not measured starch and total sugar concentrations in fruit during maturation. However, the results from the leaf:fruit ratio treatments in this study are consistent with several other types of study; these have shown that

during maturation of kiwifruit the concentration of soluble sugars increased and that of starch decreased (Wright and Heatherbell, 1967; Reid *et al.*, 1982a; MacRae *et al.*, 1989a; Beever and Hopkirk, 1990; Fig. 3.3). Hydrolysis of starch occurred in both leaf:fruit ratio treatments, but the rate of increase in total sugar concentration was higher in the 5:1 than 1:1 treatment. This faster rate of increase in sugar concentration in the 5:1 leaf:fruit ratio treatment may indicate that assimilate is still being transported into fruit in addition to sugar from hydrolysis of starch. Leaves can provide photosynthates for fruit until such time as they begin to senesce and abscise (Section 2.4). It is possible that sugar released by starch hydrolysis in the 1:1 leaf:fruit ratio treatment was utilised by priority sinks, such as for respiration, instead of remaining in the fruit flesh as in the 5:1 leaf:fruit ratio treatment.

The core may act as a carbohydrate reserve in kiwifruit. Starch and sugar concentrations were consistently found to be higher in core than outer pericarp tissue throughout this study, those in Chapters 2 and 3 and others (Hopkirk *et al.*, 1989; MacRae *et al.*, 1989a). It has been shown that there are a greater number of cells containing starch in the core than outer pericarp (Patterson *et al.*, 1991; Gould *et al.*, 1992; Hallett *et al.*, 1992) and hence, a higher starch concentration than in outer pericarp (Section 2.4).

However, vascular tissues in the fruit may also determine deposition of photosynthate. There are two networks of vascular tissue in fruit which originate from the stalk attachment (Hopping, 1976). The outer, dorsal, carpellary bundle system spreads along the fruit between the inner and outer pericarp and branches into the outer pericarp. The inner, ventral, carpellary bundle system is a ring of vascular bundles which run longitudinally in the outer tissue of the core and have direct connections to the developing seeds. However, although Hopping (1976) found no branches extending from the inner bundles into the central core, ^{14}C-labelled sugar accumulated in core tissue (MacRae and Redgwell, 1990) indicating that sugars in the vascular bundle were transported throughout core tissue. Core tissue in kiwifruit could act as a reserve of starch as the

concentration in the outer pericarp is depleted earlier than in the core both under controlled conditions (Chapter 2) and in the field (Chapter 3). Carbohydrate stored in the core may be used for seed development and growth. In its natural habitat, kiwifruit relies on seed dispersal for propagation, therefore resources for seed growth, development and maturation are a vital part of kiwifruit survival as a species. Storage of carbohydrate in core tissue may play an integral part in this role.

Rates of fruit respiration measured in this study were similar to those reported for field-grown kiwifruit (Wright and Heatherbell, 1967; Pratt and Reid, 1974; Walton and de Jong, 1990b), container-grown kiwifruit (Chapter 2), apple (Jones, 1981), cherry (Pollack *et al.*, 1961) and peach (de Jong *et al.*, 1987), but were small compared to respiration during the climacteric (Wright and Heatherbell, 1967; Pratt and Reid, 1974). This indicated that the climacteric had not been reached while the fruit were still on the vine.

There was no clear effect of the two different leaf:fruit ratio treatments on respiration, even though fruit in the 5:1 leaf:fruit ratio treatment were physiologically more mature than those in the 1:1 leaf:fruit ratio treatment. It would be anticipated that fruit in the 5:1 leaf:fruit ratio treatment would reach a climacteric before those from the 1:1 leaf:fruit ratio treatment. It is possible to estimate the maximum SSC that could be obtained after storage by fruit in the two leaf:fruit ratio treatments. Starch concentration at the end of the study was 24 and 39 mg gFW^{-1} in outer pericarp and core tissue, respectively when SSC was less than 5% in the 1:1 leaf:fruit ratio treatment. Starch concentration was 34 and 56 mg gFW^{-1} in outer pericarp and core tissue, respectively in the 5:1 leaf:fruit ratio treatment when SSC was 7.9%. Assuming that:

i) there is no further import of photosynthate from leaves due to senescence and abscission in autumn (Section 2.4),
ii) there is a residual 10 mg gFW^{-1} starch concentration in both outer pericarp and core tissue (see Fig. 2.15),
iii) the regression of total sugar concentration and SSC for field grown fruit (Fig. 3.4) can be applied to fruit from different leaf:fruit ratio treatments,
iv) the contribution of sugar from outer pericarp and core tissue is equal,

v) there is no net loss of sugar due to respiration,

hydrolysis of the remaining starch to sugar would result in a final SSC (equivalent to eating ripeness) of 10.6 and 17.5% SSC for fruit from the 1:1 and 5:1 leaf:fruit ratio treatments, respectively. Fruit with a final SSC of 10.6% are unlikely to be of acceptable eating quality as they lack the sweetness and flavour components compared to fruit with higher SSC. A predicted SSC of 17.5% for fruit from the 5:1 leaf:fruit ratio treatment indicates that the above assumptions are probably reasonable as other researchers have recorded maximum concentrations of 16% in kiwifruit (Wright and Heatherbell, 1967; Beever and Hopkirk, 1990; Mitchell *et al.*, 1992).

There are several ways of altering the carbohydrate concentration available for fruit growth and development, including manipulating the leaf:fruit ratio on a girdled kiwifruit lateral or shading of kiwifruit vines (Snelgar and Hopkirk, 1988; Snelgar *et al.*, 1991) and apple trees (Jackson *et al.*, 1971; Jackson and Palmer, 1977; Campbell and Marini, 1992). Shaded leaves produce less photosynthate than unshaded leaves, thus simulating the effect of the assimilate shortage in fruit on a girdled lateral with a low leaf:fruit ratio. Fruit size was reduced by 24 and 37 g when apple trees were shaded by 34 and 13% of full sunlight compared to those that were unshaded, respectively (Jackson *et al.*, 1971). In kiwifruit mean fruit weight was reduced by 14 g on vines which received 45% of full sunlight compared to unshaded vines (Snelgar and Hopkirk, 1988). The fruit size of both apple and kiwifruit was reduced by shading, similar to fruit in girdling treatments with a low leaf:fruit ratio. The dry matter of apple fruit was reduced by 4 and 10 g when shaded by 37 and 25% compared to unshaded trees (Jackson *et al.*, 1977). Soluble solids concentration decreased with increased shading in both apple (Campbell and Marini, 1992) and kiwifruit (Snelgar *et al.*, 1991), similar to SSC in kiwifruit from the 1:1 compared to 5:1 leaf:fruit ratio treatment. Starch concentration in apple fruit was higher when trees were unshaded compared to the shaded treatments of 34 and 13% of full sunlight (Jackson *et al.*, 1977). Respiration was highest in unshaded apple fruit and lowest in heavily shaded fruit, similar to the present study on kiwifruit (Jackson *et al.*, 1977). The low respiration rate in fruit from heavily shaded apple trees may have been partly due to the low level of carbohydrate in these fruit (Jackson *et al.*, 1977).

In this study, the reduced quantity of photosynthate in fruit from the 1:1 leaf:fruit ratio treatment resulted in fruit where increase in SSC did not appear to have been induced. Salinger and Morley-Bunker (1988) claimed that SSC increased linearly with a decrease in temperature once a threshold concentration of 5 to 5.5% had been exceeded. Further definition of this response was shown in Chapter 2 where fruit SSC increased only once a minimum SSC (6.0% in container-grown vines) had been exceeded. In the current study, SSC on 15 May in the 1:1 leaf:fruit ratio treatment was less than 5%, that is, less than the threshold SSC. The concept that there may be two components of SSC which increase differentially during maturation has been introduced previously and is discussed further in Chapter 6. It has been suggested that a basal component of SSC is present from fruit set while a maturation component is induced during maturation. It is possible that carbohydrate concentration *per se* influences maturation, in that a specific carbohydrate concentration has to be exceeded in the fruit before maturation can proceed. However, it is also likely that there is an internal gradient of carbohydrate within each fruit which is driven by priority sinks. For example, the primary sink may be for respiration essential to maintain metabolic processes, the secondary sink may be for growth, development and maturation of seeds, while the third sink may be to support fruit maturation. Hence, fruit maturation could only proceed when sufficient carbohydrate is available to fulfil the requirements of fruit for seed development and fruit respiration.

In conclusion, girdling laterals on kiwifruit to give two different leaf:fruit ratio treatments did alter carbohydrate concentration and affect rate of fruit maturation. Carbohydrate concentration was higher in fruit from the 5:1 than 1:1 leaf:fruit ratio treatment. Fruit in the 5:1 leaf:fruit ratio treatment matured similarly to fruit from ungirdled vines whereas increase in SSC in fruit from the 1:1 leaf:fruit ratio treatment was delayed. It is possible that there is insufficient storage carbohydrate (starch) in outer pericarp and core tissue from the 1:1 leaf:fruit ratio treatment for normal fruit maturation. It has been proposed that core tissue acts as a reserve tissue for starch in kiwifruit.

CHAPTER 5

DEVELOPMENT OF A MODEL TO DESCRIBE INCREASE IN SOLUBLE SOLIDS CONCENTRATION IN KIWIFRUIT

5.1 Introduction

The ability to predict events such as harvest date of crops can allow more effective scheduling of harvesting and processing operations (McPherson *et al.*, 1979). Timing of harvest will be affected by the climate experienced by the crop during growth and maturation, in particular temperature, solar radiation and rainfall. Of these, temperature has been shown to have an important influence on plant growth and phenology (McPherson *et al.*, 1979). Linear relationships between temperature and crop growth have commonly been used both in horticulture and agriculture to predict harvest date, such as in apple (Blanpied, 1964), guava (Bittenbender and Koybayashi, 1990), pear (Lombard *et al.*, 1971) and sweetcorn (Brooking and McPherson, 1989). In kiwifruit, Salinger and Morley-Bunker (1988) and Snelgar *et al.* (in prep.) predicted a linear increase in SSC with a decrease in temperature. However, if the change in SSC with temperature is not linear over the range of temperatures experienced by the vine during fruit maturation, such models will be of limited value.

A quantitative model to predict changes of fruit SSC in response to a wide range of temperatures is desirable. Prediction of harvest date in commercial orchards would benefit the New Zealand Kiwifruit Marketing Board as well as growers, packaging companies, shippers and allied support industries as they currently operate with considerable uncertainty regarding the timing of the harvest period.

Prediction of harvest date requires development and testing of a suitable quantitative model for SSC accumulation. In very general terms, a model is an analogue of the system being studied (Bell, 1981). Two types of mathematical

models are used in research; mechanistic and empirical (Thornley, 1976). Mechanistic models are used when the nature of responses of a biological system are understood. In such models, parameters have biological meaning. With empirical models, data are collected and equations are derived and fitted to data to form the model. Empirical models redescribe the data and are often a convenient method to summarise a large quantity of data. However, there is no clear dividing line between these two approaches and most models contain both mechanistic and empirical elements.

Empirical models are often used to relate plant developmental events to environmental conditions; developmental processes are complex and responses to environmental conditions are subtle and diverse. Under these circumstances, it is extremely difficult to construct mechanistic models which link physiological mechanisms to environmental factors (McNaughton *et al.*, 1985). 'Growing degree-day' and 'heat-sum' models are examples of entirely empirical approaches where no attempt is made to assign physiological significance to the model (McNaughton *et al.*, 1985). In these models, it is usually assumed that there is a linear relationship between rate of development and temperature between defined maximum and minimum temperature limits, and that no development occurs below a certain base temperature (Warrington and Kanemasu, 1983; Brooking and McPherson, 1989).

Timing of a particular phenological event can be determined by integration of daily heat-sums to some critical value. A minimum temperature of 11C and maximum temperature of 28C was used in a linear model to predict tasselling and anthesis in maize (Warrington and Kanemasu, 1983). A linear degree-day model with a base temperature of 6C accounted for 89% of the variation in crop duration (sowing to harvest) of sweetcorn grown in three regions of New Zealand (Brooking and McPherson, 1989). A base temperature of 15C was used in a growing degree-day model to predict harvest dates of guava in the Hawaiian islands (Bittenbender and Koybayashi, 1990). A similar base temperature model was used to predict harvest dates of several apple cultivars in four locations in

Europe (Kronenberg, 1988). A model based on heat-sums plus sunshine hours from 1 April to harvest was used to estimate sugar concentration of grapes at harvest (Chudyk *et al.*, 1979).

Other empirical models centre around a notional state of plant development that progresses continuously in time; for example it might be assumed that there is a unique relationship between current rate of plant development and current temperature (McNaughton *et al.*, 1985). These models have a conceptual link to physiological mechanisms and are therefore superior to completely empirical models, such as that used by McPherson *et al.* (1985) to determine effect of temperature on rate of development of pigeonpea (*Cajanus cajan* (L.) Millsp.).

The only existing models for SSC accumulation in kiwifruit are empirical ones based on a linear increase in SSC with a decrease in mean temperature (Salinger and Morley-Bunker, 1988; Kempler *et al.*, 1992; Snelgar *et al.*, in prep.). The model was only applied when SSC was greater than 5%; when less than 5% SSC, changes did not appear to be controlled by temperature (Salinger and Morley-Bunker, 1988). However, Snelgar *et al.* (in prep.) found that the linear model was not appropriate as it did not consistently predict increase in SSC between or within seasons. For example, prediction of rate of increase in SSC between 5.0 and 6.5% SSC resulted in an R^2 of 0.73 in 1981 and 0.48 in 1982. As the relationship between SSC and temperature has been shown to be curvilinear, this lack of consistency through use of an inappropriate model is not unexpected.

In this chapter, development of a physiologically-based empirical model to relate changes in SSC to temperature is described. There are two phases in this process; first, development of the model and second, testing of the model. Initially the model was developed from two controlled environment experiments with container-grown kiwifruit vines exposed to different temperature treatments. The model was then tested using data from the same population of container-grown vines, but grown outdoors in a sheltered site at Palmerston North, and also on

data from field-grown vines grown at various regions throughout New Zealand. In all of these cases a continuous time course of temperature was available. However, only daily maximum and minimum temperatures are available in many practical situations. The model was therefore modified so that daily maximum and minimum temperatures could be used to predict the increase in SSC in field-grown vines. Use of the model to predict harvest date of kiwifruit in orchards at different geographical regions in New Zealand is illustrated.

5.2 Materials and methods

Soluble solids concentration data collected from the following studies were used in development and testing of models to predict harvest date of kiwifruit, based on SSC. Container-grown vines placed in controlled environments during Experiments 1 and 2 (Section 2.2) were used in initial development of the model. The temperature treatments in Experiment 1 were 18/4, 18/8, 18/12 and 18/16C and in Experiment 2 were 14/8, 18/8, 14/12, 22/8, 26/8 and 22/12C. The model developed was tested on container-grown vines selected from the same population as those in the controlled environment treatments but grown in the sheltered outdoor site, and on field-grown vines in orchards at both Palmerston North and Riwaka. For vines at Palmerston North and Riwaka where temperature records were complete and those at Kerikeri and Te Puke, where continuous temperature records were incomplete due to operational problems with data loggers (Section 3.2), a model using daily maximum and minimum temperatures was developed and tested. The maximum/minimum model was also tested on data from field-grown vines at Te Puke and Riwaka collected in 1981.

Air temperatures were recorded in the sheltered site and at each orchard as 30 minute averages using thermistor probes coupled to Campbell Scientific CR21 data loggers; the loggers were also programmed to record daily maximum and minimum temperatures (Section 3.2.2). Temperatures in the sheltered site and orchards were recorded below the canopy (1.3 m above ground) within a louvred screen (Henshall and Snelgar, 1989); all analyses were based on these temperatures. Temperature recordings were not made until day 7 in the sheltered site in Experiment 1. Temperature records for the sheltered site and each orchard are given in Appendix 4.

The mathematical basis for the work in this chapter is given in Appendix 5. SAS and NAG (Numerical Algorithms Group Ltd., NAG Central Office, Mayfield House, 256 Banbury Road, Oxford, OX2 7DE, UK) were used for analysis of data throughout this chapter, along with appropriate purpose-written programs in

FORTRAN. Constraints were imposed on the power-law model in Experiment 1 to prevent computations becoming negative, resulting in non-convergence of the nonlinear procedure (thus standard errors were not obtained in Fig. 5.5).

A logistic equation was used to describe accumulation of SSC during kiwifruit maturation in the experiments in Chapters 2 and 3. However, only the first part of the logistic curve (up to 12% SSC for container-grown vines) was chosen for development of the model (Fig. 5.1). This portion of data was selected as being appropriate to field prediction where the nature of the final asymptote is irrelevant. Hence, data from Experiments 1 and 2 were truncated to include SSC up to a maximum of 12% and used in subsequent work in this chapter. Commercially, fruit are harvested at or above 6.2% SSC therefore, for any predicted harvest date fruit must have SSC at or above the minimum of 6.2%. Soluble solids concentration in fruit from container-grown vines was approximately 1.5% higher than that in fruit from field-grown vines at a similar stage of maturity, but in all other respects these fruit mature similarly to those from field-grown vines (cf. Chapters 2 and 3).

The focus in this study was to develop a physiologically motivated model rather than testing the statistical significance of a model. Conventional nonlinear, least squares statistical techniques were used to fit models to data. When the model is nonlinear, a number of tests such as the F-test used for regression and lack of fit used in linear regression are no longer valid (Draper and Smith, 1966). Alternative methods exist but require specialist knowledge. The usual procedure adopted by biologists was used here, that is, to use models based on minimising the residual sum of squares and to determine the relative physiological plausibility of candidate models. Once suitable parameters for the temperature-dependent rate function had been determined from controlled environment studies, these were used for the remaining fits to data from different orchard locations. In addition, the partial rate coefficient, fitted by minimising the residual sum of squares, was used to refine the fit of the model to each orchard location.

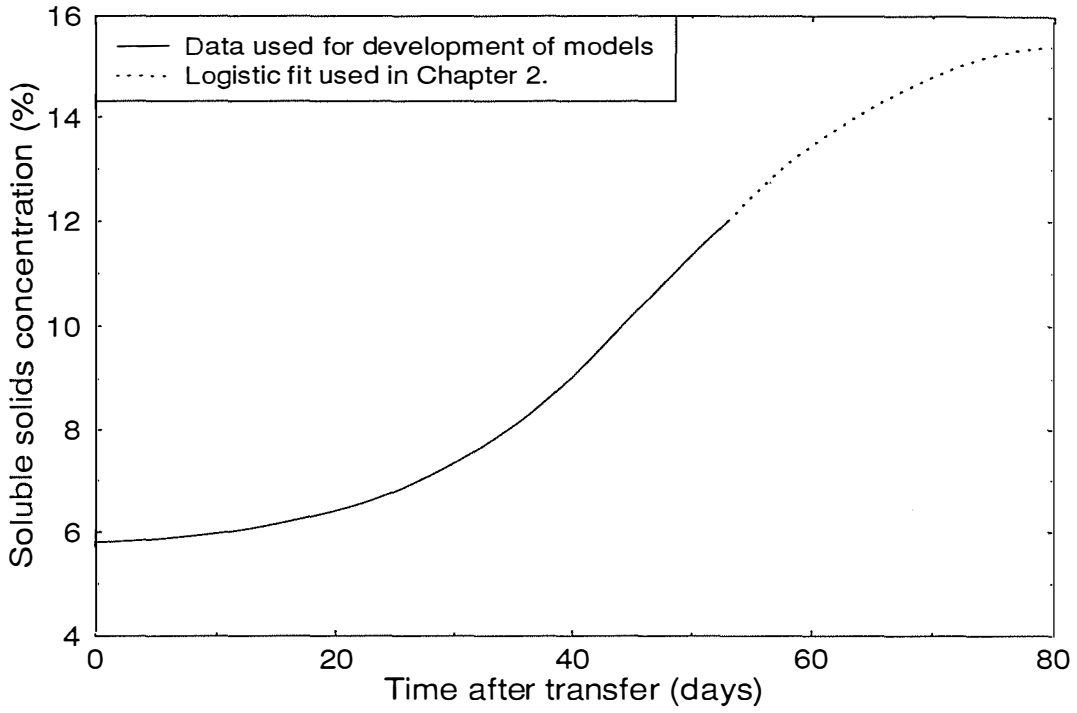


Fig. 5.1 Soluble solids concentration (%) of container-grown kiwifruit during maturation. Solid line shows soluble solids concentration up to 12%, taken from part of the logistic fit used in Chapter 2.

5.3 Results and discussion

5.3.1 Model development

Empirical models

In this section, increase in SSC with mean temperature is described firstly by use of a linear model and secondly by calculation of rate coefficients.

A descriptive approach to interpreting the rate-temperature relationship is to plot the mean rate of change of SSC against mean temperature ($\bar{T}$) (Fig. 5.2, from Fig. 2.19). Here, a linear relationship between rate of change of SSC and temperature is implied. The mean rate is

$$\bar{R} = \frac{S_2 - S_1}{t_2 - t_1}$$

where $\bar{R}$ = mean rate of change in SSC (% day^{-1})

S_1 = SSC (%) measured at t_1

S_2 = SSC (%) measured at t_2

t_1 = time (day) of measurement 1

t_2 = time (day) of measurement 2.

Rates of change in SSC were estimated for the period of rapid increase in SSC, which occurred during the final 35 days of Experiment 1 and final 33 days of Experiment 2 (Fig. 5.2). For example, the mean rate for the 18/4C temperature treatment in Experiment 1 is

$$\bar{R} = \frac{14.9 - 7.5}{56 - 21}$$

$$= 0.21$$

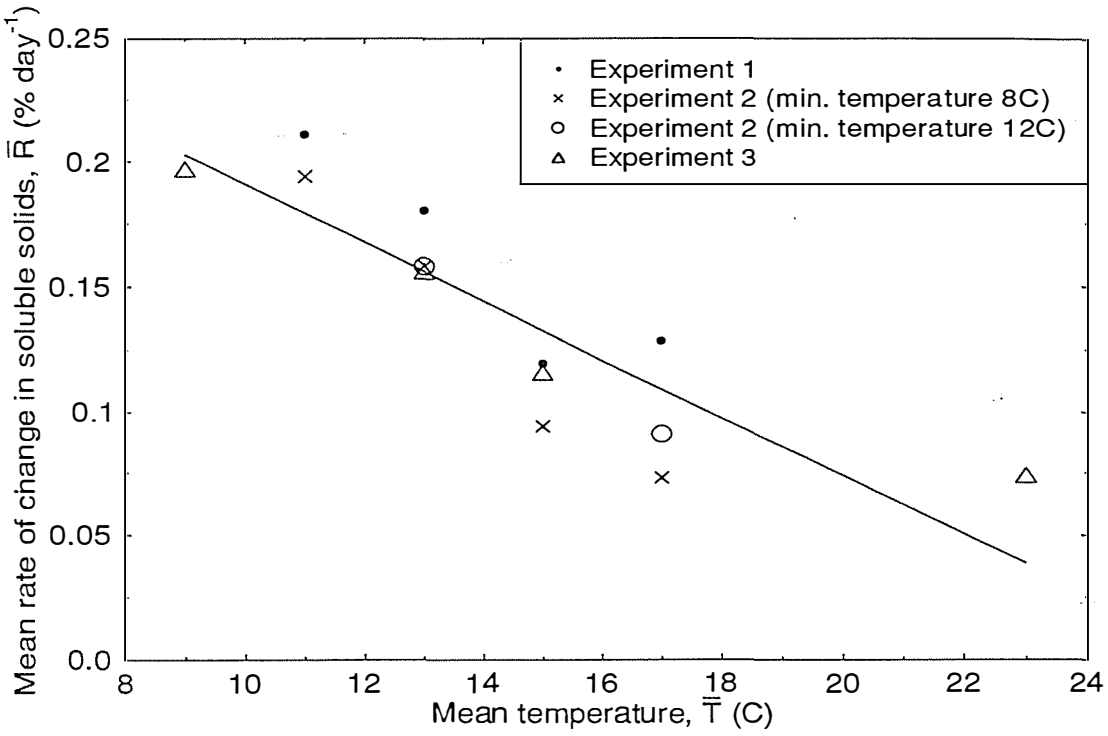


Fig. 5.2 Mean rate of change in soluble solids concentration (% day^{-1}) of kiwifruit during maturation in controlled environments at different minimum temperatures (C) (Experiment 1) or different maximum and minimum temperatures (Experiments 2 and 3), from Fig. 2.19. Regression equation is $\bar{R} = 0.308 - 0.0117\ \bar{T}$ ($R^2 = 0.75$).

The fitted equation for all the temperature treatments (from Fig. 5.2) is

$$\bar{R} = 0.308 - 0.0117\ \bar{T}$$

where $\bar{R}$ = mean rate of change in SSC (% day^{-1})

$\bar{T}$ = mean temperature (C) in treatment.

However, such a simplistic approach should be treated with caution because only about half of the total data set was used, whereas it would be preferable to incorporate the complete data set into a model. Use of selected data sets such as mean rate of increase in SSC (Fig. 5.2), night temperatures only (Wallace and Enriguez, 1980) or division of temperature records into night and day temperatures (Robertson, 1968) lead to very empirical equations and underlying physiological processes of plant development are obscure (McNaughton *et al.*, 1985).

An alternative approach that uses more data is to fit curves, derive rate coefficients and then plot these coefficients against mean temperature. A rate coefficient is the proportionality relationship between rate of increase in SSC and SSC itself. A high value implies a rapid increase in SSC at a fixed temperature and a low value a slower increase in SSC. Rate coefficients (h_j) were obtained for mean temperatures from the whole data set for Experiments 1 and 2 (Figs 5.3 and 5.4) using an exponential equation of the form of equation (2) in A.5.1 fitted to each data set. For example, in the 18/4C temperature treatment (11C mean temperature) of Experiment 1

$$S(t) = 6.2\ e^{0.013\ t}$$

where $S(t)$ = SSC (%) at a particular time, t

6.2 = SSC (%) at intercept

0.013 = rate coefficient (days^{-1})

t = time (days).

This analysis showed that highest values of rate coefficients occurred at the coolest mean temperatures and that rate coefficient values decreased linearly as

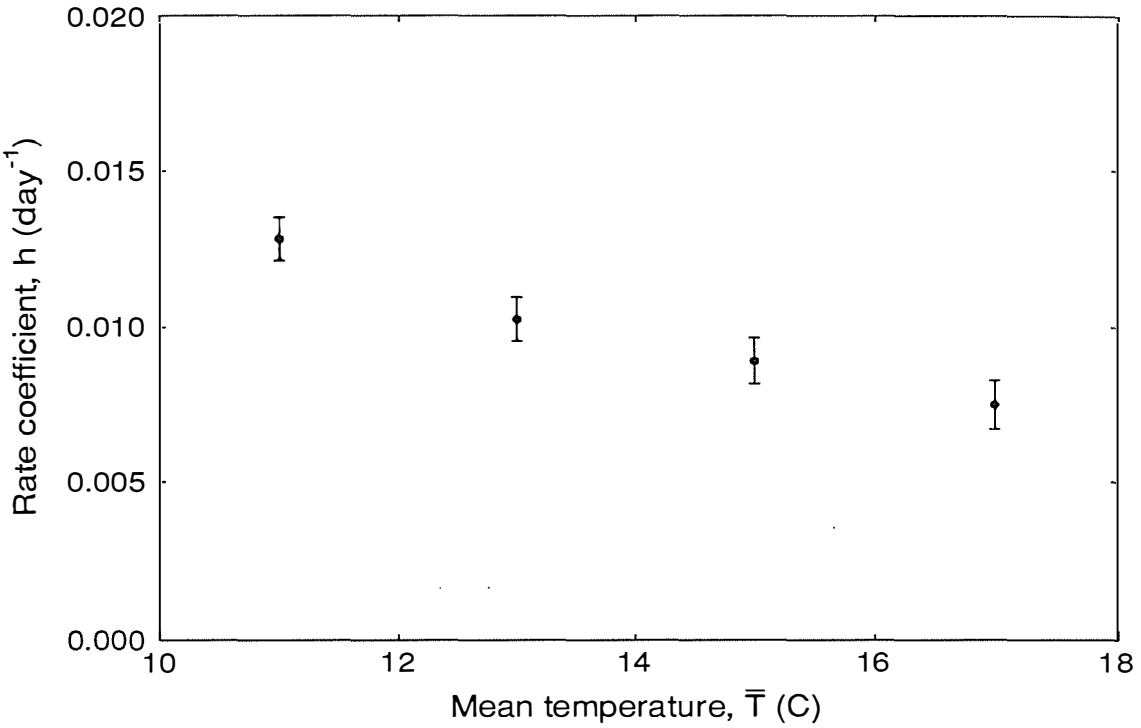


Fig. 5.3 Rate coefficient (h) values for the changes in soluble solids concentration of kiwifruit obtained by fitting exponential equations, from different temperature (C) treatments (Experiment 1). Mean ±SE.

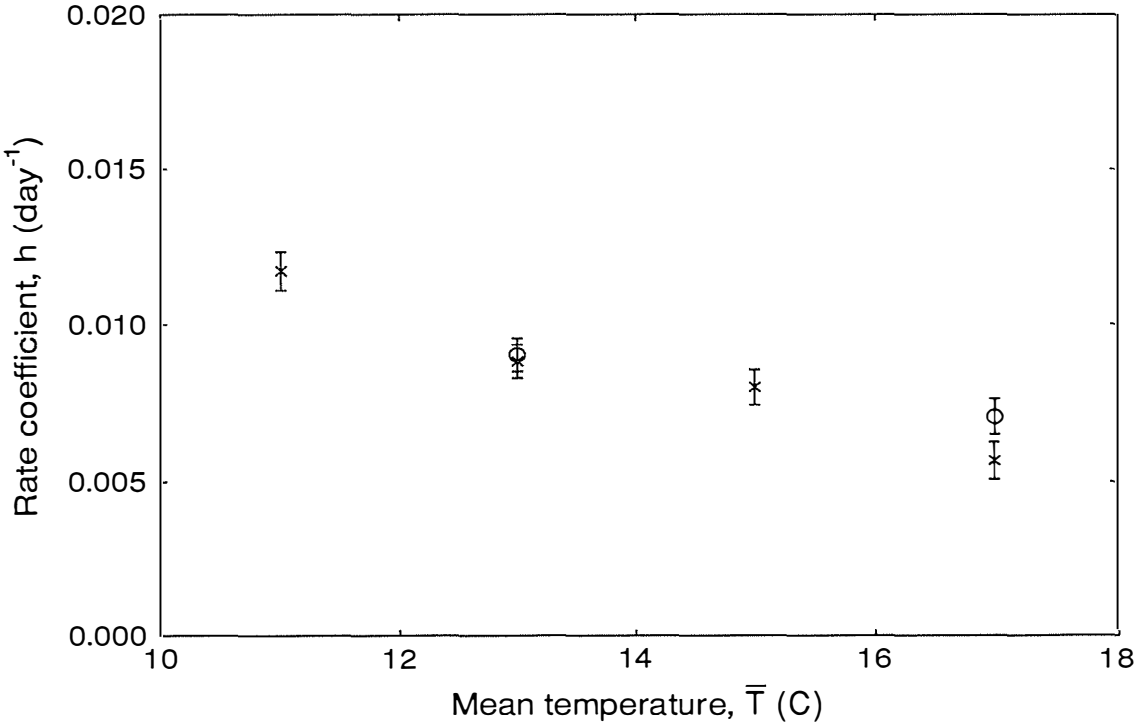


Fig. 5.4 Rate coefficient (h) values for the changes in soluble solids concentration of kiwifruit obtained by fitting exponential equations (Experiment 2). Different symbols indicate mean temperature (C) was derived from different combinations of maximum and minimum temperatures. Mean ±SE.

temperature increased to a mean of 17C (Figs 5.3 and 5.4). While this approach uses the whole data set, use of mean temperatures may not be a useful summary of the temperature response as variation in temperature between minimum and maximum temperature is ignored in the fitting. However, the use of mean temperatures is satisfactory if the rate of development varies linearly with temperature. If the response is nonlinear, there are two parts to consider; firstly, the relationship between the rate coefficient and temperature and secondly, the time course of temperature (temperature variation with time).

Improved model

Plotting rate coefficients against mean temperature (Figs 5.3 and 5.4) is a descriptive approach which may not accurately reflect the underlying rate-temperature relationship. To achieve this, a relationship is required which links the descriptive models and the physiological processes involved in fruit growth and maturation (A.5.3). In general, once fruit have commenced maturation then the rate of change in SSC might be expressed as

$$\frac{dS}{dt} = F(S,T)$$

where F = function relating rate of change in SSC to S and T

S = SSC (%)

T = temperature (C).

This equation implies that SSC accumulation is a function of both current SSC and temperature. The theory behind this equation was developed for analysis of biochemical systems (A.5.3). It is therefore possible to justify the processes involved in SSC changes, such as sugar accumulation, starch synthesis and hydrolysis, water exchange and changes in dry matter content in the fruit, as these processes are the result of biochemical reactions. This theory suggests the function *(F)* can be split (A.5.3) so that

$$\frac{dS}{dt} = g(T)\, f(S)$$

where $g(T)$ = temperature-dependent rate function

$f(S)$ = state-dependent physiological response function.

The rate of SSC accumulation is determined both by the temperature-dependent rate function and a function of the current SSC present. Examples of this equation are given in A.5.3, for example, equation (23).

State-dependent physiological response function

In fruit from container-grown vines, increase in SSC (the state-dependent physiological response function) up to 12% is positively curvilinear with time (Fig. 5.1 and Chapter 2). The exponential model described earlier is the simplest form available to account for this change and it is widely used to describe curvilinear increases in data (Fig. 4.3). However, two other models were also examined; the base + exponential and power-law models. Each of these models can be related to the logistic equation used in previous chapters (A.5.1 and A.5.3). In the base + exponential model a basal level of SSC is subtracted from every measurement. The power-law model is also similar to the exponential equation but raises each SSC measurement to a power. The latter two models give flatter curves than the exponential model, which may be an advantage as increase in SSC is slow up to 5% in field-grown vines (e.g. Fig. 3.2).

Soluble solids concentration may be separated into two components called 'basal' and 'maturation' SSC (A.5.2 and Chapter 6). Basal SSC is present from fruit set and throughout fruit development, whereas the maturation component begins at some point during development and accounts for the increase in SSC during maturation. It is possible that changes in SSC with development may be genetically determined, where basal SSC is regulated by one group of genes, while maturation SSC is determined by a separate group of genes activated during maturation. Evidence for induction of genes coding for SSC has been found in tomato (Lincoln *et al.*, 1987; Lincoln and Fischer, 1988a).

Both the magnitude of the basal component and timing of the induction of the maturation component may vary from year to year (A.5.2). In Experiments 1, 2 and 3, SSC increased fastest with cool temperatures, whereas in Experiment 4 and the first temperature perturbation in Experiment 5, SSC was not affected by any temperature treatment imposed (Figs 2.17 and 2.18). In Experiments 1, 2 and 3 where SSC was influenced by the temperature treatments (Figs 2.5, 2.7 and 2.13) it is assumed that fruit had reached a sufficient stage of maturity for the maturation component to be induced. In contrast, it was assumed that fruit in Experiments 4 and 5 were too immature for the maturation component to be induced by low temperatures (Section 2.4). Accordingly fruit were not responsive to the temperatures imposed in the different treatments. Treatments in the controlled environment rooms may have begun either before (Experiments 4 and 5) or after (Experiments 1, 2 and 3) the maturation component had been induced (A.5.2).

Temperature-dependent rate function

The temperature-dependent rate function was defined using the discrete-rate method of McNaughton *et al.* (1985) (A.5.4). This is a physiologically based approach used to determine the effect of temperature on rate of SSC accumulation in kiwifruit by allowing assessment of the rate-temperature relationship without any constraint on the form of the plant response (McNaughton *et al.*, 1985). The discrete-rate approach involves dividing the temperature regime to which plants were exposed during maturation into discrete classes (Tables 5.1 and 5.2). The mean value of the rate-coefficient function applying to each class was determined (hence use of the term 'discrete rates'). Values of the rate-coefficient function were obtained by simultaneously fitting an equation to each of the treatments in an experiment. For example, using data from Experiment 1 (Table 5.1) and the exponential model (equation (30), A.5.4), the following equations were derived for the different temperature treatments, where S°, hg_1, hg_2, hg_3 and hg_4 are determined by nonlinear least-squares regression:

Table 5.1 Fraction of time spent in each temperature (C) class in treatments in Experiment 1.

Temperature (C) max./min.	Temperature classes (C)			
	4-8	8-12	12-16	16-18
18/4	0.286	0.286	0.286	0.143
18/8	0.000	0.400	0.400	0.200
18/12	0.000	0.000	0.666	0.333
18/16	0.000	0.000	0.000	1.000

Table 5.2 Fraction of time spent in each temperature (C) class in treatments in Experiment 2.

Temperature (C) max./min.	Temperature classes (C)				
	4-8	12-14	14-18	18-22	22-26
14/8	0.666	0.333	0.000	0.000	0.000
18/8	0.400	0.200	0.400	0.000	0.000
14/12	0.000	1.000	0.000	0.000	0.000
22/8	0.286	0.143	0.286	0.286	0.000
26/8	0.222	0.111	0.222	0.222	0.222
22/12	0.000	0.200	0.400	0.400	0.000

$$S_1(t) = S^o \exp((0.286\,hg_1 + 0.286\,hg_2 + 0.286\,hg_3 + 0.143\,hg_4) * t)$$

$$S_2(t) = S^o \exp((0.400\,hg_2 + 0.400\,hg_3 + 0.200\,hg_4) * t)$$

$$S_3(t) = S^o \exp((0.666\,hg_3 + 0.333\,hg_4) * t)$$

$$S_4(t) = S^o \exp((1.000\,hg_4) * t)$$

where $S_1(t)$ = SSC (%) for 18/4C temperature treatment at a particular time, t
$S_2(t)$ = SSC (%) for 18/8C temperature treatment at a particular time, t
$S_3(t)$ = SSC (%) for 18/12C temperature treatment at a particular time, t
$S_4(t)$ = SSC (%) for 18/16C temperature treatment at a particular time, t
S^o = initial SSC (%)
hg = discrete rates when equations were simultaneously fitted to data (day^{-1})
hg_1 = 0.022 (day^{-1}) for 4-18C temperature class
hg_2 = 0.024 (day^{-1}) for 8-18C temperature class
hg_3 = 0.013 (day^{-1}) for 12-18C temperature class
hg_4 = 0.010 (day^{-1}) for 16-18C temperature class
t = time (days).

A similar approach was followed for the base + exponential and power-law models (A.5.4).

Rate coefficients from the discrete-rate method (Fig. 5.5) formed different shaped curves from those plotted using the rate coefficient values derived from mean temperature (Figs 5.3 and 5.4). The rate coefficients from the exponential, base + exponential and power-law models all decreased with an increase in temperature above 12C and there was a trend for a reduction below 8C (Fig. 5.5), indicating a curvilinear response of SSC with temperature. Although the shape of the histograms were similar for the three different models, actual values of the rate coefficients were different. The coefficients for the power-law model were an order of magnitude smaller than those for the exponential and base +

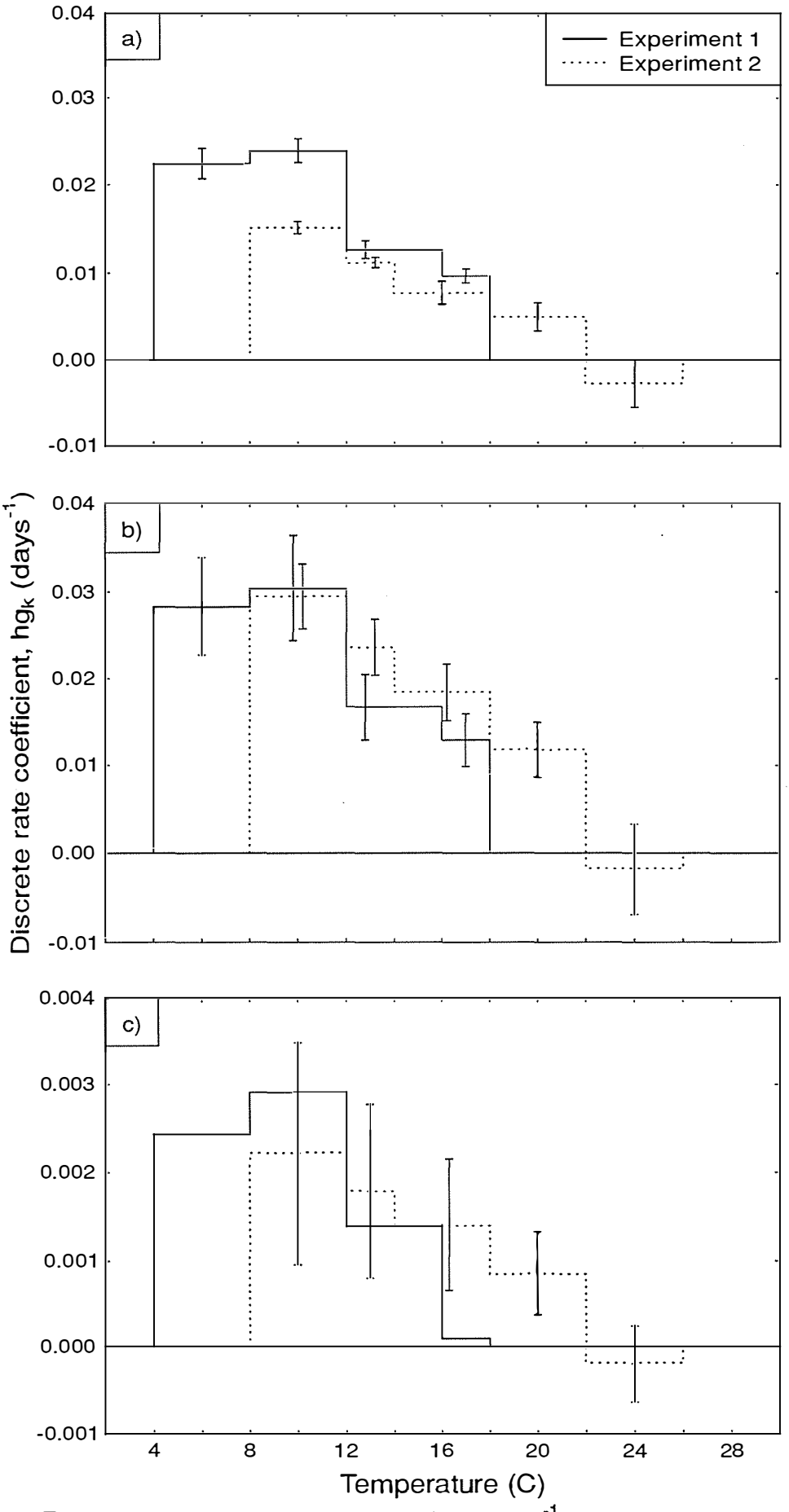


Fig. 5.5 Rate coefficient values, hg_k ($days^{-1}$) from a) exponential, b) base + exponential and c) power-law models for the changes in soluble solids concentration of kiwifruit, from different temperature (C) treatments (Experiments 1 and 2). Mean ±SE.

exponential models due to the different algebraic structure of the equation (A.5.4). Examples of the discrete-rate fits for Experiment 1 are given in Fig. 5.6.

The whole data set was used to obtain the histograms in Fig 5.5 which are discrete versions of the temperature-dependent rate function. In general, the discrete-rate approach indicated that SSC accumulation was curvilinear with temperature. A continuous description of the temperature-dependent rate function is required if these rate functions are to be applied to continuous temperature records from field environments.

Fitting a temperature-dependent rate function

The patterns of the discrete-rate coefficients in Fig. 5.5 suggest that a suitable function should rise from zero at 0C to a maximum around 10C, before falling to near zero at about 24C.

Theory developed by Johnson and Thornley (1985) has been used as a basis for the rate-coefficient function (A.5.5). An equation was developed describing the temperature response of plant processes by combining the Arrhenius equation for chemical reactions (initial exponential phase of the curve) and the Boltzmann enzyme distribution function (loss of activity at higher temperatures) (Feng *et al.*, 1990). The modified Arrhenius equation is as follows:

$$g(K) = \frac{A e^{-a/K}}{1 + B e^{-b/K}}$$

where g = rate function (dimensionless)
K = temperature (Kelvin)
A = constant (dimensionless)
a = constant (Kelvin)
B = constant (dimensionless)
b = constant (Kelvin).

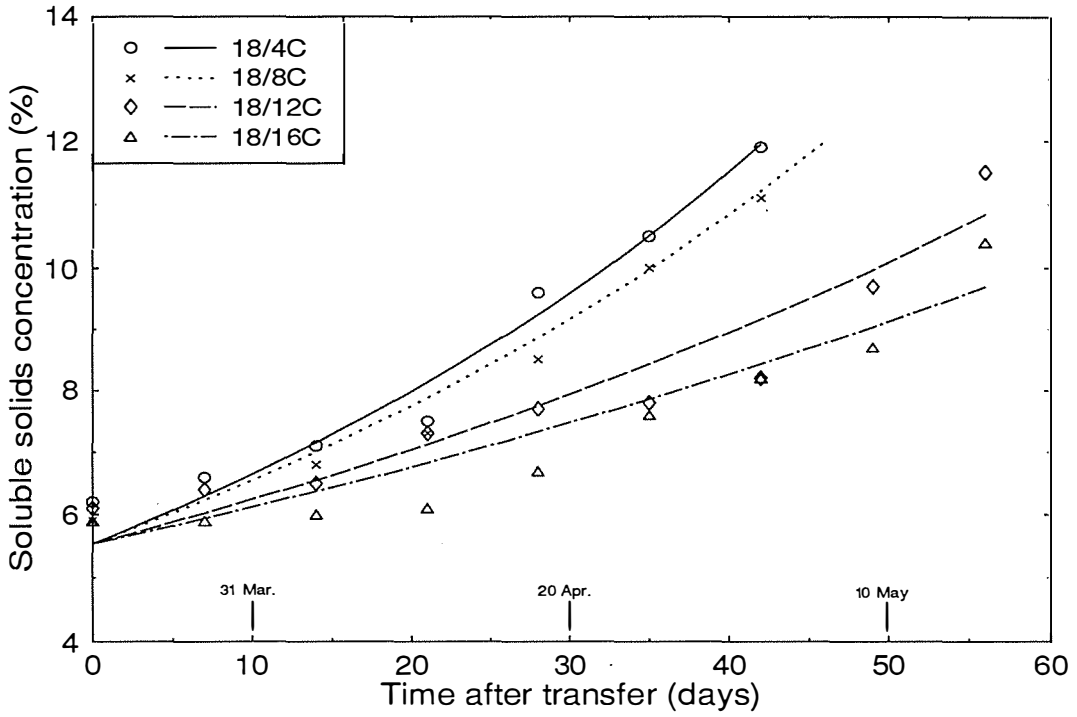


Fig. 5.6 Soluble solids concentration (%) fitted using discrete-rates (Experiment 1).

In this thesis the equation has been written in terms of degrees Celsius (A.5.5). The modified Arrhenius equation has been used to estimate temperature-dependent rate functions for growth rates of bacteria (Johnson and Thornley, 1985), leaf growth and shoot dry matter changes in spring wheat (Feng *et al.*, 1990) and colour changes in apples (Dixon, 1993). The temperature response described by this equation was zero at 0C, increased to reach a peak at intermediate temperatures and then declined at higher temperatures. Therefore, this approach appeared to be suitable for describing changes in SSC with temperature in kiwifruit. Equation (35) (A.5.6) describes the whole curve for the exponential model even though the integral in this equation cannot be evaluated algebraically.

There were several steps involved in fitting the modified Arrhenius equation to data as outlined below. In addition, a suitable form of the state-dependent physiological response function needed to be selected from one of the exponential, base + exponential and power-law models. A combined data set from Experiments 1 and 2 was used as there were insufficient data in each experiment alone; simultaneous fits of each model to all treatments in both experiments were then carried out using a combination of numerical integration in conjunction with a nonlinear fit (A.5.6). The temperature-dependent rate function was found for each of the exponential, base + exponential and power-law models and then modified by

i) including a year-dependent rate coefficient and
ii) rescaling of the temperature-dependent rate function.

These steps will now be described in more detail.

To account for the possibility that rate coefficients varied between the two experiments, a year-dependent rate coefficient was included when the model was fitted to the combined data set (A.5.6). This coefficient is the combination of the year-dependent rate coefficient (h_i) and the multiplicative coefficient (A) from modified Arrhenius equation (A.5.6). The product of these two parameters (h_iA) allows for the different physiological responses between years to be taken into

account. Parameters were obtained by fitting the modified Arrhenius equation in combination with each of the exponential, base + exponential and power-law models (Table 5.3). An example of the modified Arrhenius equation with coefficients is given for the base + exponential model for Experiment 2

$$\frac{dS}{dt} = 0.035\ (S - 4.03)\ \frac{e^{-0.72/T}}{1 + 47.05\, e^{-58.88/T}}\ ;\ S^0 = 5.77$$

where 0.035 = $(h_f A)$

S = current SSC (%)

T = temperature (C)

S^0 = initial SSC (%).

Separation of this new year-dependent rate coefficient $(h_f A)$ from the fitted temperature-dependent rate function permitted the latter to be fixed across years (experiments) (A.5.6). Hence, the year-independent temperature-dependent rate function may reflect the true underlying physiological response. At the biochemical level, the shape of temperature-dependent rate function might be explained in terms of activation and inactivation of specific enzymes in critical biochemical pathways.

The year-independent temperature-dependent rate function, based on B, a and b is:

$$g(T) = \frac{e^{-0.72/T}}{1 + 47.05\, e^{-58.88/T}}$$

The temperature-dependent rate function differed according to the model used for SSC accumulation (Fig. 5.7). The rate function increased rapidly from zero to an optimum at cool temperatures, followed by a near-linear decrease, then a slower decrease approaching zero at warmer temperatures. This is similar to the shape of the curve determined by the modified Arrhenius equation from Johnson and Thornley (1985).

The rate coefficients from each of the three models were rescaled by dividing all values by the maximum value of the rate coefficient so that $g^*(T)$ was scaled to

Table 5.3 Fitted parameters ((h_fA), B, a, b, S^0, S^b and α) from the modified Arrhenius equation for the exponential, base + exponential and power-law models, where S^0 = initial soluble solids concentration (%), S^b = basal soluble solids concentration (%) and α = power coefficient.

	Parameters										
	(h_fA)		B	a	b	S^0 (%)		S^b (%)		α	
Model	Exp 1	Exp 2				Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Exponential	0.031	0.021	71.81	1.66	60.02	5.54	5.41	-	-	-	-
Base + exponential	0.037	0.035	47.05	0.72	58.88	5.71	5.77	2.71	4.03	-	-
Power-law	0.013	0.004	55.83	0.24	62.21	5.64	5.67	-	-	1.29	1.70

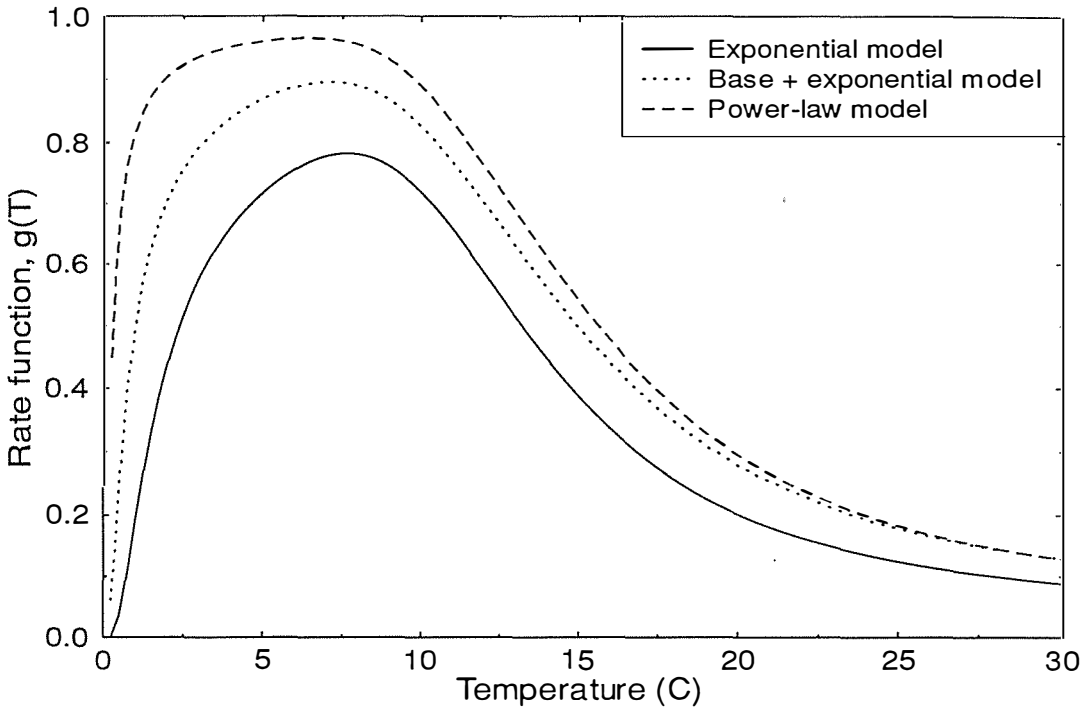


Fig. 5.7 Year-independent temperature-dependent rate function (g(T)) for exponential, base + exponential and power-law models based on a modified Arrhenius equation.

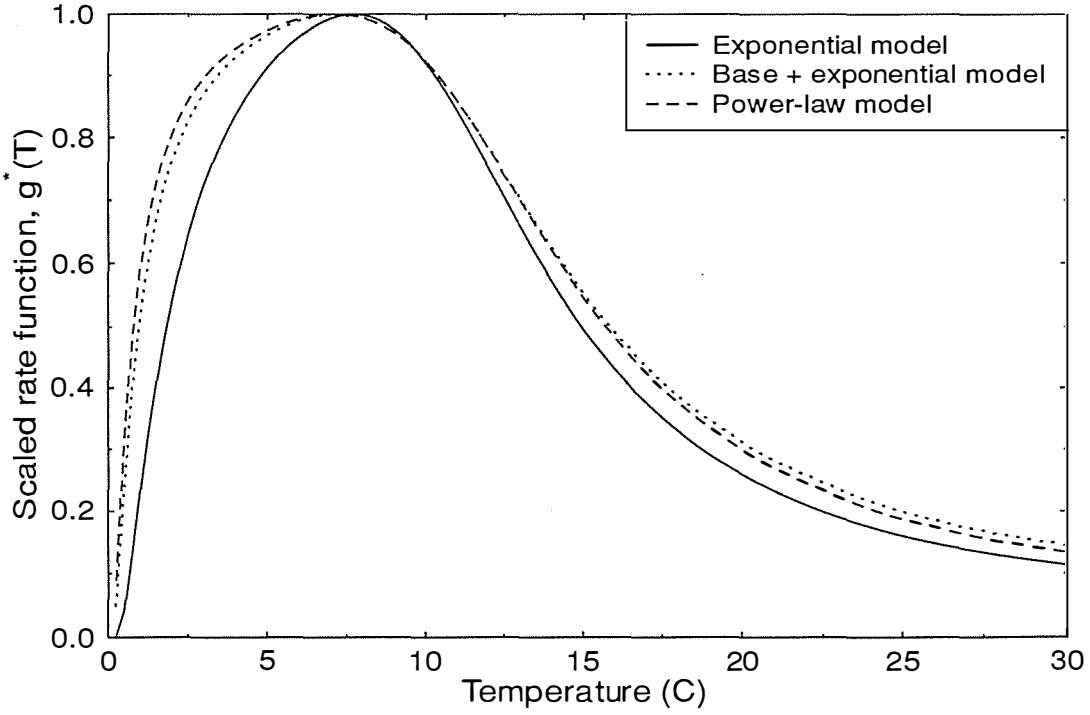


Fig. 5.8 Scaled temperature-dependent rate function ($g^*(T)$) (where g_{max} = 1) for exponential, base + exponential and power-law models based on a modified Arrhenius equation.

1.0 at the optimum temperature (Fig. 5.8). Rescaling of the rate coefficient made each of the three models similar. The maximum value (g_{max}) for the fitted function at the optimum temperature was 0.783, 0.896 and 0.966 at 7.8, 7.3 and 6.8C for the exponential, base + exponential and power-law models, respectively. The following equations from A.5.7 show incorporation of the year-dependent rate coefficient and scaled temperature-dependent rate function:

$$h_i^* = (h_i A)\ g_{max}$$

where h_i^* = scaled rate coefficient (days^{-1})

h_i = rate coefficient (days^{-1})

i = year

A = constant (dimensionless)

g_{max} = maximum value of rate function (dimensionless).

$$g^*(T) = \frac{g(T)}{g_{max}}$$

where $g^*(T)$ = scaled temperature-dependent rate function (dimensionless)

$g(T)$ = temperature-dependent rate function (dimensionless).

Contrasting temperature treatments (26/8C and 14/8C) from Experiment 2 were selected to show simultaneously fitted data to the three different models (Figs 5.9 and 5.10). It should be noted that these are two examples from a simultaneous fit to 10 data sets, therefore the fit of an individual treatment may not be particularly good. At 26/8C there was little change in SSC throughout the experiment and the fit of all models to the data was almost linear while, in contrast, SSC at 14/8C increased markedly and the base + exponential and power-law fits show more curvature in the fit than the exponential model. At this stage, it appears that the base + exponential and power-law models are more suitable (as the earlier part of the curve is flatter) than the exponential model (where the curve is steeper) for describing changes in SSC during maturation, when judged by eye. It is obviously preferable to choose one of these models to describe the response of SSC to temperature over time.

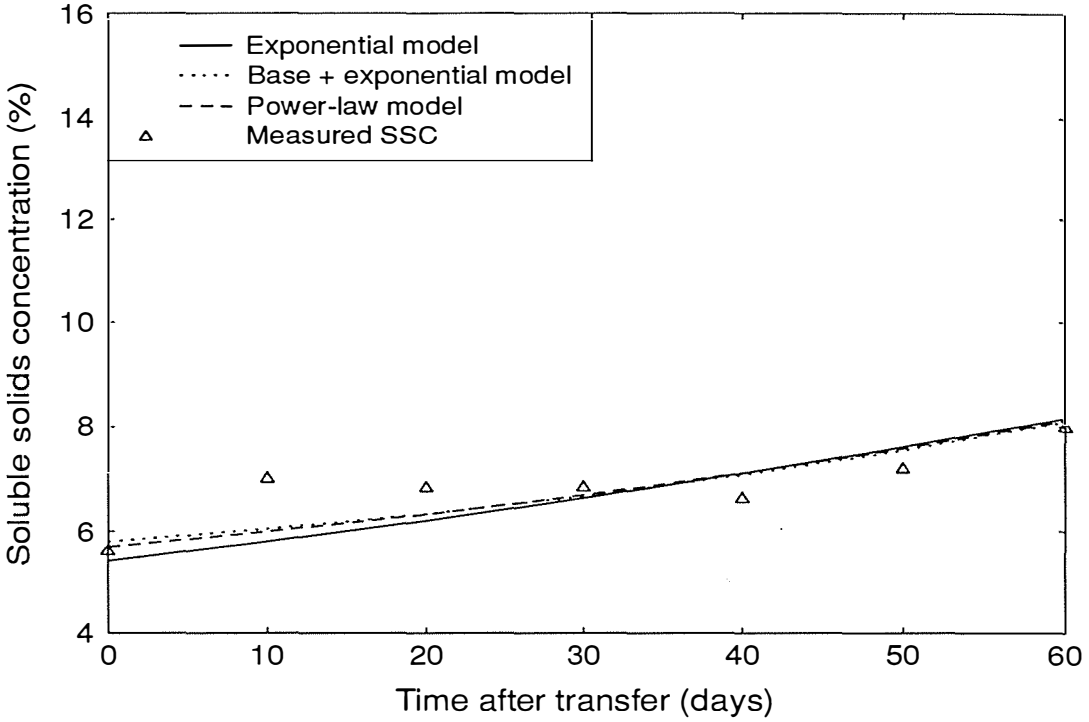


Fig. 5.9 Soluble solids concentration (%) from 26/8C temperature treatment (Experiment 2) fitted with the exponential, base + exponential and power-law models. Parameters in Table 5.3.

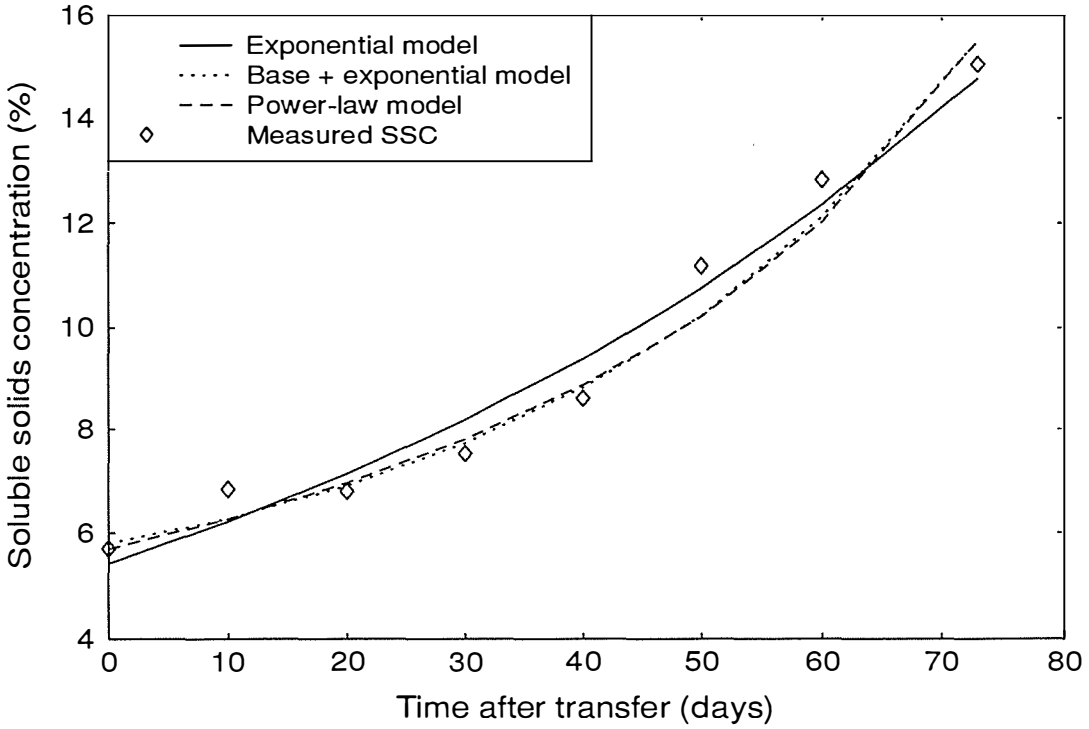


Fig. 5.10 Soluble solids concentration (%) from 14/8C temperature treatment (Experiment 2) fitted with the exponential, base + exponential and power-law models. Parameters in Table 5.3.

5.3.2 Testing the models

The models developed thus far from container-grown vines in controlled environments were tested on similar container-grown vines in the sheltered site and on field-grown vines in orchards at different geographical locations in the country. Although container-grown vines had higher SSC than field-grown vines, it was hoped that models describing the state-dependent physiological response function and/or the temperature-dependent rate function could be transported from container-grown vines to field-grown vines. However, there are three possible options: firstly, that the fitted models and parameters apply directly to other data (described above); secondly, that the fitted models apply, but new parameters are required for the kinetics of SSC accumulation or temperature-dependent rate function; thirdly, that a totally different model is required (as described in A.5.8).

Data from sheltered site

The first option was tested by assuming that fitted models and parameters apply directly to other data. Parameters for each model from the modified Arrhenius equation (Table 5.3) were applied directly to container-grown vines in the sheltered site, where fruit were harvested and measured at the same time as fruit in Experiments 1 and 2 (Figs. 5.11 and 5.12). The first observation to be used as the starting point in the simulation was day 7 in Experiment 1 and day 0 in Experiment 2. The simulated increase in SSC was similar to measured increase in SSC for all models in Experiment 1 (Fig. 5.11). In Experiment 2, the simulated increase in SSC was similar to measured increase in SSC for the base + exponential and power-law models, but not for the exponential model (Fig. 5.12). Simulated SSC from the exponential model increased at a faster rate than measured SSC during Experiment 2 and was 1.5% higher than measured SSC by the end of the study.

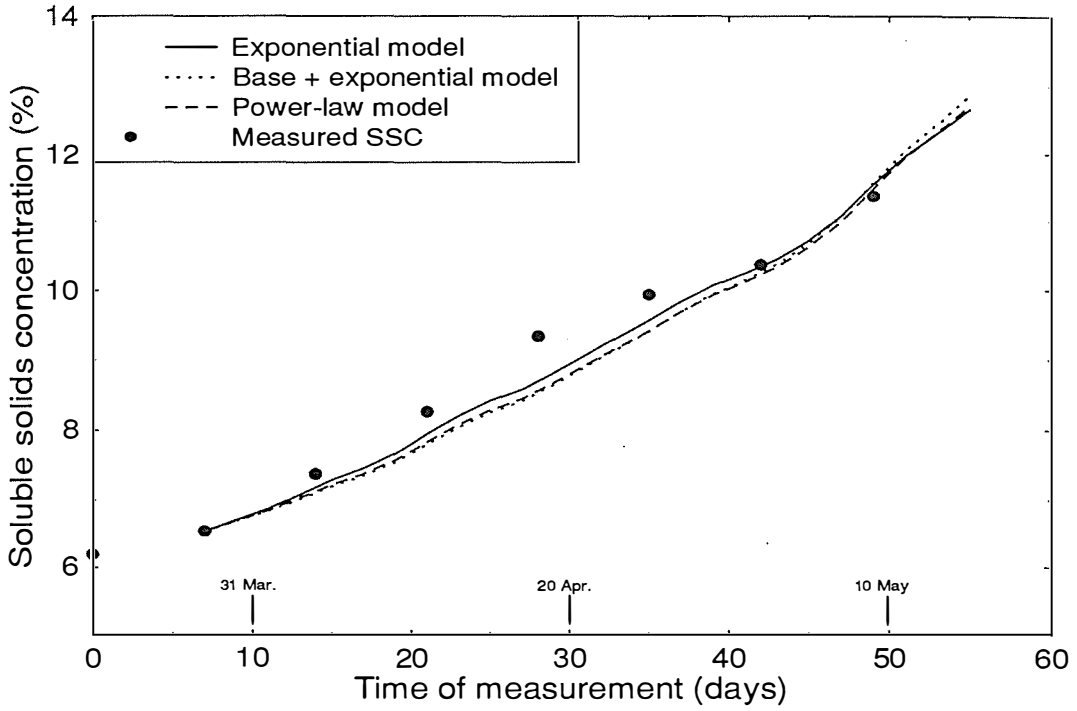


Fig. 5.11 Soluble solids concentration (%) of kiwifruit grown in the sheltered site (Experiment 1). Parameters from controlled environments used to simulate soluble solids concentration from the exponential, base + exponential or power-law models. Missing temperature records to day 7.

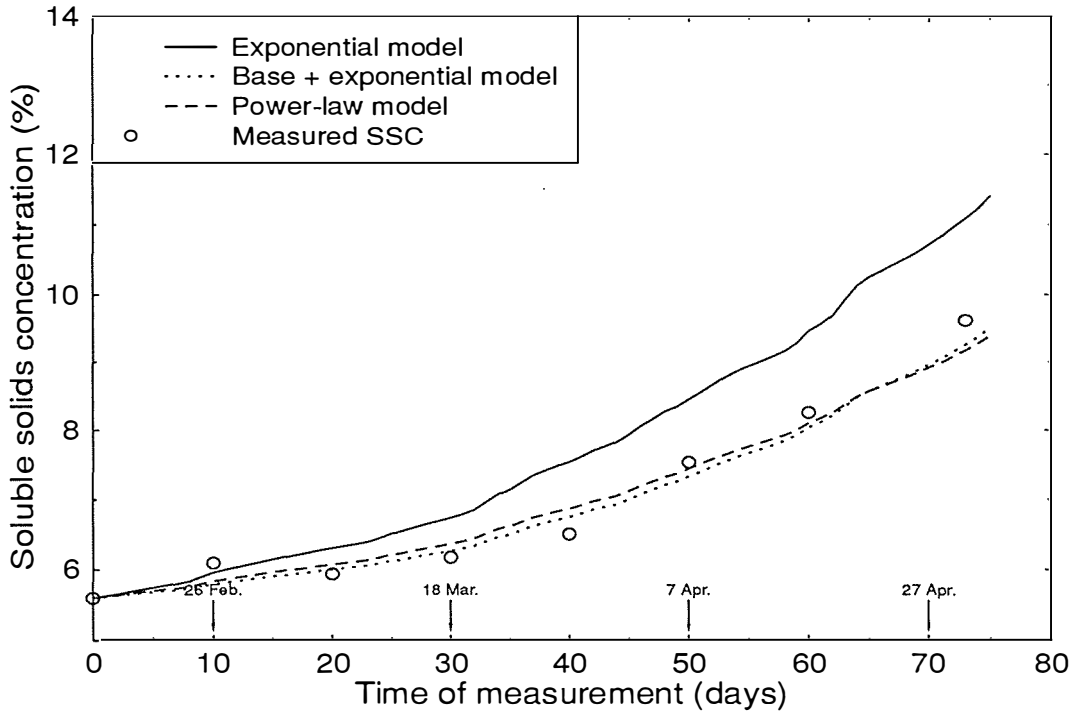


Fig. 5.12 Soluble solids concentration (%) of kiwifruit grown in the sheltered site (Experiment 2). Parameters from controlled environments used to simulate soluble solids concentration from the exponential, base + exponential or power-law models.

The second option was tested by assuming that the fitted models apply, but new parameters may be required for the kinetics of SSC accumulation or temperature-dependent rate function. Parameters for the base + exponential model from the modified Arrhenius equation (Table 5.3) were used to fit each of the exponential, base + exponential and power-law models for data from the sheltered site during Experiment 2 (Fig. 5.13). The temperature-dependent rate function for each model remained as in Table 5.3 and the fitted parameters are presented in Table 5.4. The base + exponential and power-law fits were similar and had more curvature than the exponential fit (Fig. 5.13). Use of base + exponential parameters to fit each of the three models showed that the fit of each model was similar.

Table 5.4 Fitted parameters (S^0, S^b, α and h_i) from the base + exponential model when used to fit the exponential, base + exponential and power-law models (Fig. 5.13), where S^0 = initial soluble solids concentration (%), S^b = basal soluble solids concentration (%), α = power coefficient and h_i^* = rate coefficient ($days^{-1}$).

Model	Parameters			
	S^0 (%)	S^b (%)	α	h_i^* ($days^{-1}$)
Exponential	5.31	-	-	1.64×10^{-2}
Base + exponential	5.71	4.89	-	5.07×10^{-2}
Power-law	5.61	-	2.79	4.53×10^{-4}

An example of the equation with fitted coefficients is given for the base + exponential model:

$$\frac{dS}{dt} = 5.07\ x10^{-2}\ (S - 4.89)\left(\frac{e^{-0.72/T}}{1 + 47.05e^{-58.88/T}}\right);\ S^0 = 5.71$$

where $5.07 \times 10^{-2} = h_i^*$

S = current SSC (%)

T = temperature (C)

S^0 = initial SSC (%).

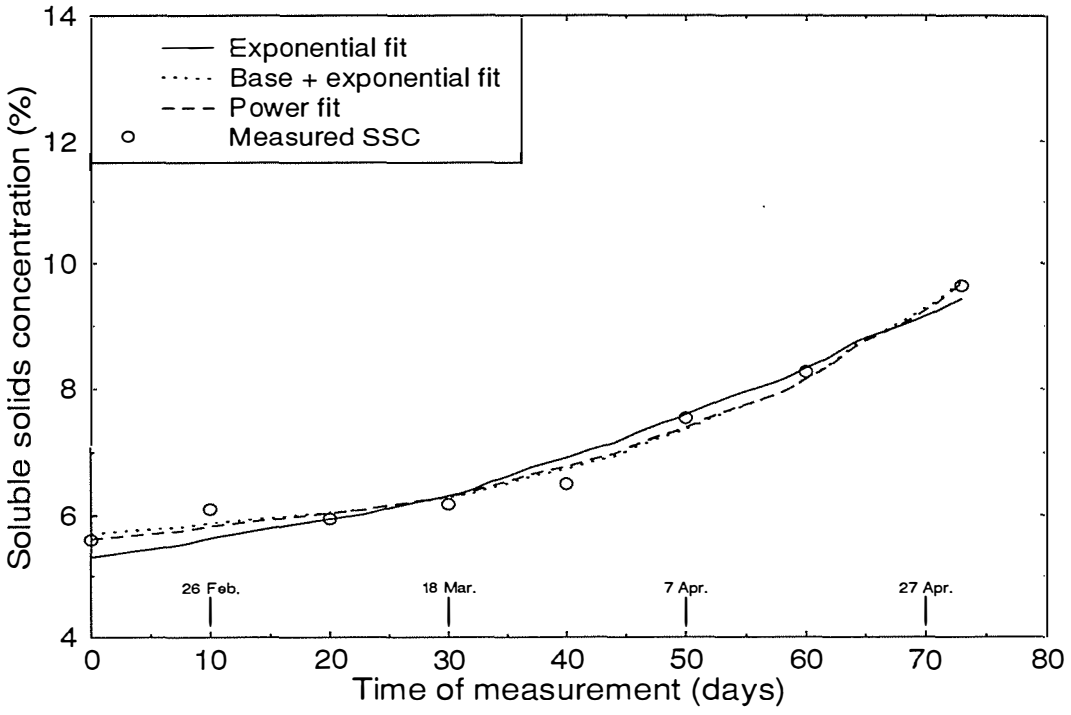


Fig. 5.13 Soluble solids concentration (%) of kiwifruit grown in the sheltered site (Experiment 2). Parameters from the base + exponential model were used to fit the exponential, base + exponential and power-law models. Parameters in Table 5.4.

Fitting of these parameters resulted in a better fit of the data than when parameters were transported directly from controlled environments (cf. Fig. 5.12). Results from the base + exponential and power-law models were similar and appeared to fit the data well, when judged by eye, whereas the exponential fit was less precise. As the exponential model was the least sensitive on several occasions (Figs 5.10, 5.12 and 5.13), it was discarded.

In conclusion, the fitted models and parameters from container-grown vines in controlled environments could not be applied directly to accurately predict SSC (first option) of fruit from container-grown vines in the sheltered site. When the temperature-dependent rate function was transported (second option), the fitting of some parameters was required. Hence the third option, of developing a totally different model was not necessary. In conclusion, the temperature-dependent rate function was successfully transported from container-grown vines in controlled environments to container-grown vines in the sheltered site but new parameters were required to adequately describe SSC accumulation. The exponential model to describe the kinetics of SSC accumulation, however, was discarded in favour of the base + exponential and power-law models as these appear more sensitive.

Data from orchards

Parameters from the base + exponential and power-law models from the modified Arrhenius equation (Table 5.3) were fitted to data obtained from two orchard locations (Palmerston North and Riwaka) where temperature records from the data logger were complete (Table 5.5). Both models fitted data from the two locations very well (Figs 5.14 and 5.15), even though measured SSC increased faster at Riwaka than at Palmerston North. The fits to data from the base + exponential model were virtually identical to those from the power-law model (Figs 5.10, 5.12, 5.13, 5.14 and 5.15); the base + exponential model was selected for all future analyses as only one model is required.

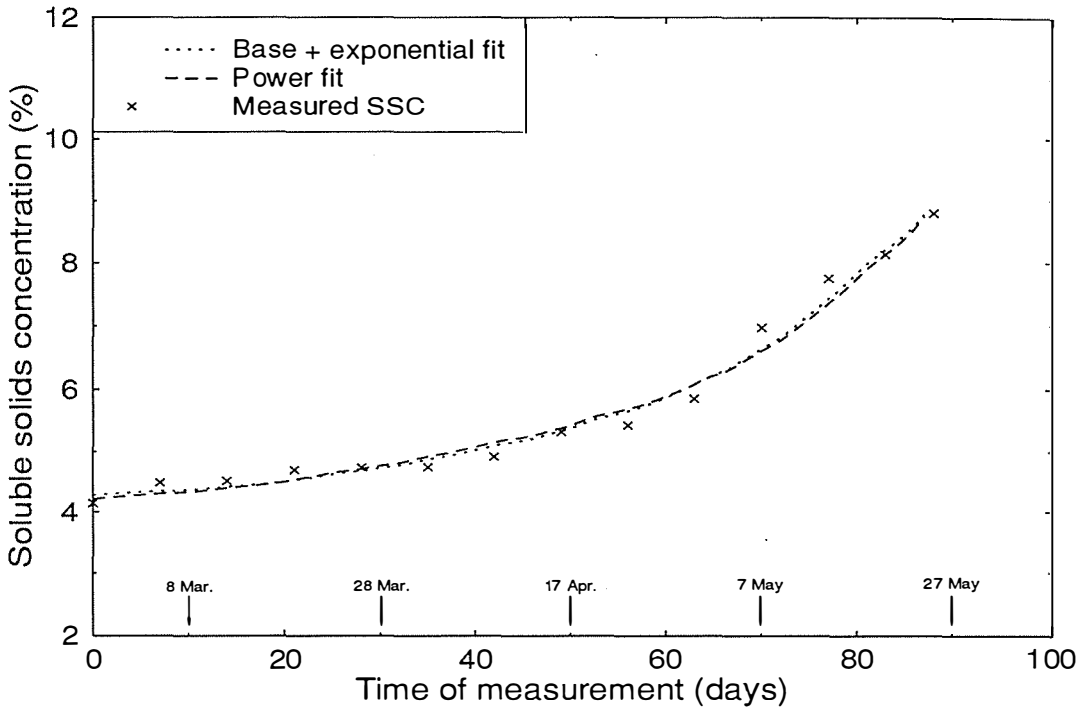


Fig. 5.14 Soluble solids concentration (%) of kiwifruit grown at Palmerston North and fitted with the base + exponential or power-law models. Parameters in Table 5.5.

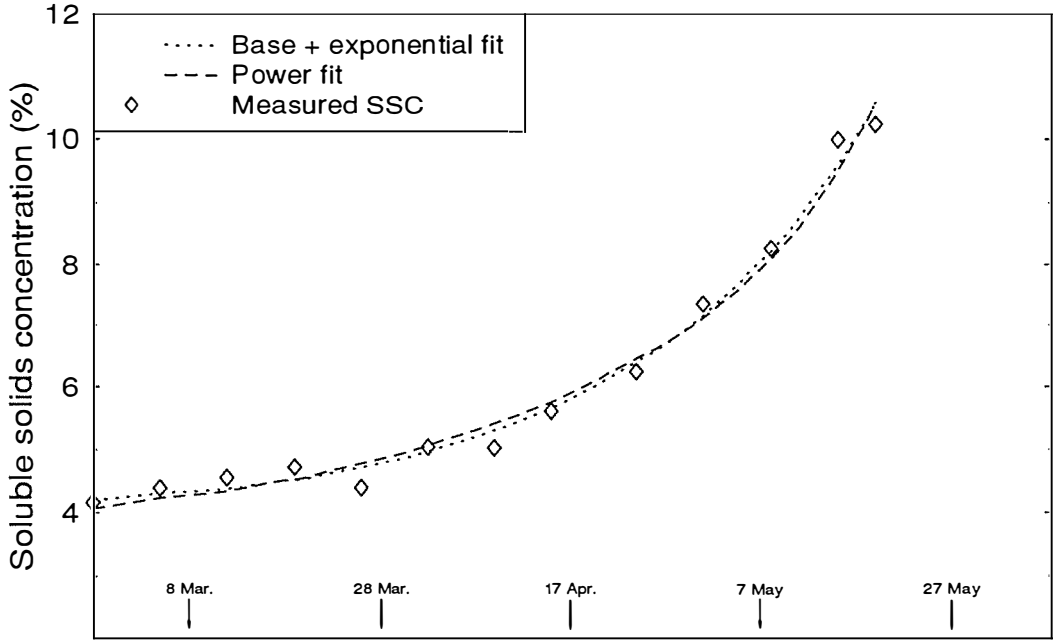


Fig. 5.15 Soluble solids concentration (%) of kiwifruit grown at Riwaka and fitted with the base + exponential or power-law models. Parameters in Table 5.5.

Table 5.5 Fitted parameters from the base + exponential and power-law models for Palmerston North and Riwaka (Figs 5.14 and 5.15), where S^0 = initial soluble solids concentration (%), S^b = basal soluble solids concentration (%), h_i^* = rate coefficient ($days^{-1}$) and α = power coefficient.

Orchard location	Parameters from base + exponential model		
	S^0 (%)	S^b (%)	h_i^* ($days^{-1}$)
Palmerston North	4.28	3.73	4.15×10^{-2}
Riwaka	4.19	3.71	4.89×10^{-2}
	Parameters from power-law model		
	S^0 (%)	α	h_i^* ($days^{-1}$)
Palmerston North	4.21	2.70	6.91×10^{-4}
Riwaka	4.06	2.30	1.64×10^{-3}

At this stage several conclusions can be drawn from the testing of the model:

i) the temperature-dependent rate function can be successfully transported from container-grown vines in controlled environments both to container-grown vines in the sheltered site and to field-grown vines in different geographical locations within New Zealand.

ii) even though the temperature dependence of the biochemical steps involved in SSC accumulation is not known, there appears to be an optimum temperature around 7 to 10C where these processes occur fastest. The biochemical steps are presumably slowed at temperatures less than 7C and greater than 10C.

iii) different fitted rate coefficients, basal SSC and power coefficients were required at the different orchards.

Use of maximum and minimum temperatures

The methods used thus far for fitting and testing the models are only feasible when temperature records are complete and frequent. The numerical integration

scheme described in A.5.6 estimates SSC at the end of each half-hour, based on temperatures measured during the half-hour. Therefore, temperature records must be complete during maturation for the method to work. This was not the case at Kerikeri and Te Puke where there were periods of missing data greater than one day. Also, the models cannot be used when temperature records are restricted to daily maximum and minimum temperatures as measured at meteorological stations. As continuous temperature records are often unavailable, the method of integration needs to be modified to ensure it can be used when only daily maximum and minimum temperatures are available. Modifications to the method of integration were developed (A.5.9) and used with SSC and temperature records collected from orchards at Kerikeri, Te Puke, Palmerston North and Riwaka in 1990 and tested on data collected at Te Puke and Riwaka in 1981.

Altering the model from using continuous temperature records to a daily maximum and minimum temperature is described in A.5.9. The time step in the integration needs to be changed from half-hours to days as only daily maximum and minimum temperatures are available. Instead of basing the integral on an unknown daily time course of temperature, a temperature-based average is used. This creates an error and necessitates introduction of a term, the daily correction value (k_d) to account for the error. The daily correction factor accounts for the error when a temperature-based mean is used in place of the daily integral.

The following equation shows that the exponent includes h^*, k_d and $\overline{g^*(T)}$, therefore h^* is a component of the rate coefficient and is now called a partial rate coefficient.

$$S(d) = S^{b+} + (S(d-1) - S^{b+}) \exp\left(h^* k_d \overline{g^*(T)}\right)$$

where $S(d)$ = SSC (%) at day d

S^{b+} = basal SSC (%) after maturation induction point

$S(d-1)$ = SSC (%) at previous day

h^* = partial rate coefficient (day^{-1})

k_d = daily correction factor (dimensionless)

$\overline{g^*(T)}$ = mean value of scaled temperature function (g^*) for maximum to minimum temperature (C) range. This is calculated using Simpson's rule (a simple method of integration), that is:

$$\overline{g^*(T)} = 1/6\,[g^*(T_{min}) + 4g^*(\overline{T}) + g^*(T_{max})$$

where $g^*(T)$ = scaled temperature-dependent rate function (dimensionless)

T_{min} = minimum temperature (C)

$\overline{T} = 0.5(T_{min} + T_{max})$ (C)

T_{max} = maximum temperature (C).

The partial rate coefficient takes into account most of the physiological differences between years, orchards and experiments.

Studies of the daily correction factor

Estimates of values for the daily correction factor are required to use the equation above. The daily correction factor was calculated and plotted against maximum and minimum temperatures, when data were available, from Kerikeri, Te Puke, Palmerston North and Riwaka for the whole study in 1990 (Fig. 5.16). There was a decrease in the daily correction factor during the studies at all orchard locations. Use of a time-based function is not ideal because it may differ between years. A temperature-based function may have a more direct relationship with SSC and be transportable between years. The daily correction factor was therefore regressed against mean temperature for each of the orchards (Fig. 5.17 and Table 5.6). The daily correction factor was either greater than, equal to, or less than 1.0 depending on mean temperature (Fig. 5.17) but, on average, it was close to 1.0, hence the low R^2 values in Table 5.6. As the daily correction factor is close to 1.0, this indicates that using it as a function of temperature may be suitable.

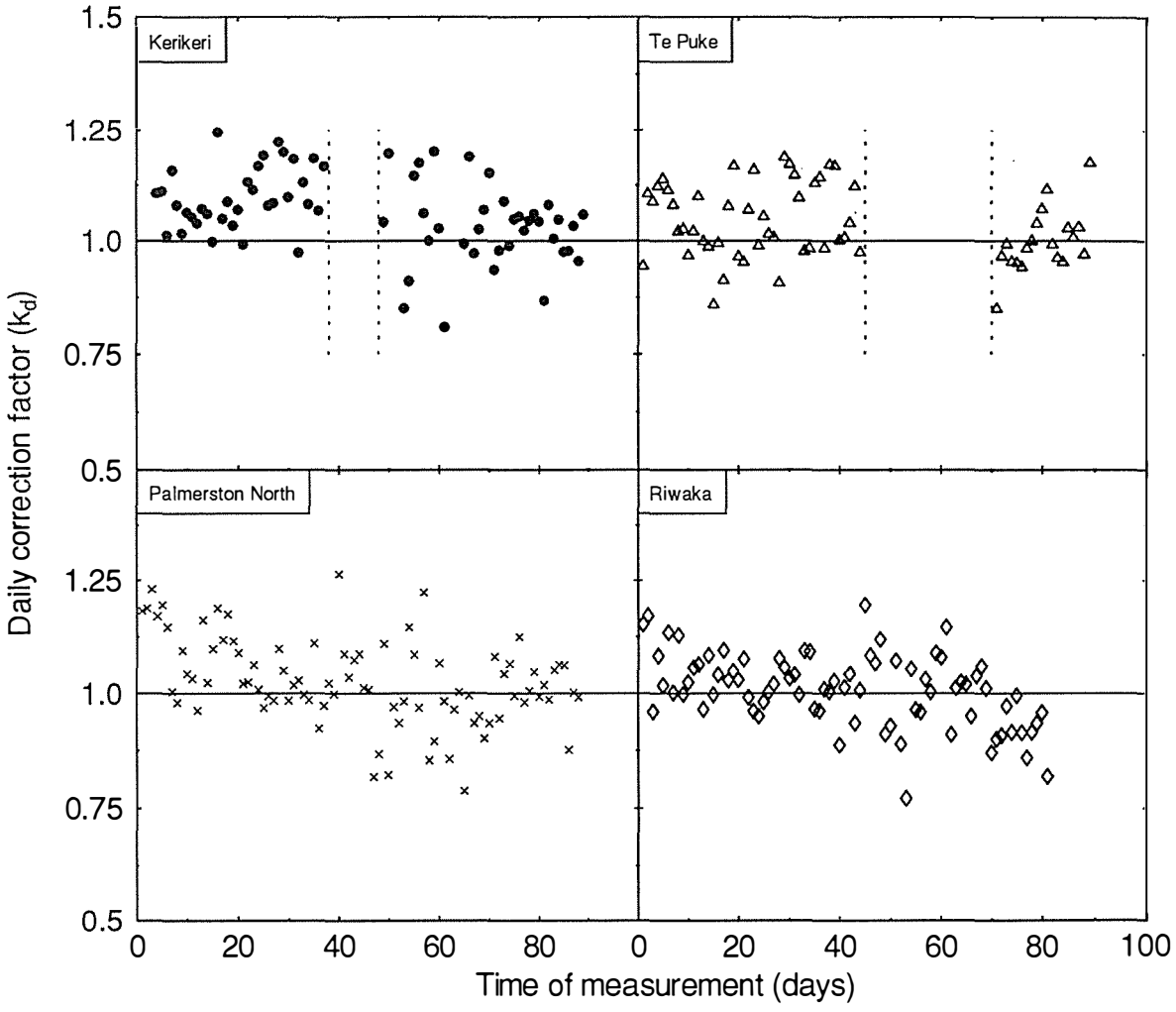


Fig. 5.16 Seasonal trend of the daily correction factor (k_d) at Kerkeri, Te Puke, Palmerston North and Riwaka. Missing data at Kerikeri and Te Puke between vertical lines.

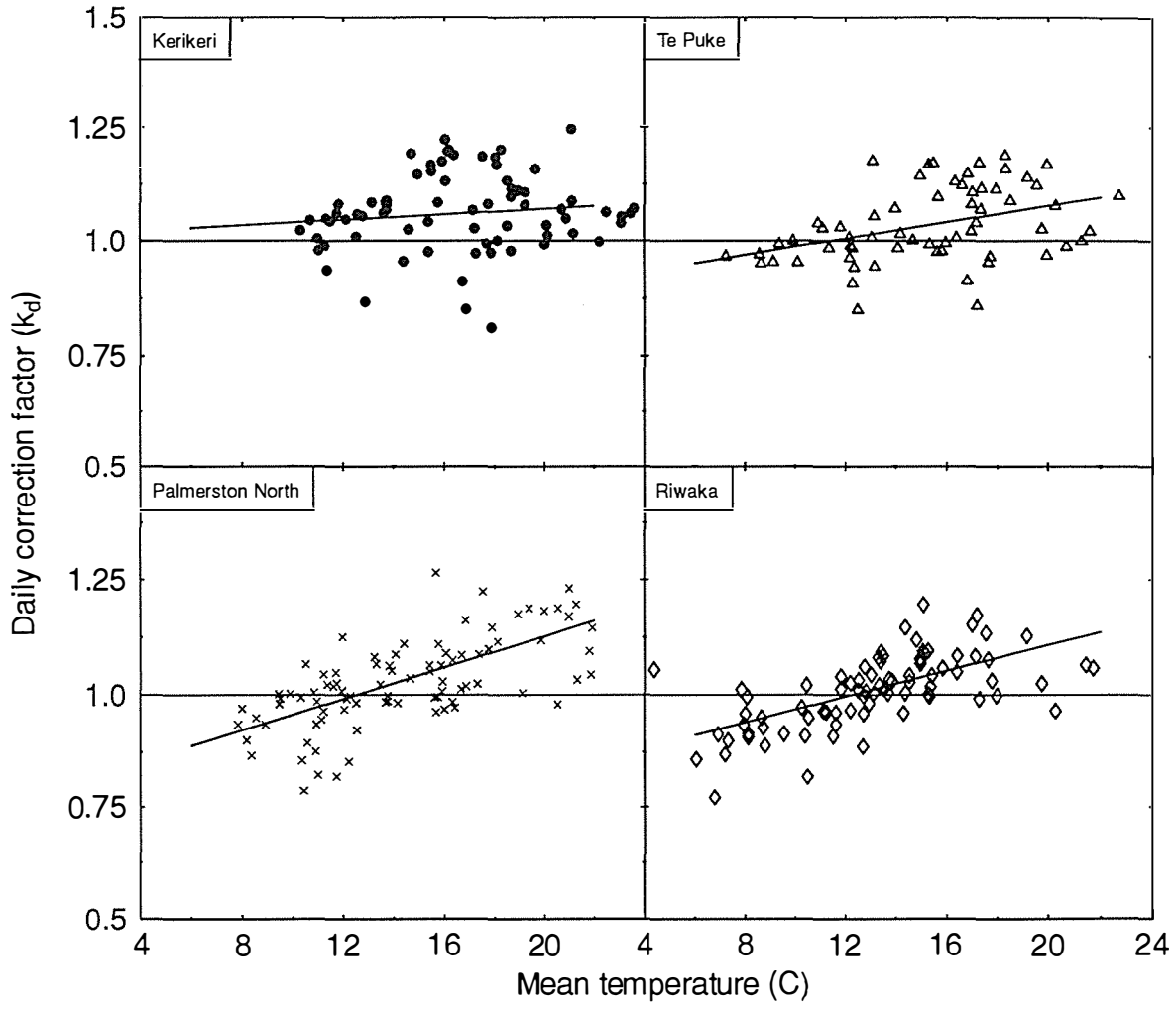


Fig. 5.17 Trend of the daily correction factor (k_d) with mean temperature at Kerikeri, Te Puke, Palmerston North and Riwaka. Regression coefficients in Table 5.6.

Table 5.6 Regression coefficients (b_0, b_1) and R^2 for the daily correction factor (k_d) versus mean temperature (C) at Kerikeri, Te Puke, Palmerston North and Riwaka (Fig. 5.17), where b_0 = intercept and b_1 = slope.

Orchard location	Coefficients		
	b_0	b_1	R^2
Kerikeri	1.010	0.003	0.02
Te Puke	0.897	0.009	0.16
Palmerston North	0.787	0.017	0.44
Riwaka	0.828	0.014	0.40

Testing of the daily correction factor at Palmerston North and Riwaka

The option of transporting the temperature-dependent rate function, including the daily correction factor, and fitting parameters from the base + exponential model was applied to data from Palmerston North and Riwaka. The temperature-dependent rate function was expected to be transportable from vines in controlled environments to those in orchards as shown previously from data with continuous temperature records. Fitting of

i) the partial rate coefficient *(h^*)*,
ii) the partial rate coefficient and basal SSC *(S^b)* or
iii) the partial rate coefficient, basal SSC and initial SSC *(S^0)*

resulted in similar fitted SSC at Palmerston North (Fig. 5.18) and Riwaka (Fig. 5.19). Hence it was decided to use the simplest option, that is, to fit only one parameter - the partial rate coefficient. So far, the daily correction factor obtained from the regression against mean temperature has been used (Table 5.6). However, the model may be further simplified by using a value of 1.0 instead. Comparison of use of regression coefficients or 1.0 for the daily correction factor for Palmerston North and Riwaka showed that there was little difference between the two fitted curves of SSC (Figs 5.20 and 5.21). Again, the simplest option (k_d = 1.0) was chosen for the remaining work.

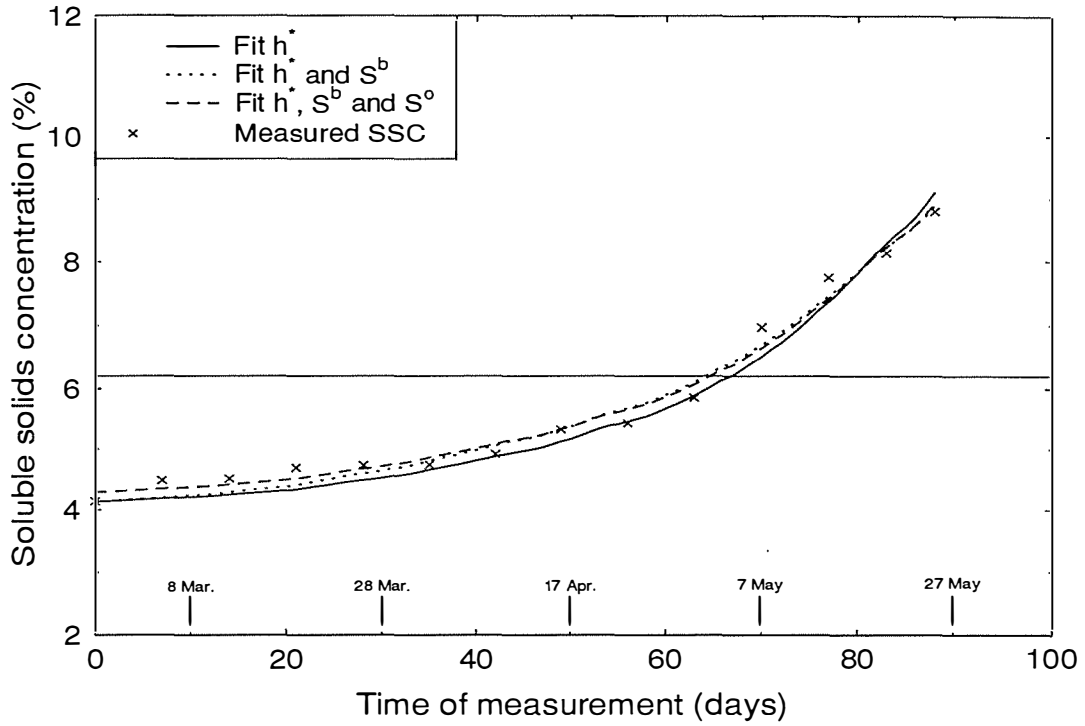


Fig. 5.18 Soluble solids concentration (%) of kiwifruit grown at Palmerston North and fitted with one, two or three parameters (h^*, S^b, S^o) and use of regression values for k_d (0.787 + 0.017 $\overline{T}$). Parameters in Table 5.5.

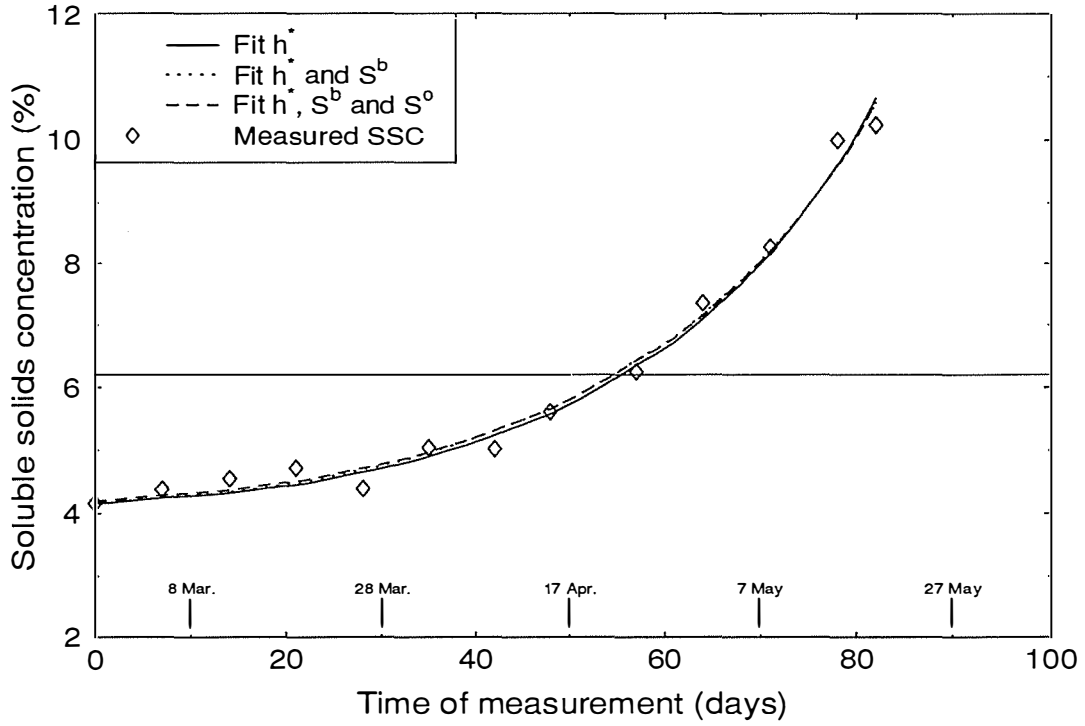


Fig. 5.19 Soluble solids concentration (%) of kiwifruit grown at Riwaka and fitted with one, two or three parameters (h^*, S^b, S^o) and use of regression values for k_d (0.828 + 0.014 $\overline{T}$). Parameters in Table 5.5.

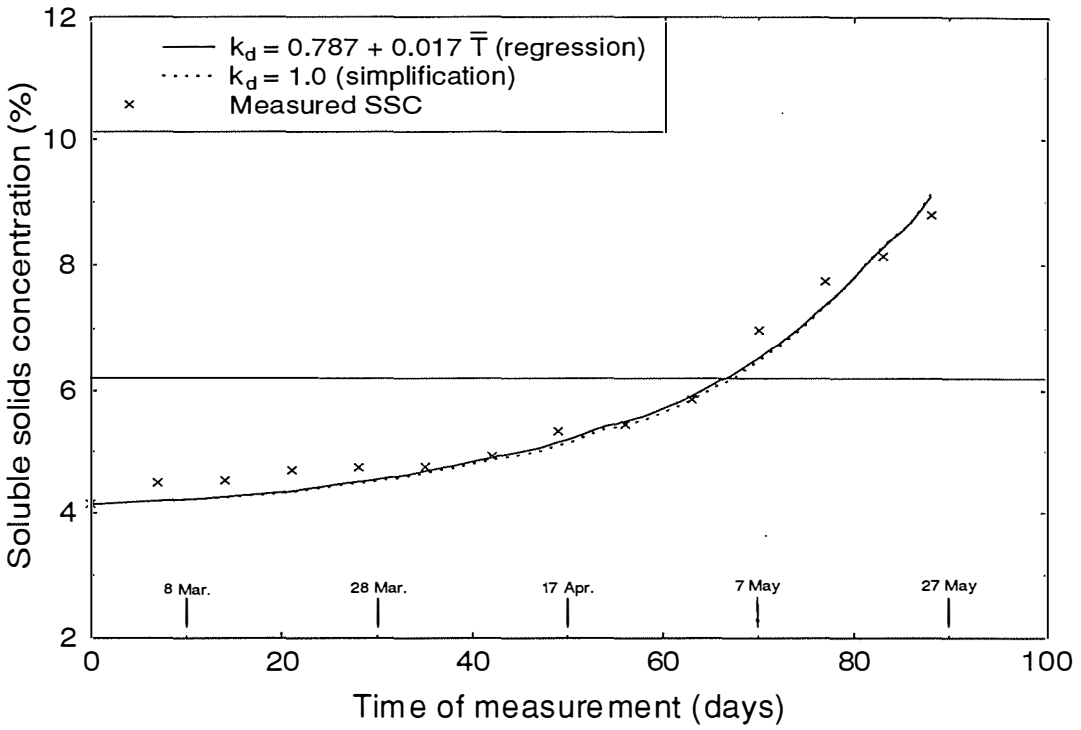


Fig. 5.20 Soluble solids concentration (%) of kiwifruit grown at Palmerston North and fitted with one parameter (h^*) ($h^* = 4.66 \times 10^{-2}$) and different values for the daily correction factor (k_d).

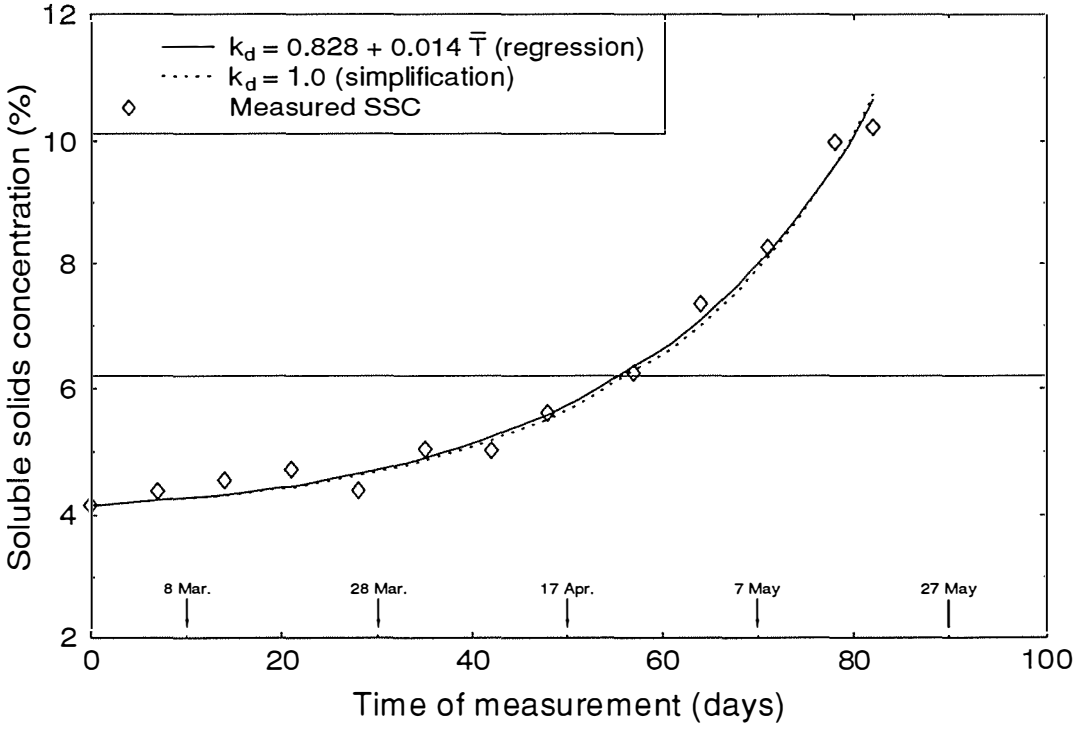


Fig. 5.21 Soluble solids concentration (%) of kiwifruit grown at Riwaka and fitted with one parameter (h^*) ($h^* = 4.70 \times 10^{-2}$) and different values for the daily correction factor (k_d).

Application of the daily correction factor to Kerikeri and Te Puke

The main test of the model was to apply it to data from Kerikeri and Te Puke, where the only temperature records available were daily minimum and maximum temperatures. As explained above, only one parameter (partial rate coefficient) was required as the daily correction factor was set at 1.0. The partial rate coefficient was used in two ways, either fitted or used as a parameter in the model. The value of the partial rate coefficient when used as a parameter was calculated as a mean from the values from fitted data from Palmerston North and Riwaka (Figs 5.20 and 5.21), that is, $h^* = 4.68 \times 10^{-2}$. Simulation of SSC from a fixed value of the partial rate coefficient was not a suitable method as simulated SSC was too high at both Kerikeri (Fig. 5.22) and Te Puke (Fig. 5.23). However, fitting the partial rate coefficient resulted in a good simulation of SSC compared to the measured SSC at both orchards (Figs 5.22 and 5.23). Again, this shows that the second option was appropriate; that the fitted models apply, but a new parameter was required for the partial rate coefficient.

Fitted values of the partial rate coefficient were lower at Te Puke and Kerikeri (4.12 and 4.20×10^{-2}, respectively) compared to 4.66 and 4.70×10^{-2} at Palmerston North and Riwaka, respectively. However, fitted values of the partial rate coefficient did not correspond to increase in SSC at each orchard location (Fig. 3.2). The partial rate coefficient encompasses all the physiological differences between years, orchards and regions, hence factors other than temperature may also influence the partial rate coefficient, such as daylength, daily light integral (sunshine hours) and rainfall.

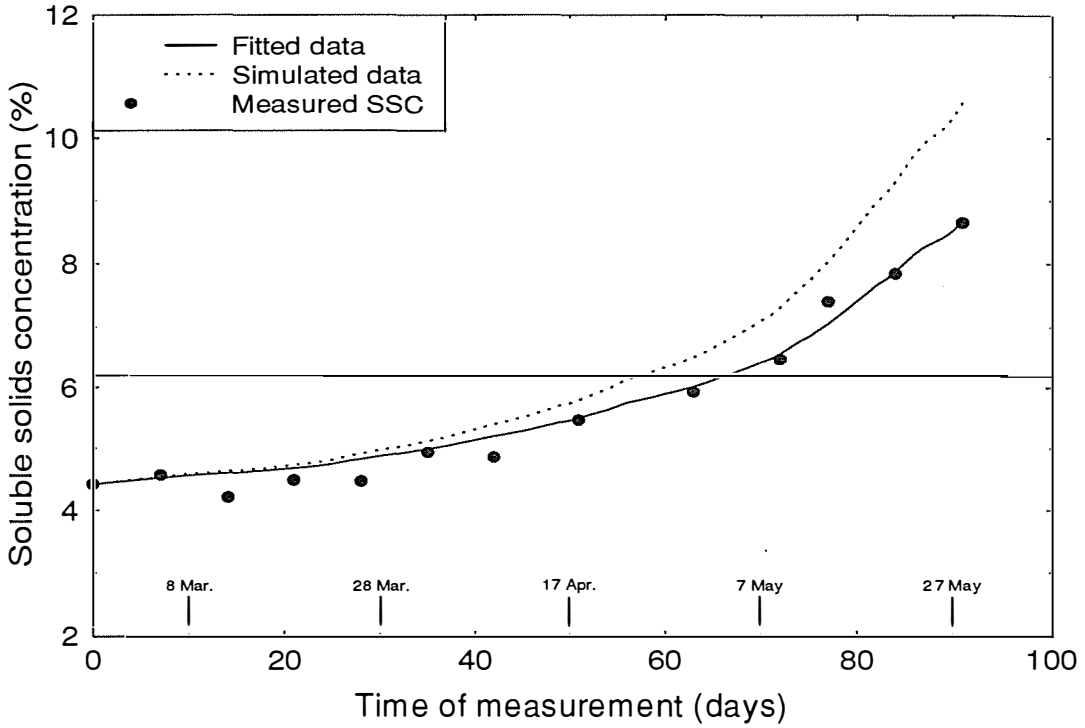


Fig. 5.22 Soluble solids concentration (%) of kiwifruit grown at Kerikeri and simulated with one parameter (h^* = 4.68 x10^{-2}, mean h^* from Palmerston North and Riwaka) or fitted with one parameter (h^* = 4.20 x10^{-2}).

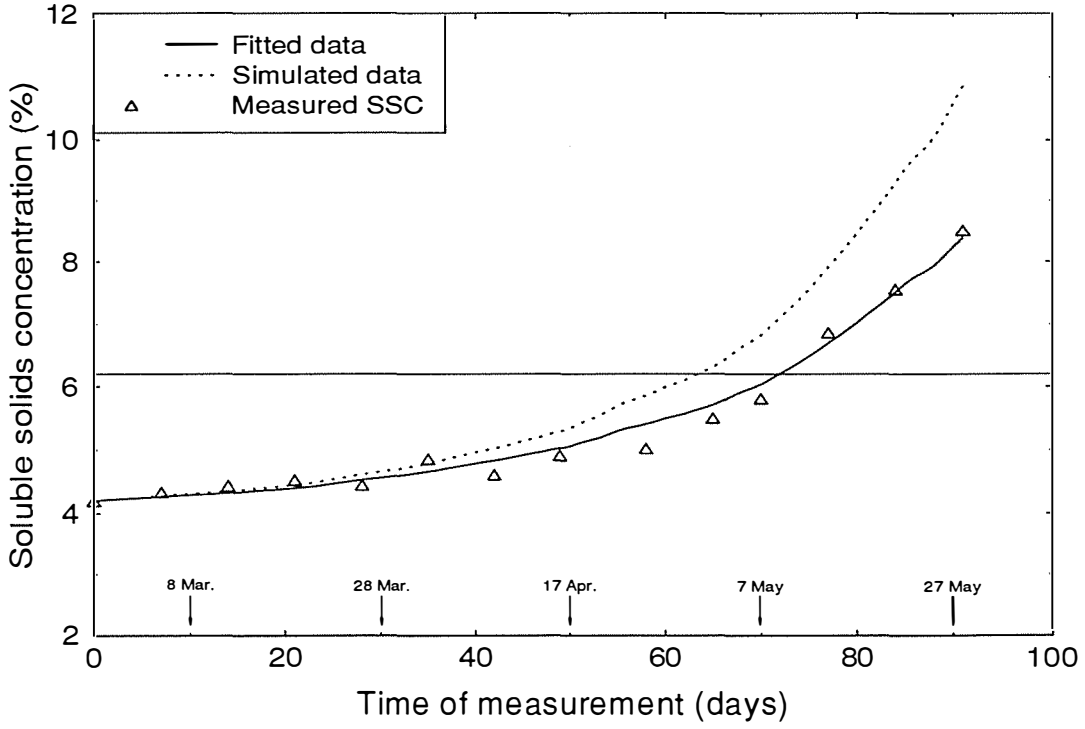


Fig. 5.23 Soluble solids concentration (%) of kiwifruit grown at Te Puke and simulated with one parameter (h^* = 4.68 x10^{-2}, mean h^* from Palmerston North and Riwaka) or fitted with one parameter (h^* = 4.12 x10^{-2}).

In summary, the basic equation for the base + exponential model used for field-grown vines at Palmerston North is:

$$S(d) = 3.73 + (S(d-1) - 3.73) \exp\left(4.66\, x10^{-2}\, \overline{g^*(T)}\right)$$

where the components are made up as follows:

S(d) = current SSC (%) on day *(d)* of measurement

3.73 = basal SSC (%)

S(d-1) = SSC (%) at previous day

4.66 $x10^{-2}$ = the partial rate coefficient (day^{-1}). This was 4.12, 4.20 and 4.70 $x10^{-2}$ for Te Puke, Kerikeri and Riwaka, respectively.

$\overline{g^*(T)}$ = the mean value of scaled temperature function (g^*) for maximum to minimum temperature (C) range (Section 5.3.2 Use of maximum and minimum temperatures).

A further test of the transportability of the partial rate coefficient was possible with data from four different orchards in Te Puke and six near Riwaka, but collected in a different year (1981). Mean SSC was used as the starting point (day 0) for the simulation as the reliability of the model is dependent on the starting point. The fitted partial rate coefficient from Te Puke and Riwaka was used to simulate SSC at the four orchards (Figs 5.24 and 5.25). The prediction of increase in SSC at orchards in Te Puke and Riwaka was good. This result is important because it indicates that the partial rate coefficient may be transportable between years within a region. In terms of the practical use of the whole model, it appears that the partial rate coefficient is the only parameter that is required to be fitted, but that once it has been found for a region it may apply to any orchard within that location.

The predictive capacity of the model developed in this chapter was shown when it was used to predict harvest date at Te Puke and Riwaka in a different year (1981). However, to accurately predict harvest date for kiwifruit, a sensitivity analysis is required using historical records of maximum and minimum temperatures and SSC measurements. In addition, interpolation of these temperature records is necessary between the orchard location and closest meteorological station.

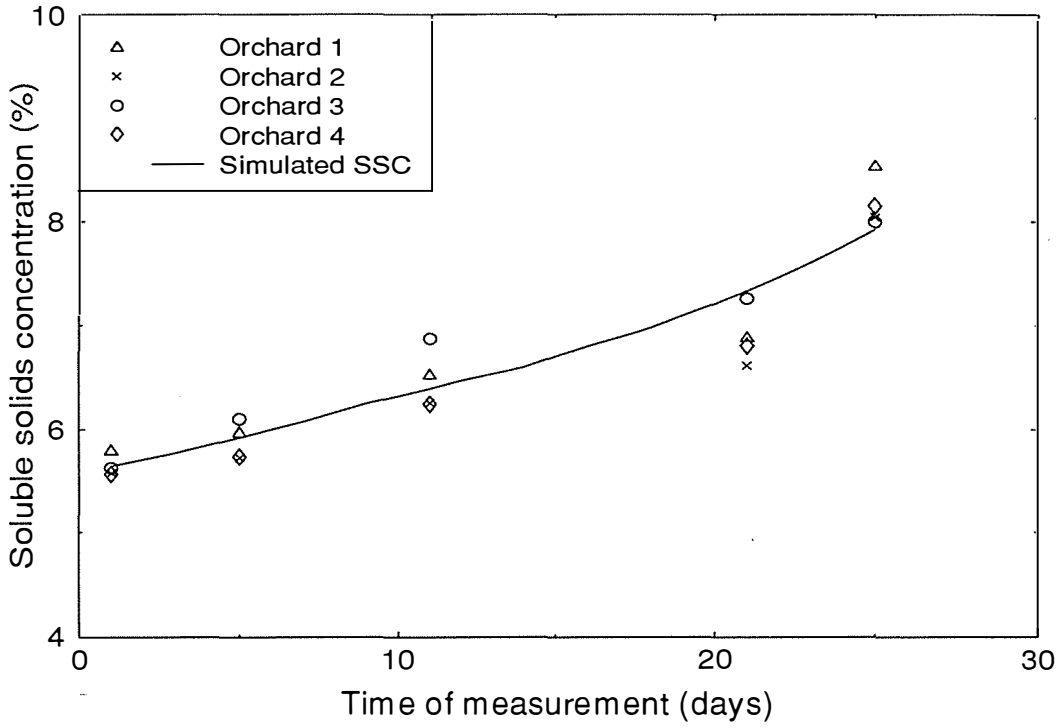


Fig. 5.24 Soluble solids concentration (%) of kiwifruit grown at four different orchards in Te Puke (1981) simulated using fitted parameters from Te Puke ($h^* = 4.12 \times 10^{-2}$).

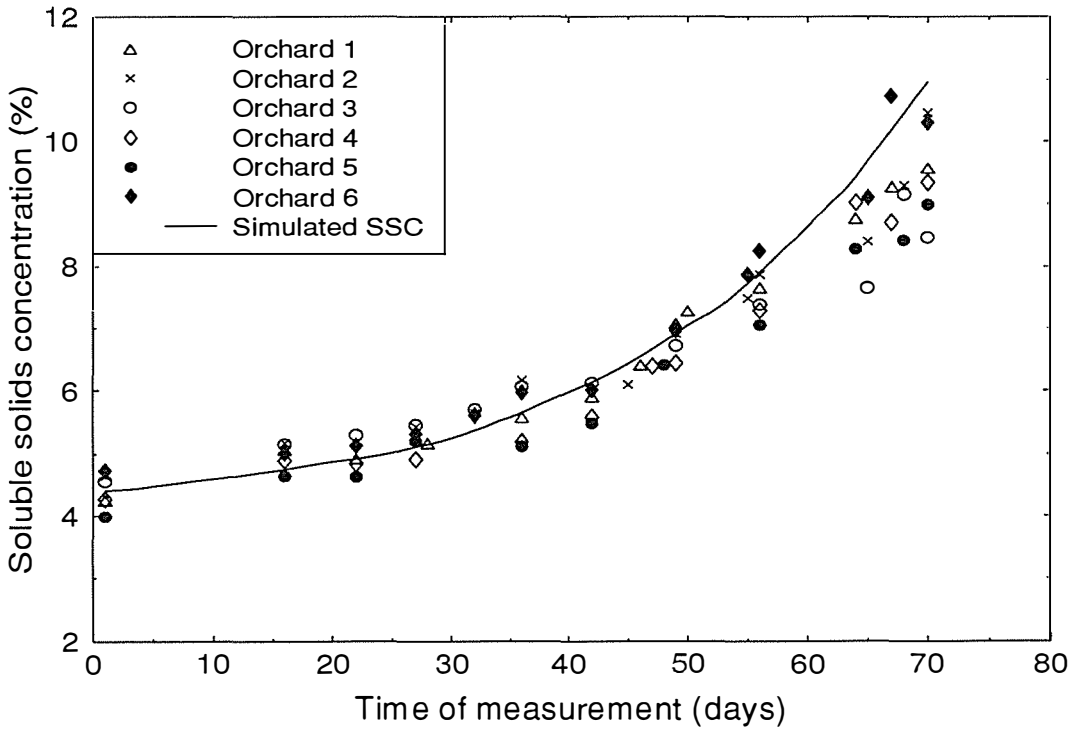


Fig. 5.25 Soluble solids concentration (%) of kiwifruit grown at six different orchards in Riwaka (1981) simulated using fitted parameters from Riwaka ($h^* = 4.70 \times 10^{-2}$).

5.4 Summary and conclusions

The model developed to predict the rate of change in SSC during kiwifruit maturation is made up of two components. Firstly, the state-dependent physiological response function and secondly the temperature-dependent rate function. The overall model was developed using combined data from container-grown vines in two controlled environment studies. The base + exponential and power-law models both fitted data equally well. However, the base + exponential model was chosen to represent the function of SSC and a temperature-dependent rate function was selected based on a modified Arrhenius equation. This model was then tested on data from similar container-grown vines in the sheltered site and on field-grown vines at a range of contrasting sites.

The temperature-dependent rate function from controlled environments was successfully transported to fit SSC measured from similar container-grown vines grown in the sheltered site exposed to uncontrolled ambient temperatures. A similar approach also resulted in good simulation of SSC in field-grown vines during maturation. These results showed the benefits of using controlled environments to obtain the temperature function which could then be applied to vines in other situations. Initial SSC was different between container-grown and field-grown vines, but the transportability of the temperature-dependent rate function showed that increase in SSC during kiwifruit maturation was similar, even though the measured values were different.

The temperature-dependent rate function was modified to use daily maximum and minimum temperatures, allowing greater practical use of the model for prediction of SSC without affecting fit of data. This is very significant as only maximum and minimum temperature records are available from standard meteorological stations. There were differences among locations in the value of the partial rate coefficient, hence the best method was to fit the model for each region. Transportability of the partial rate coefficient between years in the same region was shown for data from Te Puke and Riwaka. This shows that the model has

great potential for prediction of harvest date in different regions and seasons, but access to additional data for regions in different years will be necessary to further validate the model.

The model developed and used to describe prediction of harvest date in kiwifruit in this chapter has a stronger physiological basis than models used by Salinger and Morley-Bunker (1988), Kempler *et al.* (1992) and Snelgar *et al.* (in prep.). These other models are based simply on a linear relationship between mean rate of increase in SSC and mean temperatures. For example, Kempler *et al.* (1992) calculated daily heat-units with a 5C base temperature, but gave no justification for selection of 5C as a base temperature. The analysis in this chapter suggests that increase in SSC can occur at temperatures below 5C. From a physiological perspective, it is unlikely that inactivation of enzymes in biochemical pathways will occur at exactly 5C; inactivation is more likely to be gradual with a decrease in temperature. Base temperatures have been used for other crops, for example, 15C for guava (Bittenbender and Kobayashi, 1990) and a similar physiological argument may apply.

Salinger and Morley-Bunker (1988) and Snelgar *et al.* (in prep.) based their approaches on mean rate of increase in SSC between 5.0 and 6.5% regressed against mean temperature for that period. The regressions showed an increased rate of increase in SSC with decrease in temperature which are similar to results from controlled environment studies (Chapter 2). Snelgar *et al.* (in prep.) found that 73 and 48% of the variation could be accounted for by the regression in 1981 and 1982, respectively. However, the temperature coefficients for the two years were significantly different, resulting in a model where the precision of prediction is likely to be poor. The form of the model used by Snelgar *et al.* (in prep.) is not appropriate as this study clearly showed that change in SSC is curvilinear with temperature. These authors concluded that i) factors other than temperature may influence rate of increase in SSC and ii) that the form of the model relating rate of increase in SSC to temperature may not be appropriate. Use of a curvilinear model as in this study allowed prediction of increase in SSC

both among different orchard locations and between two different years. The present study also suggests that factors other that temperature influenced rate of increase in SSC as there were differences in the partial rate coefficient among the four orchard locations studied. This also justifies use of a more complex model than that by Salinger and Morley-Bunker (1988) and Snelgar *et al.* (in prep.) for description of the effect of temperature on increase in SSC in kiwifruit.

While Salinger and Morley-Bunker (1988) and Snelgar *et al.* (in prep.) developed models to describe the effect of temperature on increase in SSC in kiwifruit, they did not attempt to predict the date of harvest. Kempler *et al.* (1992) did predict the number of days from anthesis to maturity, but with the same data that was used to derive the model, and hence it was not a true predictive test. In this study, the model developed was used to predict harvest date at two orchard locations in a different year to that used in development of the model. However, substantial further work is required to validate the predictive capacity of this model. Prediction of SSC appears to be possible for orchards from different regions once the partial rate coefficient has been determined and where daily maximum and minimum temperatures are available. Prediction of harvest date would give the Kiwifruit Marketing Board, growers, packaging companies, shippers and allied support industries a greater certainty of the timing of harvest for kiwifruit.

CHAPTER 6

GENERAL DISCUSSION

6.1 Introduction

The primary objective of this study was to determine the effect of temperature on the rate of SSC and carbohydrate changes in kiwifruit during maturation. Early studies by Reid *et al.* (1982a) and Harman (1981), described changes in SSC and flesh firmness during maturation, but did not attempt to evaluate the effect of temperature on these processes. Considerable interest has been shown in SSC and carbohydrate changes during maturation, initially to discover the rates of these changes (Okuse and Ryugo, 1981; Reid *et al.*, 1982a). Later investigations examined differences in maturation at different orchard locations (MacRae *et al.*, 1989a) but again, the effect of temperature on these processes was not considered.

Recently, Snelgar *et al.* (1988) developed relocatable greenhouses; when placed over field-grown kiwifruit vines allowed the effect of temperature during growth, development and maturation to be evaluated (Hopkirk *et al.*, 1989). Such studies while valuable, are limited because it was only possible to increase air temperature around vines in such greenhouses by 3 to 4C above ambient conditions. To fully evaluate the influence of temperature on kiwifruit maturation, and to be able to develop an accurate predictive model of increase in SSC during maturation, a more accurate determination of the response of kiwifruit to a wide range of temperature conditions is required. Controlled environments allow the effect of temperature on kiwifruit maturation to be investigated over a wide range of temperature treatments, and also allow SSC and carbohydrate changes during development to be quantified. Results showed that the fastest rate of increase in SSC and total sugar concentration and decrease in starch concentration occurred at the coolest mean temperatures. In addition, temperature was found to effect

an increase in SSC only when a critical, as yet unidentified, stage of development had been reached.

Another aim of this study was to alter carbohydrate status of field-grown fruit and to determine if this affected rate of accumulation of SSC and fruit maturation. It should be possible to alter carbohydrate status in fruit by manipulation of leaf:fruit ratio, which can markedly influence fruit weight (Snelgar *et al.*, 1986; Lai *et al.*, 1989; Woolley *et al.*, 1992) and SSC (Snelgar and Thorp, 1988). Other changes that occur in kiwifruit during maturation, such as flesh firmness, starch and sugar concentrations were not measured in previous studies. In the present work, increase in SSC was delayed in fruit with a low compared to a high carbohydrate concentration.

Other aims were to obtain temperature records and details of kiwifruit maturation from four orchards at different geographical locations, selected to provide a range of temperatures, and to use this data to develop a mathematical model to relate changes in SSC to temperature. Previously, various components of maturation of field-grown fruit have been compared among orchards (MacRae *et al.*, 1989a; Walton and de Jong, 1990a), but temperatures were either not recorded or were not used for incorporation into a model to predict harvest date. A linear relationship between mean temperature and increase in SSC has been proposed as a method to predict harvest date in kiwifruit (Salinger and Morley-Bunker, 1988; Kempler *et al.*, 1992; Snelgar *et al.*, in prep.). However, the present study has shown that the relationship between mean temperature and increase in SSC was curvilinear, hence a linear approach is unlikely to permit sufficient accuracy or precision in prediction of harvest date (Snelgar *et al.*, in prep.). The nonlinear approach adopted in the current study allowed increase in SSC of kiwifruit to be described at four orchards at different geographical locations with a reasonable degree of accuracy.

The remaining part of this chapter discusses several issues in greater detail than has been possible in previous chapters. These include:

i) physiological reasons for smaller fruit and higher SSC in fruit from container-grown vines compared to fruit from field-grown vines
ii) a possible mechanism to account for the induction of maturity in fruit
iii) the possible biochemical pathways involved in increase in sugar and decrease in starch concentrations at low temperatures
iv) the strength and weaknesses of the mathematical model developed to predict increase in SSC.

6.2 Differences between container-grown and field-grown vines

The main differences between container-grown and field-grown vines were smaller fruit and higher SSC in fruit from container-grown vines. Fruit typically weighed 40 to 75 g from container-grown vines compared to 69 to 129 g from field-grown vines. Soluble solids concentration of container-grown vines was 1 to 1.5% higher at the beginning of each controlled environment experiment than for field-grown vines at a similar date. Similarly, fruit from container-grown vines placed in an orchard next to field-grown vines also had higher SSC (G. Lupton, HortResearch, Riwaka, pers. comm.). Fruit from vines grown in containers in a sheltered site, pruned to have a main stem 1 m high with 4-5 fruiting canes and irrigated with Hoagland's A solution will not necessarily be identical to fruit from field-grown vines where the training system, pruning and available nutrients are different. The reasons for the difference in SSC between container-grown and field-grown vines were not investigated, but a possible explanation for the differences may, in part, be due to restriction of the root system and a high leaf:fruit ratio.

Restriction of roots for example, by growing the plant in a pot, does not allow the root system to spread beyond the soil volume in that pot (Geisler and Ferree, 1984). Richards and Rowe (1977a, 1977b) restricted one-year-old peach seedlings in small containers and plant size decreased due to a reduction in leaf number and area and shoot length. Such seedlings developed a reduced number

of roots and shorter roots than unrestricted plants. A direct relationship was found between the size of the root system and size of the above ground portion of the plant. The regulation of top growth may be determined by the production of hormones from the root apices (Richards and Rowe, 1977a) as cytokinins are produced in roots and translocated to shoots (Mika, 1986). Application of a foliar spray of the synthetic cytokinin (6-benzylaminopurine) overcame the reduced growth of restricted plants which suggests that production of cytokinin from root tips exerts considerable control over top growth (Richards and Rowe, 1977a, 1977b). Additional evidence for the effect of cytokinins was shown by another synthetic cytokinin, CPPU (N-(2-chloro-4-pyridyl)-N-phenylurea), which increased fruit size of kiwifruit when applied directly to fruit or as a foliar spray (Henzell *et al.*, 1992; Lawes *et al.*, 1992; Lötter, 1992).

Assuming that root restriction occurred in the container-grown kiwifruit vines, it would be expected that both the number of root tips and root length would be reduced, resulting in a reduction in cytokinin production. Furthermore, it is likely that a reduction in cytokinin would result in smaller fruit compared to those from field-grown vines where root growth is unrestricted.

Fruit growth depends on the ability of fruit to compete with concurrent vegetative growth for a supply of assimilates from leaves (Lai *et al.*, 1989). Girdling of laterals has been used as a technique to alter the leaf:fruit ratio in kiwifruit, and hence alter fruit growth. On girdled laterals, an increase in leaf area resulted in an increase in fruit weight (Snelgar *et al.*, 1986; Snelgar and Thorp, 1988; Lai *et al.*, 1989). High SSC was associated with a high leaf:fruit ratio on girdled laterals (Snelgar and Thorp, 1988) and was higher in the 5:1 than the 1:1 leaf:fruit ratio treatment in the study reported in this thesis. The minimum leaf:fruit ratio required to support maximum fruit growth in the kiwifruit lateral was 2:1 (Lai *et al.*, 1989). Container-grown vines have a leaf:fruit ratio of approximately 10:1.

The high leaf:fruit ratio in container-grown vines may affect SSC in fruit and account for higher SSC when compared to field-grown vines. Photosynthates flow

from the source (leaves) to the sink (fruit) down a concentration gradient. A leaf:fruit ratio of 10:1 means there is a high ratio of source to sink. Total (non-structural) carbohydrate was higher in fruit from container-grown than field-grown vines, being 49 and 81 mg gFW^{-1} at the beginning of Experiments 2 and 3, respectively compared to 39 mg gFW^{-1} in field-grown vines. By the end of the experiment the concentrations had risen to 96 and 93 mg gFW^{-1} from Experiments 2 and 3, respectively and 67 mg gFW^{-1} in field-grown vines. In this situation it is possible that the sinks are filled to near capacity in fruit from container-grown vines, resulting in a low gradient between the source and sink. If leaves cannot release the photosynthates produced then they will also fill with starch. Unfortunately starch concentration in leaves was not measured in this study. However, Schupp *et al.* (1992) found that if the sink (blossom) was removed from apple trees then leaves had a higher starch concentration than those from apple trees where blossom was not removed. Starch concentration also increased in leaves of pot-bound tobacco (*Nicotiana tabacum* L.) plants, but decreased rapidly after transfer of the root restricted plants to larger pots (Herold and McNeil, 1979). Increased starch concentration in leaves of pot-bound tobacco plants may be the result of the lowered requirement for photosynthates when growth was restricted.

Snelgar *et al.* (1988) suggested that results obtained from container-grown vines in phytotrons may not be directly applicable to kiwifruit vines in a commercial orchard. The small size of fruit and higher SSC in container-grown than field-grown vines may result from both a restricted root volume and high leaf:fruit ratio. Nevertheless, fruit from container-grown vines appeared to be typical of field-grown vines during maturation in other respects, such as decrease in flesh firmness and starch concentration, increase in sugar concentration, fresh weight and dry weight. Also, the temperature-dependent rate function from the mathematical model for container-grown vines in controlled environments was successfully fitted to the increase in SSC in field-grown vines. Therefore, this study clearly showed that results obtained from container-grown vines placed in

controlled environments were applicable to field-grown vines, given that there may be differences in the magnitude of specific responses.

6.3 Induction of maturation

Soluble solids concentration of kiwifruit increased irrespective of whether vines were grown in containers or in the field. The rate of SSC increase was temperature-dependent, with the fastest rate of increase occurring at the coolest mean temperature. However, in one controlled environment study designed to test the influence of alternating temperature, it was found that fruit did not respond to any temperature treatment imposed. These fruit were found to have an initial SSC about 0.5% lower than fruit measured in previous studies. In addition, SSC was higher at the proximal than distal ends of fruit for the majority of measurements and seed colour was white at the beginning of the study, gradually changing to brown and black with succeeding measurements. A similar effect was found in a subsequent study when fruit were subjected initially to 7 days of cool temperatures, but SSC increased in response to cool temperatures when a second perturbation was applied 22 days later. By this stage, SSC had increased by 0.5% in both the fixed temperature and temperature perturbation treatments. It is suggested that fruit which did not respond to the perturbations to cool temperatures may have been physiologically immature and had not reached a stage of development where they could respond to external stimuli, such as a decrease in temperature. Salinger and Morley-Bunker (1988) claimed that SSC increased linearly with a decrease in temperature once a threshold concentration of 5 to 5.5% had been exceeded. It is possible that a specific carbohydrate concentration in the fruit has to be exceeded before maturation can be induced. However, there are also likely to be prioritised requirements for carbohydrates, creating gradients within each fruit. If this happens, fruit maturation as a whole may only proceed when available carbohydrates have fulfilled the priority requirements of the fruit for maintenance respiration and growth, development and maturation of seeds.

Physiologically, this may be regarded as a change or switch from the immature to mature stage. The concept of a threshold or critical point in the developmental process, after which cool temperatures could induce an increase in SSC, was used in the modelling work to describe the difference between the two components of SSC; the basal and maturation components. In this thesis it has been proposed that the process of maturation is induced at some point in the developmental pathway of kiwifruit; the precise cause or development stage of this induction point is unknown. The decrease in starch and increase in sugar concentrations measured in this study during maturation of kiwifruit have shown that hydrolysis of starch sometimes commences after the increase in sugar concentration. The two processes may therefore not be concomitant, but the increase in sugar concentration may be due to direct inflow of photosynthate (sucrose) from leaves or some other factor. Only relatively simple carbohydrate concentrations were measured in this study, but there are many other molecular and biochemical changes occurring in fruit during maturation and ripening. An obvious candidate to be considered for involvement in induction of the maturation process is ethylene. Ethylene is a plant hormone that influences development and in particular has been implicated in controlling fruit ripening (Brady, 1987).

During maturation there is a progressive decrease of resistance to ethylene action (or increase in sensitivity to ethylene action) and this process is thought to be controlled by ripening inhibitors, availability and/or activity of ethylene receptors (Yang, 1987). The sensitivity of banana fruit to ethylene changes during fruit maturation (Peacock, 1972). Two systems of ethylene production were proposed by McMurchie *et al.* (1972). System I is common to nonclimacteric and climacteric fruit until ripening, where a low concentration of ethylene is present in fruit. System II operates in climacteric fruit only and is the autocatalytic burst of ethylene production accompanying the ripening process. The low level of system I ethylene gradually destroys the ripening inhibitor which either suppresses the development of system II receptor or interferes with ethylene binding to the receptor (Yang, 1987). As the ripening inhibitor is inactivated, fruit tissue develops a functional system II receptor and hence, the autocatalytic

production of system II ethylene and synthesis of ripening-specific enzymes. Thus, there is a transition from a nonclimacteric tissue which lacks the system II receptor to a climacteric tissue, where system II receptor develops.

In kiwifruit, a marked increase in ethylene production occurs during ripening (Paterson, V.J. *et al.*, 1991). However, this increase in ethylene production occurs when flesh firmness is below 0.6 N, that is, when fruit are at eating ripeness and a considerable time after the increase in SSC during maturation. MacRae *et al.* (1989b) found that internal ethylene concentration of fruit at harvest increased with increasing maturity of fruit. Therefore, if ethylene is involved in switching fruit from the unresponsive to a responsive state in relation to cool temperatures, fruit cells would need to react to low internal ethylene concentrations. Fruit cells must be ready to ripen before they can respond to ethylene (Romani, 1987) so that factors other than ethylene may determine whether a fruit will ripen (Romani, 1984). Some ripening changes in tomato are apparently independent of ethylene stimulation (Jeffery *et al.*, 1984) and ripening changes can still occur in pear even when ethylene production was blocked (Romani *et al.*, 1983). Irrespective of the involvement of ethylene during kiwifruit maturation, the rise in soluble solids and total sugar concentrations and decrease in starch concentration is a biochemical response to the stage in fruit development. This stage may be related to the (as yet unidentified) genes induced and/or expressed during maturation. Once appropriate genes are active, the documented changes in carbohydrates and flesh firmness can occur. The rates of these reactions will be regulated by the enzyme and substrate concentrations while the direction of the reaction will depend on temperature.

The cells of ripening fruit undergo a series of physiological and biochemical changes which render them attractive to eat. Ripening used to be considered as a degradative process caused by the breakdown of cellular organisation and the liberation of hydrolytic enzymes. However, there is increasing evidence that the expression of specific genes is required for normal ripening (Grierson *et al.*, 1985). Most plant genes are expressed in a highly regulated manner. Gene

products may be present only in certain cell types, at specific stages of development or only following the application of distinct environmental stimuli (Kuhlemeier *et al.*, 1987). For example, the transcription of many plant genes involved in photosynthesis is controlled by light (Apel and Kloppstech, 1978). Similarly in soybean plants, production of mRNAs for low molecular weight proteins was induced by heat stress (Gurley *et al.*, 1986). Likewise, when plantlets of *Solanum commersonii* Dun. were acclimatized for 14 days at 5/5C maximum/minimum temperature after being grown at 20/15C maximum/minimum temperature (Tseng and Li, 1990), 23 cold-induced polypeptides were identified that were thought to be important in the development of cold-hardiness.

In tomato, polygalacturonase (PG) mRNA was virtually absent from immature-green fruit, but increased 2000-fold during ripening to reach its highest concentration in red-ripe fruit (DellaPenna *et al.*, 1986). Two translation products of mRNA were found in ripening tomato fruit that were present in a small amount or absent from mature-green fruit (Rattanapanone *et al.*, 1978). Grierson *et al.* (1985) proposed that ripening related mRNAs are products of a group of genes that code for enzymes important in the ripening process. A ripening-related gene (*AVOe3*) was found in avocado fruit where the protein and mRNA appeared before endogenous ethylene production; the gene coded for ACC oxidase (McGarvey *et al.*, 1992), a key enzyme involved in ethylene biosynthesis (Tucker and Grierson, 1982). During tomato fruit ripening, gene expression is regulated by ethylene concentration (Grierson *et al.*, 1985; Maunders *et al.*, 1987). Low concentrations of ethylene were measured in unripe tomato, but higher concentrations in ripe fruit (Lincoln *et al.*, 1987), similar to increasing ethylene concentration measured in kiwifruit during maturation (MacRae *et al.*, 1989b). Exogenous ethylene applied to mature-green fruit induced an increase in four different mRNAs (E4, E8, E17 and J49) within 0.5 to 2 hours (Lincoln *et al.*, 1987). An increase in ethylene concentration led to the onset of gene expression. Lincoln and Fischer (1988a) also showed that onset of *E4* and *E8* gene transcription coincided with the increase in ethylene concentration; whereas onset

of *E17* and *J49* gene transcription preceded the increase in ethylene concentration.

Ripening-impaired mutant lines of tomato were used to further investigate the effect of ethylene during ripening. Ethylene concentrations in the tomato fruit ripening mutant (*rin*) remained at basal levels throughout fruit development (Lincoln and Fischer, 1988b). The low concentration of ethylene in *rin* fruit inhibited transcriptional activation of the *E4* gene and significantly reduced *E8* and *J49* gene transcription. *E17* gene transcription took place but E17 mRNA accumulation was inhibited. The functions of the *E4*, *E8*, *E17* and *J49* genes may not have been elucidated but, in the context of this discussion, it is sufficient to know that induction of genes occurs during maturation in mature-green tomato. The responsiveness of these genes to ethylene is different, that is, some respond to a basal ethylene concentration and others to increased concentrations of ethylene. MacRae *et al.* (1989b) measured 6.9 nl l^{-1} gFW^{-1} internal ethylene on 25 March 1985 at 4.4% soluble solids but this concentration had increased to 16.1 nl l^{-1} gFW^{-1} ethylene by 7 May at 6.4% soluble solids. It is therefore possible that there is a sufficient basal concentration of ethylene in kiwifruit to allow the relevant genes to respond.

Based on the above information for tomato and avocado, it is probable that new genetic material is induced in kiwifruit as they develop from the immature to mature stage. As far as the author is aware, there is no such information available for kiwifruit. However, considerable research has been conducted with tomato at the genetic, physiological and biochemical levels to determine the mechanisms of fruit ripening. It is possible that similar mechanisms operate in kiwifruit during maturation. If genes for SSC could be identified and found to be present in mature, but absent in immature, fruit then this may give additional evidence for the induction of maturation. Six different cultivars of *A. deliciosa* ('Abbott', 'Bruno', 'Constricted', 'Gracie', 'Hayward' and 'Wilkins Super') commenced maturation at different times and had different SSC at harvest and

at eating ripeness after storage (Cotter *et al.*, 1991). It would be interesting to determine the genetic differences among these cultivars during maturation.

Many important heritable characters are a consequence of the joint action of several genes. Such characters are often referred to as polygenic or quantitative traits and are more difficult to manipulate in breeding programs than are single gene traits (Tanksley *et al.*, 1989). Soluble solids concentration of tomato is quantitatively inherited and genetic variation with respect to this character is low among commercial cultivars (Ibarbia and Lambeth, 1969). In contrast, other wild species of tomato such as *L. chmielewskii* have very high SSC (Rick, 1974). Recombinant DNA technology has been used to clone genes, but few of these have been directly associated with characters of major agricultural importance, such as yield or product quality (Tanksley *et al.*, 1989). Restriction fragment length polymorphism (RFLP) markers enabled identification, mapping and measurement of the effects of genes underlying quantitative traits (Tanksley *et al.*, 1989). In tomato, genes effecting changes in SSC have been marked with RFLPs (Tanksley and Hewitt, 1988). The F3 generation (selfed F2) from a cross between *L. esculentum* and a wild type, *L. cheesmanii*, was used to determine the chromosomal location of quantitative trait loci (QTL) affecting fruit SSC (Paterson, A.H. *et al.*, 1991). The F3 population was grown in both California and Israel. The positions of QTLs on chromosomes 1, 3 and 6 were similar for SSC from fruit grown in the two locations. However, the Israeli population revealed two new QTLs, on chromosomes 7 and 9, although the gene actions of these QTLs were thought to be recessive and additive/recessive, respectively. The gene action on chromosome 1 was dominant/additive, chromosome 3 was additive/recessive and on chromosome 6 was additive (Israel) or dominant/additive (California). Paterson, A.H. *et al.* (1991) suggested that differences in adaptation of plant genotypes may be due to environment-sensitive QTLs.

Several points will be discussed from work on tomato (Paterson, A.H. *et al.*, 1991) that could be applied to kiwifruit. Firstly, a number of QTLs have been

identified in tomato that code for SSC and these appear to have different gene actions. As measurement of the QTLs was not made at different growth stages, it cannot be determined whether genes are induced during maturation. However, some genes may account for 'basal' SSC and others for 'maturation' SSC in kiwifruit as used in the mathematical model developed to predict harvest date. Secondly, in tomato individual QTLs appear to show a range of sensitivity to the environment. Two new QTLs were discovered in the F3 population of tomatoes grown in Israel compared with those grown in California. However, SSC in ripe fruit from the Israel population was lower than for those grown in California and perhaps, this is in some way connected to the predominantly recessive gene action of the new QTLs.

Kiwifruit grown at different geographical locations in NZ matured at different rates. Fruit on these vines experienced different environmental conditions, for example, temperature, daylength and daily light integral (sunshine hours) during the growing season. It is possible that increase in SSC of tomato and kiwifruit is affected by the environment in two ways; genetically by induction of environment sensitive QTLs and biochemically by temperatures experienced during the maturation period. Paterson, A.H. *et al.* (1991) acknowledge that the QTLs mapped thus far almost certainly do not comprise the entire set of genes which affect SSC. As molecular genetics becomes a more established technique, it will be interesting to discover whether additional genes for SSC are induced during maturation and the extent to which the environment determines the genetic composition of a fruit. Further work will be needed to establish whether the basal and maturation components of SSC are genetically determined in kiwifruit.

6.4 Carbohydrate metabolism at low temperatures

Controlled environments were used to determine the effect of temperature on rate of kiwifruit maturation. The main response to different temperature treatments was a change in SSC and carbohydrate where, for example, SSC increased

fastest at the coolest mean temperatures. During maturation, increase in SSC was similar to the increase in total sugar concentration and coincident with a decrease in starch concentration. A similar pattern of decrease in starch concentration and increase in sugar concentration occurred in fruit from field-grown vines during maturation. Hydrolysis of starch to sugar accounted for part of these changes in carbohydrates, where the rate of hydrolysis was enhanced at low temperatures.

Why is starch hydrolysis stimulated at low temperatures? Processes occurring at the subcellular level need to be investigated in order to explain the increase in sugar concentration and decrease in starch concentration at low temperatures. Much research has been undertaken on harvested potato tubers to investigate the cause of cold induced sweetening in potato (an increase in sugar when tubers are stored at low temperatures). Cold sweetening is a complex metabolic process influenced by several levels of cellular regulation and the mechanism(s) remains unexplained at the molecular level (Sowokinos, 1990a). However, knowledge of the mechanism of cold sweetening in potato might help to explain partitioning in carbohydrate that occurs in kiwifruit at low temperatures.

Pathways and enzymes involved in glycolysis and gluconeogenesis were described in Section 1.3.2. Gluconeogenesis usually refers to formation of glucose from various non-carbohydrate precursors, but in a more narrow sense it relates to the reversal of the glycolytic pathway (Hall *et al.*, 1974). The concept of regulatory enzymes is important because they control inflow or outflow from a particular step in a pathway and are strategic points for control (Copeland and Turner, 1987). Available evidence suggests that phosphofructokinase (PFK) and pyruvate kinase are the main control points of glycolysis in plant tissues. Both of these enzymes catalyse reactions that are strongly exergonic and effectively irreversible, so that a different enzyme is required for the reverse reaction (Hall *et al.*, 1974). The enzyme fructose 1,6-bisphosphatase (F16BPase) catalyses the reverse reaction of PFK and is considered a regulatory enzyme in the gluconeogenic pathway. Experiments with mature potato tubers showed that entry

into glycolysis was regulated by PFK, while pyruvate kinase controlled movement of carbon out of glycolysis and into the oxidative pentose phosphate pathway. Activity of PFK and pyruvate kinase was reduced at temperatures of 2 to 10C compared to those of 10 to 25C, indicating that these enzymes were cold labile (Pollock and ap Rees, 1975). The instability of PFK and pyruvate kinase at low temperatures would probably favour the reverse gluconeogenic reaction.

In the early 1980s a new hexose, fructose 2,6-bisphosphate (F26BP) was discovered, which modulates sugar metabolism (Stitt, 1987). At about the same time it was shown that plants contain two sets of PFKs that catalyse the conversion of fructose 6-phosphate (F6P) to fructose 1,6-bisphosphate (F16BP) (Black *et al.*, 1985). These two PFKs are: an ATP-dependent enzyme (ATP-phosphofructokinase or PFKase) and a PPi-dependent enzyme (pyrophosphate: fructose 6-phosphate phosphotransferase or PFPase). There are two interconvertible molecular forms of PFPase; F26BP regulates the enzyme into either the form active in glycolysis or the form active in gluconeogenesis (Section 1.3.2). The concentration of F26BP influences the direction of the step F6P to F16BP, that is, either towards glycolysis or towards gluconeogenesis. There is good evidence that changing concentrations of F26BP strongly regulate glycolytic and gluconeogenic carbon flow in the cytoplasm of plant cells (Sung *et al.*, 1988). As concentration of F26BP rises, glycolysis is favoured and when concentration of F26BP falls, gluconeogenesis will proceed because inhibition of an enzyme controlling production of F16BP is relieved (Black *et al.*, 1985). Thus low concentrations of F26BP promote the interconversion of starch to sucrose (Stitt, 1987).

Although there are few direct links between starch and the gluconeogenic pathway (Fig. 1.1) another enzyme, sucrose 6-phosphate synthase (SPS) is a key regulator of the sucrose biosynthetic pathway. Properties of SPS indicate that it is a regulatory enzyme, as SPS activity is promoted by high concentrations of substrate, particularly UDP-glucose and inhibited by end-products of sucrose synthesis (Sowokinos, 1990b). Extractable SPS activity in barley leaves was

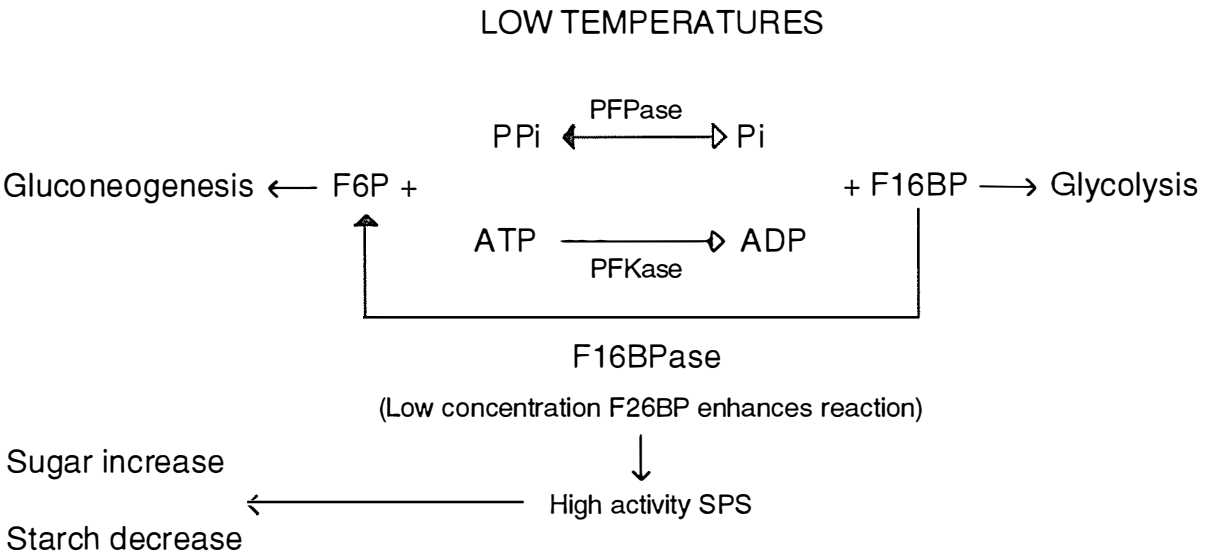
inversely related to F26BP concentration (Sicher, 1986). Since fructose 1,6-bisphosphatase (F16BPase) activity also increases with low concentrations of F26BP, it appears that both SPS and F16BPase may be correlated to control sucrose synthesis (Sicher, 1986; Sicher *et al.*, 1986). Hence, hydrolysis of starch to sugar is likely to occur when F26BP concentration is low as gluconeogenesis is favoured in the cytoplasm. In addition, starch may be phosphorolysed to glucose 1-phosphate (G1P) in the starch granule, transported to the cytoplasm and phosphorolysed to UDP-glucose which is catalysed by SPS to form sucrose and later, glucose and fructose. It is possible that a low concentration of F26BP results in starch degradation due to enhancement of several reactions that favour the formation of sucrose, resulting in a concentration gradient towards sucrose and hence glucose and fructose.

Concentration of F26BP is also affected by temperature. A low concentration of F26BP was found in potato tubers stored at 4 compared to 20C (Morrell and ap Rees, 1986b) and in green pepper at 3 compared to 30C (Phelps and McDonald, 1989). In contrast, the higher concentration of F26BP found in potato at 2 and 4 compared to 8C (Claassen *et al.*, 1991) may have been due to different experimental techniques used among the studies. At low temperatures, the low concentration of F26BP and/or the elevated PPi levels found in cold stressed potato increased the form of PFPase active in gluconeogenesis (Black *et al.*, 1985). Another contribution to starch degradation at low temperatures is high PPi concentration, as this is thought to enhance the speed of the glucose phosphorylase catalysed reaction towards G1P (ap Rees *et al.*, 1985). The PPi:G1P ratio in isolated starch granules from potato tubers was higher (250:1) than the PPi:G6P (glucose 6-phosphate) ratio which was 15:1 (Isherwood, 1976). In contrast, at high temperatures there is an increase in concentration of F26BP so that PFPase converts to the form where glycolysis is favoured, especially as F16BPase is inhibited by high concentrations of F26BP. The reaction catalysed by PFKase can also proceed. The enhanced gluconeogenic pathway at low temperatures links into the mathematical model developed to relate changes in

SSC to temperature during maturation; the temperature-dependent rate function was highest between 7 and 8C compared to lower or higher temperatures.

Concentration of F26BP increased in carrot (*Daucus carota* L.) and potato treated with ethylene and in naturally ripened avocado (Stitt *et al.*, 1986). Assuming that ethylene is associated with maturation and ripening, the increase in F26BP in response to ethylene treatment provides evidence that this regulatory metabolite is promoting carbohydrate mobilisation and breakdown, as glycolysis is favoured. It remains to be seen how F26BP brings about this stimulation. Tissues of avocado, carrot and potato are of different morphological origins and may not exhibit similar patterns of response, also avocado does not store starch. The effects of applied exogenous ethylene or naturally occurring ethylene invoke a different biochemical response (high F26BP concentration) compared to the effect of low temperature (low F26BP concentration). Similarly, there may be a difference between cold induced and senescent sweetening in potato tubers. Cold induced sweetening has already been described, whereas senescent sweetening is induced during maturation and perhaps by the application of exogenous ethylene. The amyloplast membrane was intact in tubers stored at low temperatures, but broken in senescent tubers (depending on cultivar) (Sowokinos *et al.*, 1985). A broken membrane around the amyloplast would increase vulnerability of the starch granule to degradative enzymes from outside the amyloplast, and cytoplasmic levels of PPi could be elevated by leaky membranes (Sowokinos, 1990a). Hence, the PPi concentration may increase in potato and in fruit during maturation and ripening. In this situation, gluconeogenesis would be favoured and starch may be hydrolysed to sugar. It is possible that this mechanism is necessary for fruit to ripen and sweeten.

A summary is given of the proposed biochemical pathways that may describe the effect of temperature on the rate of carbohydrate changes that occur in kiwifruit during maturation and influences SSC concentration (Fig. 6.1). It is assumed that the maturation component of SSC has been induced in the fruit and therefore, the fruit are responsive to temperature. When temperatures are low starch is



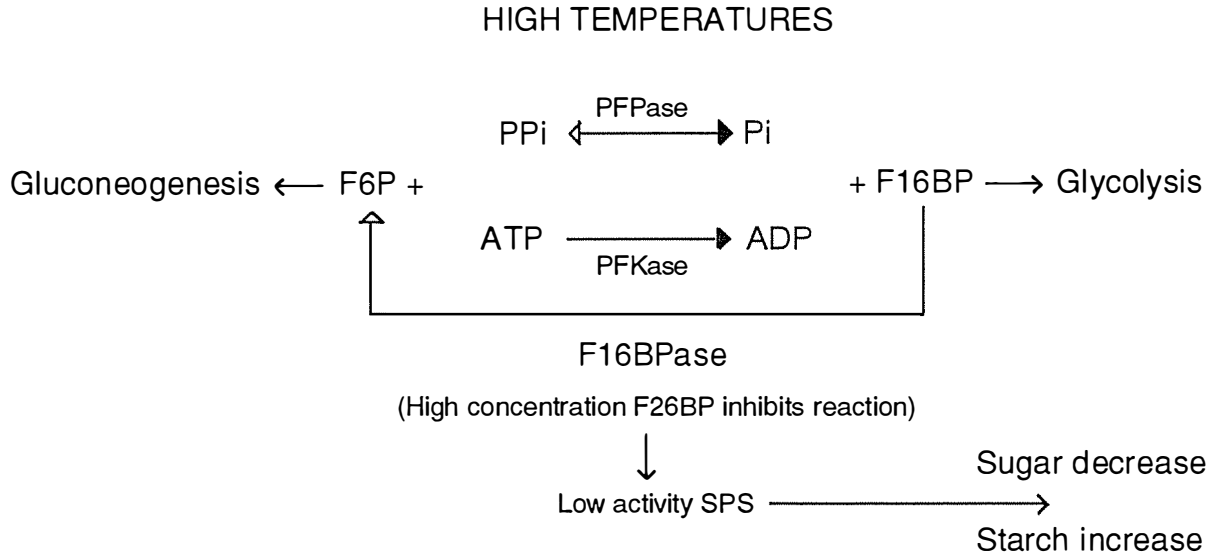


Fig. 6.1 A theoretical scheme to demonstrate that temperature may affect the direction of the glycolytic pathway. → inhibited reaction → enhanced reaction

hydrolysed to sugar through regulation of gluconeogenesis and other pathways by F26BP concentrations. A low F26BP concentration and/or high PPi concentration would increase the concentration of the form of PFPase active in gluconeogenesis and F16BP is converted to F6P. In addition, a low concentration of F26BP results in high activity of SPS leading to formation of sucrose and perhaps a concentration gradient from starch to sugar. At high temperatures sugar is converted to starch through control of glycolysis and other pathways regulated by F26BP. Concentration of F26BP is high and PFPase is converted to the form active in glycolysis, catalysing conversion of F6P to F16BP. In addition, SPS and F16BPase are inhibited by high concentrations of F26BP, so that the reaction catalysed by PFKase can proceed. As the glycolytic and gluconeogenic pathways play a central role in cellular metabolism and F26BP concentration strongly regulates glycolytic and gluconeogenic carbon flow in plants (Sung *et al.*, 1988), it would be expected that the biochemical pathways in other starch containing fruit, such as apple, pear and tomato, would respond to temperature during maturation in a similar way to that proposed for kiwifruit.

For an enzyme-catalysed chemical reaction to proceed there must be sufficient concentrations of both substrate and enzymes present. The law of mass action states that the rate of a chemical reaction is proportional to the concentration of the reactants (Goodwin and Mercer, 1972). The rate of an enzyme-catalysed reaction shows a saturation effect with increasing substrate concentration (Hall *et al.*, 1974). At low substrate concentrations the reaction rate is directly proportional to the substrate concentration, but as the substrate concentration is increased further, the reaction rate declines giving a hyperbolic relationship characteristic of all enzyme reactions. However, there is considerable variation in the substrate concentration needed to produce saturation.

The Michaelis-Menten equation describes the relationship between the enzyme reaction rate and substrate concentration (Hall *et al.*, 1974). Glycolysis and gluconeogenesis are networks of reactions, not single pathways (Sung *et al.*, 1988). Hence, at each step in the glycolytic and gluconeogenic pathways the rate

of reaction will be proportional to the concentration of substrate and enzyme present. However, some steps may be slower than others, resulting in rate-limiting steps (Goodwin and Mercer, 1972). Dunn (1974) used an equation to describe the effect of α-amylase concentration on degradation of starch. The equation is

$$v = k A E^n$$

where v = velocity of reaction

k = constant

A = total surface area of granules

E = total α-amylase concentration

n = constant

A higher concentration of α-amylase resulted in a higher rate of reaction. It should be possible to calculate the rates of reaction for each step in the glycolytic and gluconeogenic pathways. However, the Michaelis-Menten equation and its relationship with temperature would need to be known for each step. If such information was available, it may be possible to incorporate it into a physiologically based model in order to predict changes in starch and sugar concentrations with changes in temperature during maturation of kiwifruit.

How quickly can a metabolic pathway respond to a change in temperature? At the subcellular level, if F26BP is a regulator of PFPase, then its concentration must be able to be altered in plant tissues (Black *et al.*, 1987). To be effective in coping immediately with rapid environmental changes, the change in F26BP concentration should occur in minutes. Paz *et al.* (1985) flooded roots of pea plants and measured an increase in F26BP concentration in leaves within 15 to 30 seconds. A change in F26BP concentration within a similar period of time may also be expected in response to a change in temperature. However, changes in concentrations of starch, total sugar and soluble solids were not recorded until several days after a temperature perturbation was imposed on kiwifruit or in potato tubers (Pressey and Shaw, 1966). This is probably because changes in concentrations of these carbohydrates are the result of many metabolic steps and, realistically are gross measurements. Measurement of the concentration of

key enzymes and sugar substrates of kiwifruit subjected to cool and warm temperatures is necessary to determine the effect of temperature on individual metabolic steps.

In potato tubers the effect of a temperature perturbation on starch and sugar concentrations appeared to be reversible. For example, when tubers were transferred from 10 to 2C, the concentration of total sugar increased; after transfer back to 10C starch concentration increased and sugar concentration decreased (Pressey and Shaw, 1966). In this thesis, some treatments were designed to investigate the reversibility of starch and sugar conversions in kiwifruit with temperature perturbations. Unfortunately, results were not obtained after the transfer from 15/11 to 26/20C due to a leak of ethylene into that controlled environment treatment which resulted in ripe fruit. However, when the temperature was changed back to 11/7C after 18 days at 20/13C the decrease in starch concentration and increase in sugar and soluble solids concentrations were faster than at 20/13C. These results indicate that the dynamic temperature-dependent relationship in starch and sugar concentration, in both kiwifruit and potato tubers, is dependent on ambient temperature experienced. The central role of glycolysis and gluconeogenesis in plant metabolism appears to give plants their unique ability to respond dynamically to their changing environment.

Biochemical pathways influencing cold sweetening in potato have been explained. It is suggested that similar pathways may operate in kiwifruit accounting for the rapid decrease in starch concentration and increase in sugar concentration that occur at low temperatures. Detailed knowledge of the biochemical reactions involved in starch degradation and sugar accumulation in kiwifruit may be useful when attempting to include these into a mathematical model. Further work is needed to ascertain whether F26BP is present in kiwifruit, whether it changes in response to temperature and if it plays a central role during maturation.

6.5 Description of increase in soluble solids concentration

The model developed to predict rate of change in SSC during kiwifruit maturation has two components. Firstly, the state-dependent physiological response function as described by the base + exponential model. Secondly, the temperature-dependent rate function based on a modified Arrhenius equation. The model was developed using continuous temperature records and a combined data set from container-grown vines in two controlled environment studies. Testing of this model was undertaken on data from similar container-grown vines in the sheltered site and field-grown orchard vines from sites where continuous temperature records were available. As only daily maximum and minimum temperatures are available in many practical situations, the model was modified to use these temperatures to describe the increase in SSC in field-grown vines and hence predict harvest date when based on a specified SSC. The model was suitable for describing the rate of change in SSC at different sites as increase in SSC was similar to measured SSC at four orchards in different locations, and for two of these sites in two different years. A wide range of controlled temperature treatments were used in the development of this model. Data collected from previous controlled temperature studies (Hopkirk *et al.*, 1989; Kempler *et al.*, 1992) or when temperatures were not controlled (Salinger and Morley-Bunker, 1988; Snelgar *et al.*, in prep.) are more difficult to use for developing a model, but could be used for validation of other models.

Prediction of harvest date would be of benefit to the kiwifruit industry in terms of planning harvesting operations and subsequent transport of fruit. The model is envisaged being used as follows. Soluble solids concentration should be measured from fruit harvested weekly from, for example, the end of March in the same orchard block. Once SSC begins to increase during maturation, a measured value can be inserted into the model as the starting point. At this stage maximum and minimum temperature data from previous years could be used to predict the most likely earliest and latest date of harvest. As the season progresses, further SSC measurements can be inserted into the model as well

as current maximum and minimum temperatures for that particular season. Hence, prediction of harvest date by early April should be more accurate than that from the first prediction in March. Updating of predicted harvest date could continue until actual harvest date is reached.

Although the model developed has not been rigorously tested in a predictive capacity, an example of simulating SSC has been prepared where maximum temperature was increased and minimum temperature was decreased by 1C from measured temperatures (Figs 6.2 and 6.3). Under these conditions, when SSC was simulated from 26 February, the possible time of harvest (minimum of 6.2% SSC) ranged over an 8 day period (Fig. 6.2). However, if SSC simulation commenced 35 days later, the possible time of harvest ranged over a 4 day period (Fig. 6.3). These simulations show that i) the time of harvest is affected by different temperatures during the growing season and ii) prediction of harvest date improves when the prediction is made closer to the time of harvest. At present, commercial harvest in kiwifruit is when fruit SSC exceeds a minimum of 6.2%, but it is possible that this minimum SSC may be increased as fruit harvested at higher SSC store better and fruit are more guaranteed to ripen to an acceptable flavour after storage (Beever and Hopkirk, 1990). If this happens the model developed here will still be suitable because it can predict the time of harvest at any predetermined SSC.

There are some limitations of the model. Prediction of the exact harvest date cannot be made a long time before harvest. This is a disadvantage to exporting companies and packhouses as ships to collect export fruit are ordered up to 6 months in advance of actual harvest in April/May. Timing of harvest will also affect the time that new season fruit arrive in the export markets and hence, may affect marketing strategies. However, a prediction of harvest date 4 to 10 weeks before harvest may be better than relying on estimates from previous years. The base + exponential was chosen to describe the kinetics of SSC increase during kiwifruit maturation as there was no apparent difference between this model and

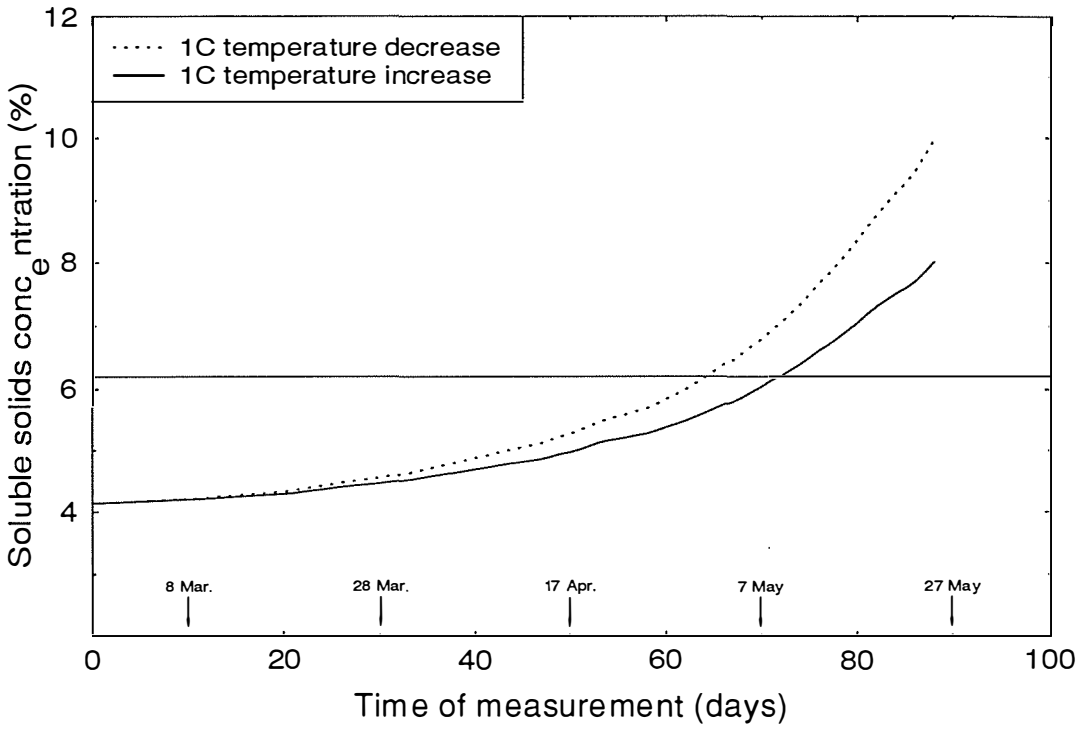


Fig. 6.2 Simulated soluble solids concentration (%) of kiwifruit grown at Palmerston North using fitted parameters from Palmerston North ($h^* = 4.66 \times 10^{-2}$), where maximum and minimum temperatures increased or decreased by 1C from measured temperatures in 1990.

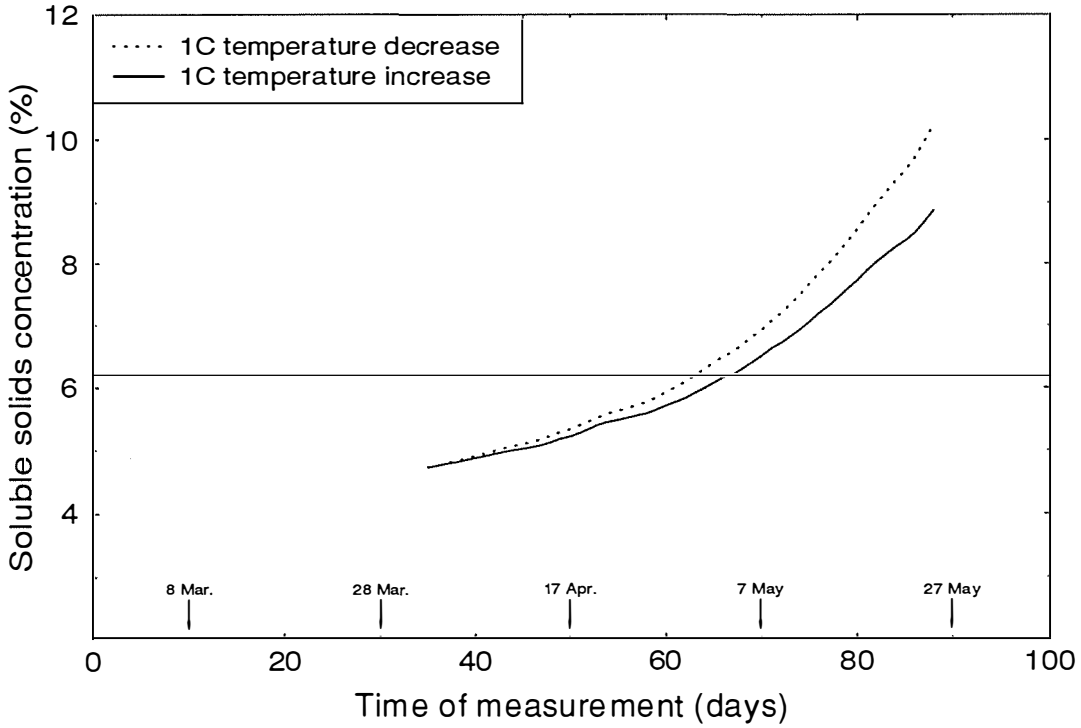


Fig. 6.3 Simulated soluble solids concentration (%) of kiwifruit grown at Palmerston North using fitted parameters from Palmerston North ($h^* = 4.66 \times 10^{-2}$), where maximum and minimum temperatures increased or decreased by 1C from measured temperatures in 1990.

the power-law model. Whichever model is used, the fitted parameters will vary between controlled environment experiments or different field studies. An interesting feature of this model is the use of a site specific coefficient, the partial rate coefficient. This may mean that SSC and temperature data has to be collected for a new site in order to derive the specific partial rate coefficient for that site. Nevertheless, it is possible that the country could be split into localities or regions where the rate coefficient is similar and can be used over a wider area than the specific orchard measured. For example, the northern region could comprise of Northland and a central region comprising of the Bay of Plenty, while the southern region could include Horowhenua and Nelson. Analysis of suitable data at different sites will confirm whether an average value of the partial rate coefficient can be used for a region. However, it appears that the partial rate coefficient is consistent between years, although analysis of previous data will establish whether this is correct. Also, the physiological basis of the partial rate coefficient needs to be determined by examining other factors, such as daily light integral and daylength at the different orchard locations, to fully understand those factors influencing the maturation process.

Future work should involve testing this model against data from other sites and the same sites in different years, and attempts should be made to model changes in starch and sugar concentrations during maturation, as carbohydrate concentration may determine the potential of a fruit for storage and eating quality after storage. It would also be interesting to determine the effect of temperature on carbohydrate partitioning before maturation, and to develop a suitable model to predict maximum carbohydrate concentration or carbohydrate concentration at harvest. It is possible that this model could be incorporated into a larger crop prediction model such as Orchard 2000 (Atkins *et al.*, 1992).

APPENDIX 1

RAPID ESTIMATION OF FRUIT STARCH AND SOLUBLE SUGAR CONCENTRATIONS IN KIWIFRUIT

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Abstract. Experiments investigating kiwifruit (*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson var. deliciosa) maturation were undertaken requiring the analysis of total soluble sugars and starch in large numbers of fruit samples. Current analytical methods were found to be cumbersome, slow and not suited for use with fruit tissue. Existing techniques were modified to allow larger numbers of samples to be processed more rapidly. The phenol-sulfuric acid assay was judged to be a convenient method for determination of total soluble sugars from tissue extracts and gave results similar to those obtained by high pressure liquid chromatography. The starch procedure adopted involved gelatinisation of fruit tissue using hot water and a thermostable α-amylase (Termamyl); starch hydrolysis using amyloglucosidase; and determination of glucose using glucose oxidase. The methods enabled up to 40 kiwifruit samples to be analysed for total sugar and starch concentrations by one person over a nine-hour period and should be applicable to research areas concerning fruit development and maturation.

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The predominant storage carbohydrate in kiwifruit is starch which is stored in both the outer pericarp and core tissue of the fruit. Fruit starch concentrations increase and reach a peak about one month before harvest and then rapidly decrease with a concomitant rise in free sugars during the latter phases of maturation (Beever and Hopkirk, 1990). The major sugars in kiwifruit at eating ripeness are glucose (2-6% of fresh weight), fructose (1.5-8%) and sucrose (2%) (Beever and Hopkirk, 1990) with traces of polyols such as inositol (Kawamata, 1977).

Studies have been conducted to investigate the changes in partitioning of carbohydrate between starch and sugars in response to temperature during kiwifruit maturation (Seager et al., 1991). Mature fruit are regarded as those having reached eating ripeness. This work required analysis of sugars and starch in fruit samples which were collected regularly during fruit development from plants subjected to different temperature treatments. However, as it was not feasible to analyse the carbohydrates at the time of sampling, it was necessary to preserve the fresh tissue at harvest. The very large numbers of samples accumulated (6000) meant the analytical methods adopted were required to have as high a sample throughput as possible.

Sugar and starch concentrations have been determined in a wide range of different types of fresh and dried plant tissue. Typically, sugars are quantified using colorimetric (e.g., Hodge and Hofreiter, 1962) or chromatographic procedures following extraction of the fresh or dried tissue with alcohol-based solvents. After soluble sugars have been removed, starch is usually hydrolysed enzymatically following pretreatment to render the substrate available to enzyme attack, and the released glucose is measured using specific enzyme systems (e.g., Haslemore and Roughan, 1976; MacRae et al., 1974). None of the published methods appear to have been specifically designed or adapted for fresh kiwifruit tissue or other fruit types. Our colleagues have applied the Boehringer Mannheim starch kit (Boehringer Mannheim GmbH, International Division, PO Box 310120, D6800, Mannheim 31, Germany) based on the methods of Keppler and Decker (1974) for the analysis of starch in fresh kiwifruit tissue (Bowen et al., 1988; MacRae et al., 1989a), but the method was found to be unsuitable for the rapid analysis of large numbers of fruit samples.

The aim of this work was to develop a simple and practical method for determining total soluble sugar and starch concentrations in the fruit tissue of kiwifruit with sufficient throughput to undertake large numbers of analyses during each fruit growing season.

Preparation of samples for storage and analysis. Approximately 4-5 g samples were taken from the outer pericarp or core tissue of each 'Hayward' kiwifruit. The tissue was chopped into approximately 5 mm cubes, weighed and steeped in 20 ml methanol: water: formic acid (12:2:1 v/v) (E.A. MacRae, pers. com.) in capped vials prior to storage at 3C for up to one year. For analysis, the extractant was decanted into a beaker, the tissue transferred into a pre-weighed 50 ml centrifuge tube and homogenised with 20 ml distilled water using a Polytron homogeniser, then centrifuged at 2000x g for 15 mins. The supernatant was combined with the original extractant and used for soluble sugar analysis. The weight of residual fruit tissue was obtained by reweighing the tube plus contents and set aside for starch analysis.

Total sugar analysis. Calculations were based on the mean total volume of extractant estimated from at least 10 replicate measurements from outer pericarp or core tissue samples. These volumes varied according to the type of kiwifruit tissue being extracted but were consistent among either outer pericarp or core fractions. Aliquots of one ml were removed from the extract solutions and diluted by a factor of between 10 and 120 with distilled water depending on sugar concentration, and 1 ml of diluted extract was assayed for total carbohydrate (by comparison with a range of glucose standards) by the phenol-sulfuric acid method of Dubois et al. (1956).

A comparison of the phenol-sulfuric acid assay with a high pressure liquid chromatography (HPLC) procedure was undertaken to independently evaluate the recovery of sugars from the fruit extracts. Subsamples from selected sugar extracts were evaporated to dryness under reduced pressure at 40C, reconstituted to the original volume with distilled water and filtered through a 0.2 μm nylon membrane. Samples were injected into a HPX-87C Bio-Rad column (300 x 7.8 mm) and eluted with water at 85C for 90 mins. Component peaks were detected by refractive index.

Starch analysis. Subsamples of the residual tissue (300 to 900 mg depending on the maturity of the fruit sample) and three starch standards of approximately 10, 35 and 65 mg were weighed accurately into screw-capped culture tubes (Kimax 45066-A; 16 x 125 mm). The starch standard used was a purified wheat preparation (Sigma S-2760) and weights were corrected for moisture content (10.6% of DM determined at 105C). A 10 ml aliquot of distilled water and 50 µl of a thermostable α-amylase (Termamyl, activity 120 KNu g^{-1}; supplied by Novo Industri, A/S Copenhagen, Denmark) were added to each sample, mixed and the tubes were then heated in a boiling water bath for 90 min. The tubes were firmly capped after 5 min heating, and remixed after 45 min. After cooling, the tube contents were poured into a 130 ml container, the tubes rinsed with 10 ml 0.25 M sodium acetate buffer (pH 4.5), and the buffer plus 80 ml distilled water was added to the container with thorough mixing to give a final solution of 100 ml with a variation in volume of ±0.5 ml.

Approximately 1 ml from each sample was centrifuged in a 1.5 ml micro-centrifuge tube at 8000x g for 2 min. Omitting the centrifuging step had no effect on starch recovery but gave rise to blocked pipette tips when subsampling for further analysis. A 200 µl aliquot of supernatant from each sample was added to a culture tube (16x125 mm) followed by 400 µl sodium citrate buffer (pH 4.6), 350 µl distilled water and 50 µl amyloglucosidase (Boehringer Mannheim 737160, 14 U mg^{-1}). The contents were mixed and then hydrolysed by incubating at 60C for 20 min. Released glucose was determined by a glucose oxidase method (e.g., Kilburn and Taylor, 1969).

Calculations. Soluble sugar and starch concentrations were calculated as mg per g of fresh tissue and corrected for the appropriate dilutions. Starch concentration was also corrected for the amount of residual tissue subsampled for analysis and multiplied by 0.9. This factor (x0.9) accounted for the mass of glucose theoretically hydrolysed from a unit mass of starch.

Total sugar analysis. The major requirement for this procedure was to obtain a reliable estimate of total soluble sugars in fruit samples harvested sequentially and preserved in an acidic alcoholic extractant. The number of samples generated meant that the method chosen needed to balance analytical accuracy with sample throughput. Examination of numerous samples, particularly mature fruit high in sugar levels, indicated that the original steeping extraction

procedure removed nearly 80% of all soluble sugars and accounted for 96% of all sugars when combined with a cold water wash. Washing the residual tissue a second and third time released the last 3% and 1% of the remaining soluble sugars, respectively. The additional time needed for these steps was not justified for our work, but might possibly be required in other situations. Also, to save time, combined extract volumes were not measured individually but were based on mean volumes estimated from replicated measurements.

Examination of extracts of mature fruit using HPLC identified the major components as fructose (48%), glucose (39%) and sucrose (13%). These mean proportions are similar to those reported elsewhere (MacRae et al., 1989b). None of the extracts contained other di- or oligosaccharides. The presence of sugar alcohols such as inositol were not detected using this system. The phenol-sulfuric acid assay proved ideal for the colorimetric determination of total soluble sugars in kiwifruit tissue extracts. This procedure gave a similar response to glucose (100%), fructose (114%) and sucrose (96%) when normalised to glucose on a mass basis. It is a quick, convenient and considerably more cost effective method than that available using HPLC. Furthermore, the results obtained were comparable with those using HPLC for measuring total sugars in extracts. For example, the analysis of a typical mature kiwifruit sample by the colorimetric and HPLC procedures gave 44.4 ±1.0 and 42.8 ±2.2 mg sugar.g fresh weight^{-1}, respectively (mean and standard deviation for three replicated tissue analyses within a fruit sample).

Starch analysis. Initially, fruit starch assays were carried out using the Boehringer Mannheim method (1979) with the aim of modifying some of the steps to increase throughput above the 15 samples per day achieved by our colleagues. This analytical procedure involves starch solubilisation using hot acidic DMSO, neutralisation to pH 4.5 monitored by a pH meter, hydrolysis of the solubilised substrate using amyloglucosidase, and specific determination of the released glucose using the UV/hexokinase method. Most of these steps are time consuming and unsuited to reductions in scale to improve efficiency and throughput.

Solubilising starch by steeping fruit tissue at 60C in HCl/DMSO in conical flasks (Boehringer Mannheim, 1979) did not permit rapid analysis. The recovery of hydrolysed glucose from starch standards was some 5% higher, when flasks

were constantly swirled in a shaking water bath compared to a non shaking water treatment. However, the efficiency of sample processing was substantially improved when the solubilisation step was carried out in screw capped tubes. Here, preliminary results with fruit samples indicated that starch levels were similar to those obtained with flasks, shaken or otherwise. Following steeping in HCl/DMSO, the neutralisation procedure recommended by Boehringer Mannheim (1979) used dropwise addition of 5 M sodium hydroxide to achieve a final pH of 4.5. Attempts were made to neutralise these acidic solutions by adding constant volumes of sodium acetate buffer (pH 4.5), but these were not completely successful.

We gelatinised starch by treating fruit tissue in water at 100C as an alternative to the solubilisation/neutralisation step. Gelatinisation is commonly used to render starch accessible to enzymic hydrolysis (e.g., Haslemore and Roughan, 1976) and in recent years, this procedure has been extended by boiling tissue in the presence of a thermostable α-amylase preparation such as Termamyl (Aman and Hesselman, 1984; Bengtsson and Larsson, 1990) which converts the substrate to a mixture of soluble oligosaccharides. Addition of Termamyl gave higher starch levels with improved precision for both standard and fruit tissue samples compared to samples gelatinised without Termamyl. For example, starch levels measured from a typical ripe fruit sample were almost doubled when 50 µl Termamyl was used compared to gelatinisation without the enzyme (Table 1), but were not increased further when enzyme volumes larger than 50 µl were added.

Table 1 The effect of using a thermostable α-amylase (Termamyl) during starch gelatinisation in standard and fruit samples.

Sample type	Volume of Termamyl (µl)		
	0	25	50
	Theoretical recovery of released glucose (%)[z]		
10 mg starch standard	92.8±12.7	101.2±2.4	106.6±1.5
35 mg starch standard	86.7±1.5	97.9±2.3	101.2±0.9
65 mg starch standard	69.9±2.0	81.2±0.9	80.7±0.6
	Starch level (mg g FW^{-1})[z]		
Outer pericarp tissue from ripe kiwifruit	1.9±0.7	3.0±0.3	3.5±0.2

[z]Data are the means and standard deviations of three replicate analyses from the same fruit using the methods described above.

A variant of the commonly used glucose oxidase procedure (Kilburn and Taylor, 1969) was substituted for the hexokinase method employed by Boehringer Mannheim (1979) for measuring hydrolysed glucose. Both methods gave similar theoretical recoveries of glucose from several standard starch hydrolysates (ranging from 15 to 70 mg starch) namely 99.6 and 96.6% determined from the glucose oxidase and glucose hexokinase methods, respectively. The glucose oxidase method was thus adopted routinely because of its advantages of speed, convenience and economy.

Both the soluble sugar and starch methods described here have given similar results for common or comparable fruit samples to those obtained with alternative procedures. For soluble sugars, this involved quantitative HPLC on common extracts as described above. For starch, the comparisons were made with fruit of similar maturity using the method reported here (Seager et al., 1992) and the Boehringer Mannheim method (MacRae et al., 1989b). The proposed starch method is similar to procedures developed specifically for cereal grains (Aman and Hesselman, 1984) and for non-fruit horticultural material (Rasmussen and Henry, 1990), but both the sugar and starch methods described in this paper

should be applicable to a wide range of different types of fruit tissue. Specifically, the use of a thermostable α-amylase during gelatinisation of starch in kiwifruit samples resulted in much improved recoveries of starch from these fruit samples. Furthermore, the use of Termamyl may have a similar benefit in the analysis and quantitative recovery of starch from other fleshy fruit types and this approach may warrant further study.

Kiwifruit, like most other fruit tissue, is inherently variable. Repeated analyses within the outer pericarp tissue of individual fruit gave coefficients of variation in the order of 2-10% and 5-15% for sugar and starch analyses respectively, depending on the stage of fruit maturity. Typically, variation between fruit for both analyses was 15% or greater.

The development of these methods arose from the need to analyse the gross carbohydrate fractions of numerous fruit samples from kiwifruit vines. Whilst the procedures are practical and do not require expensive equipment, there may however, be opportunities to improve or refine them further. Currently, their key advantages over existing methods, particularly the Boehringer Mannheim starch procedure, are simplicity, convenience, and speed as one analyst can process 40 samples daily. We suggest that these procedures may have applications in all aspects of kiwifruit research concerning the relationship between fruit carbohydrate composition and fruit development.

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APPENDIX 2

SOLUBLE SOLIDS CONCENTRATION AT PROXIMAL AND DISTAL ENDS OF FRUIT

A.2.1 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown under treatments of different minimum temperatures (C) (Section 2.1) (Experiment 1).

Time after transfer (days)	Temperature (max./min.) (C)							
	18/4		18/8		18/12		18/16	
	Soluble solids concentration (%)							
	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.
0	6.1	6.2	5.9	5.9	6.1	6.2	5.8	6.0
7	6.4	6.7	6.5	6.5	6.3	6.5	5.8	5.9
14	6.8	7.3	6.9	7.3	6.3	6.6	5.8	6.1
21	7.2	7.7	7.2	7.4	7.1	7.5	5.7	6.1
28	9.3	9.8	8.3	8.6	7.4	7.9	6.4	7.0
35	10.2	10.8	9.7	10.3	7.5	8.0	7.3	7.9
42	11.4	12.4	10.8	11.5	7.9	8.5	7.9	8.5
49	13.5	15.1	12.1	14.1	9.1	10.2	8.4	8.9
56	14.0	15.8	12.8	14.5	11.0	12.0	10.0	10.7

A.2.2 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown under treatments of different maximum and minimum temperatures (C) (Section 2.1) (Experiment 2).

Time after transfer (days)	Temperature (max./min.) (C)											
	14/8		14/12		18/8		22/8		22/12		26/8	
	Soluble solids concentration (%)											
	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.
0	5.7	5.7	5.7	5.8	5.4	5.5	5.7	5.7	5.8	5.6	5.6	5.7
10	6.8	6.9	5.8	5.9	6.1	6.1	6.6	6.8	6.3	6.3	7.1	7.1
20	6.7	6.9	6.2	6.2	6.4	6.4	7.2	7.3	6.3	6.2	6.9	6.8
30	7.3	7.7	6.5	6.8	7.2	7.3	7.6	7.8	6.7	6.8	6.8	6.9
40	8.3	8.9	7.2	7.9	7.9	7.9	7.1	7.3	6.6	6.8	6.7	6.7
50	10.8	11.6	8.8	9.5	9.8	9.6	8.0	8.6	7.5	7.9	7.1	7.3
60	12.2	13.4	10.3	11.3	10.2	11.0	8.9	9.3	9.3	9.9	7.8	8.3
73	14.3	15.8	12.4	13.4	12.4	13.6	9.9	10.8	9.2	10.2	9.0	9.3

A.2.3 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown under fixed temperature (C) treatments (Section 2.2) (Experiment 3).

Time after transfer (days)	Temperature (max./min.) (C)							
	11/7		15/11		15/15		26/20	
	Soluble solids concentration (%)							
	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.
0	6.1	5.9	6.0	5.7	6.9	6.1	5.9	5.5
3	6.3	6.4	6.2	6.4	-	-	-	-
5	6.5	6.2	6.4	6.0	-	-	-	-
8	7.0	6.6	6.2	5.9	-	-	7.0	7.0
11	6.6	6.5	6.2	6.1	-	-	6.0	6.2
14	7.4	7.2	6.4	6.3	-	-	6.1	6.2
17	7.5	7.4	6.7	6.6	7.1	7.2	6.1	6.2
20	8.5	8.1	7.4	6.9	9.4	8.9	6.5	5.7
23	8.2	8.5	7.3	7.3	7.7	8.2	6.1	6.0
26	9.1	9.6	7.3	7.4	8.2	9.1	6.3	6.4
29	9.5	10.2	7.6	7.5	8.0	8.6	6.3	6.3
32	9.1	10.5	8.4	8.8	9.0	9.7	6.2	6.2
35	9.9	12.5	8.2	8.2	8.2	9.3	6.2	6.3
38	11.0	13.8	7.7	8.2	8.4	9.7	6.2	6.2
41	10.7	12.4	9.4	9.9	8.7	9.5	6.7	6.8
44	11.4	14.7	8.3	9.3	9.2	9.9	6.9	6.9
47	11.0	14.4	9.3	11.3	8.7	10.0	6.7	6.7
50	10.6	15.6	9.3	10.2	9.6	11.6	6.9	7.2
53	10.8	14.5	11.2	12.7	9.5	11.7	6.3	6.7
56	11.6	15.0	10.0	12.2	10.0	11.7	7.0	7.3
59	10.6	14.8	12.0	14.2	10.9	13.3	7.7	8.0
62	12.6	14.7	11.2	12.1	11.7	13.7	7.6	8.3
65	13.0	15.7	12.0	15.0	12.2	13.9	7.4	9.0
68	12.1	15.4	12.2	14.7	12.4	15.2	7.6	9.3
71	11.9	15.8	12.7	15.5	12.6	16.1	8.2	10.0
75	11.7	15.9	14.3	17.0	12.7	14.9	8.0	9.9

A.2.4 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown under temperature (C) perturbation treatments (Section 2.2) (Experiment 3).

Time after transfer (days)	Temperature (max./min.) (C)			
	11/7-→2013-→11/7		26/20-→1511-→26/20	
	Soluble solids concentration (%)			
	Prox.	Dist.	Prox.	Dist.
0	6.2	6.0	6.5	6.4
5	6.3	6.0	6.6	6.3
10	6.3	6.5	6.3	6.4
15	7.0	7.0	6.1	6.1
20	7.8	7.3	6.4	5.9
25	8.2	8.3	5.9	5.9
28	8.3	8.6	5.8	5.5
31	9.3	10.0	5.9	5.9
34	11.0	11.9	6.4	6.5
37	10.6	11.5	6.1	6.6
40	9.8	10.9	6.5	6.3
42	9.3	10.5	6.4	6.7
44	10.0	12.2	7.0	7.4
46	11.0	13.0	7.4	7.7
48	11.2	14.3	7.9	7.8
50	10.4	12.4	7.8	7.9
52	9.9	12.1	7.5	8.2
54	10.6	12.1	8.1	8.4
56	10.6	13.6	7.9	8.8
58	10.4	12.2		
60	12.3	14.1		
62	12.8	14.5		
64	12.0	14.4		
66	13.1	15.4		
68	12.0	14.8		
70	12.6	15.4		
72	13.1	14.8		
75	12.5	15.4		

A.2.5 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown under temperature perturbation treatments (C) on immature fruit (Section 2.2) (Experiment 4).

Time after transfer (days)	Time (days) until perturbation to 14/10C							
	0		2		6		13	
	Soluble solids concentration (%)							
	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.
0	5.8	5.3	6.0	5.6	6.1	5.0	6.0	5.5
3	5.9	5.1	6.2	5.5	5.9	5.0	6.0	5.1
6	5.7	5.0	5.8	5.0	6.0	5.4	5.7	5.1
9	5.4	4.8	5.7	5.0	5.9	5.4	5.4	4.7
13	5.6	5.1	5.8	5.2	6.4	5.4	5.5	4.9
15			5.6	5.1	5.9	5.5	5.5	5.2
16	5.7	5.3						
17			5.9	5.3	6.0	5.5	5.6	5.2
20	6.0	5.6	5.6	5.2	6.1	5.6	5.7	5.2
22			5.5	5.0	6.0	5.4	5.5	5.0
23	5.8	5.4						
24			5.7	5.4	6.1	5.8	5.7	5.3
27	5.6	5.3	5.5	5.1	6.3	5.7	5.9	5.4
29			5.7	5.3	6.4	5.8	5.7	5.4
30	5.9	5.5						
31			5.8	5.4	6.4	6.0	5.5	5.3
34	6.1	6.0	5.7	5.4	6.1	5.6	5.5	5.1
41	6.1	5.8	6.4	5.4	6.3	5.8	5.6	5.4
48	6.0	5.7	5.7	5.1	6.5	6.0	5.6	5.3
56	6.9	6.6	7.1	6.2	6.5	6.1	5.5	5.2
58			6.7	6.1				
59	6.8	6.8	6.7	6.4	7.4	7.0	6.6	6.3
62	6.9	6.8	7.0	6.4	7.5	7.2	7.1	6.7
65	7.5	7.3	7.3	6.6	7.0	6.7	7.0	6.6
66	7.4	7.0	8.5	7.8	7.4	6.9	7.4	6.8
69	7.6	7.4	7.4	6.9	7.6	7.3	7.7	7.3
72	7.6	7.3	7.6	7.1	7.5	7.3	8.2	7.9
73					7.3	7.1	7.8	7.7
76	7.4	7.4	8.2	7.8	7.2	7.1	7.1	6.8

A.2.6 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown under temperature perturbation treatments (C) on immature fruit (Section 2.2) (Experiment 5).

Time after transfer (days)	Time (days) until perturbation to 14/10C					
	0		2		22	
	Soluble solids concentration (%)					
	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.
1	5.5	5.4			5.4	5.3
2			5.7	5.8		
7	5.4	5.3	5.3	5.3		
15	5.5	5.5				
22	6.4	6.2			6.2	6.1
27	6.5	6.1			7.0	7.1
36	6.4	6.3			7.4	7.3

A.2.7 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown at different orchard locations (Chapter 3).

Time of measurement (weeks)	Orchard location							
	Riwaka		Kerikeri		Palmerston North		Te Puke	
	Soluble solids concentration (%)							
	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.	Prox.	Dist.
1	4.2	4.1	4.4	4.4	4.2	4.1	4.3	4.1
2	4.4	4.3	4.5	4.6	4.5	4.5	4.4	4.3
3	4.6	4.5	4.2	4.2	4.5	4.5	4.5	4.4
4	4.8	4.7	4.5	4.5	4.7	4.7	4.6	4.5
5	4.4	4.4	4.4	4.5	4.7	4.8	4.5	4.4
6	5.0	5.1	4.8	5.1	4.7	4.8	4.8	4.9
7	4.9	5.1	4.7	5.0	4.7	5.1	4.5	4.7
8	5.4	5.8	5.2	5.7	5.1	5.5	4.8	5.1
9	5.9	6.6	5.8	6.6	5.2	5.7	4.8	5.2
10	7.0	7.7	5.6	6.3	5.5	6.2	5.3	5.7
11	7.7	8.8	6.0	7.0	6.5	7.4	5.5	6.1
12	9.2	10.8	6.8	8.0	7.3	8.2	6.5	7.2
13	9.5	11.0	7.2	8.5	7.6	8.7	7.1	8.0
14	-	-	7.9	9.4	8.2	9.4	8.0	9.0

A.2.8 Soluble solids concentration (%) at proximal and distal ends of kiwifruit grown under different leaf:fruit ratio treatments (Chapter 4).

Time of measurement (days)	Leaf:fruit ratio treatment			
	1:1		5:1	
	Soluble solids concentration (%)			
	Prox.	Dist.	Prox.	Dist.
0	3.8	4.2	4.4	4.6
21	3.8	4.2	4.3	4.7
36	4.6	5.3	5.1	6.0
46	4.4	5.3	6.8	7.7
55	4.0	5.6	7.2	9.1

APPENDIX 3

TABLES OF REGRESSION COEFFICIENTS

A.3.1 Regression coefficients from fitted linear equations for decrease in flesh firmness of kiwifruit grown under different minimum temperatures (C) (Experiment 1). From Fig. 2.4. Intercept = 98.9 N, R^2 = 0.89.

Temperature (max./min.) (C)	Mean temperature (C)	Slope (N day^{-1})
18/4	11	-0.892
18/8	13	-0.921
18/12	15	-0.540
18/16	17	-0.724

A.3.2 Regression coefficients from fitted logistic curves for increase in soluble solids concentration of kiwifruit grown under different minimum temperatures (C) (Experiment 1). From Fig. 2.5.

Temperature (max./min.) (C)	Mean temperature (C)	Intercept (%)	Slope (% day^{-1})	R^2
18/4	11	4.07	0.112	0.98
18/8	13	3.99	0.098	0.97
18/12	15	4.36	0.081	0.91
18/16	17	5.38	0.091	0.91

A.3.3 Regression coefficients from fitted linear equations for decrease in flesh firmness of kiwifruit grown under different maximum and minimum temperatures (C) (Experiment 2). From Fig. 2.6. Intercept = 86.5 N, R^2 = 0.95.

Temperature (max./min.) (C)	Mean temperature (C)	Slope (N day^{-1})
14/8	11	-0.757
18/8	13	-0.651
14/12	13	-0.613
22/8	15	-0.451
26/8	17	-0.493
22/12	17	-0.527

A.3.4 Regression coefficients from fitted logistic curves for increase in soluble solids concentration of kiwifruit grown under different maximum and minimum temperatures (C) (Experiment 2). From Fig. 2.7.

Temperature (max./min.) (C)	Mean temperature (C)	Intercept (%)	Slope (% day^{-1})	R^2
14/8	11	4.40	0.092	0.89
18/8	13	4.14	0.070	0.97
14/12	13	4.74	0.079	0.99
22/8	15	3.59	0.051	0.71
26/8	17	3.36	0.037	0.62
22/12	17	4.71	0.067	0.75

A.3.5 Regression coefficients from fitted linear equations for decrease in flesh firmness of kiwifruit grown under fixed temperature treatments (C) (Experiment 3). From Fig. 2.11. Intercept = 89.3 N, R^2 = 0.93.

Temperature (max./min.) (C)	Mean temperature (C)	Slope (N day^{-1})
11/7	9	-1.128
15/11	13	-0.914
15/15	15	-0.879
26/20	23	-0.587

A.3.6 Regression coefficients from fitted logistic curves for increase in soluble solids concentration of kiwifruit grown under fixed temperature treatments (C) (Experiment 3). From Fig. 2.13.

Temperature (max./min.) (C)	Mean temperature (C)	Intercept (%)	Slope (% day^{-1})	R^2
11/7	9	3.29	0.101	0.95
15/11	13	4.53	0.089	0.92
15/15	15	2.77	0.055	0.92
26/20	23	5.72	0.064	0.50

A.3.7 Regression coefficients from fitted linear equations for decrease in flesh firmness of kiwifruit grown at four different orchard locations. From Fig. 3.1. Intercept = 110.0 N, R^2 = 0.95.

Orchard location	Slope (N day^{-1})
Kerikeri	-0.624
Te Puke	-0.533
Palmerston North	-0.533
Riwaka	-0.568

A.3.8 Regression coefficients from fitted logistic curves for increase in soluble solids concentration of kiwifruit grown at four different orchard locations. From Fig. 3.2.

Orchard location	Intercept (%)	Slope (% day^{-1})	R^2
Kerikeri	4.54	0.044	0.92
Te Puke	4.90	0.045	0.96
Palmerston North	4.59	0.044	0.98
Riwaka	4.91	0.058	0.95

A.3.9 Regression coefficients from fitted linear equations for decrease in flesh firmness of kiwifruit grown at two different leaf:fruit ratios. From Fig. 4.2. Intercept = 100.4 N, R^2 = 0.91.

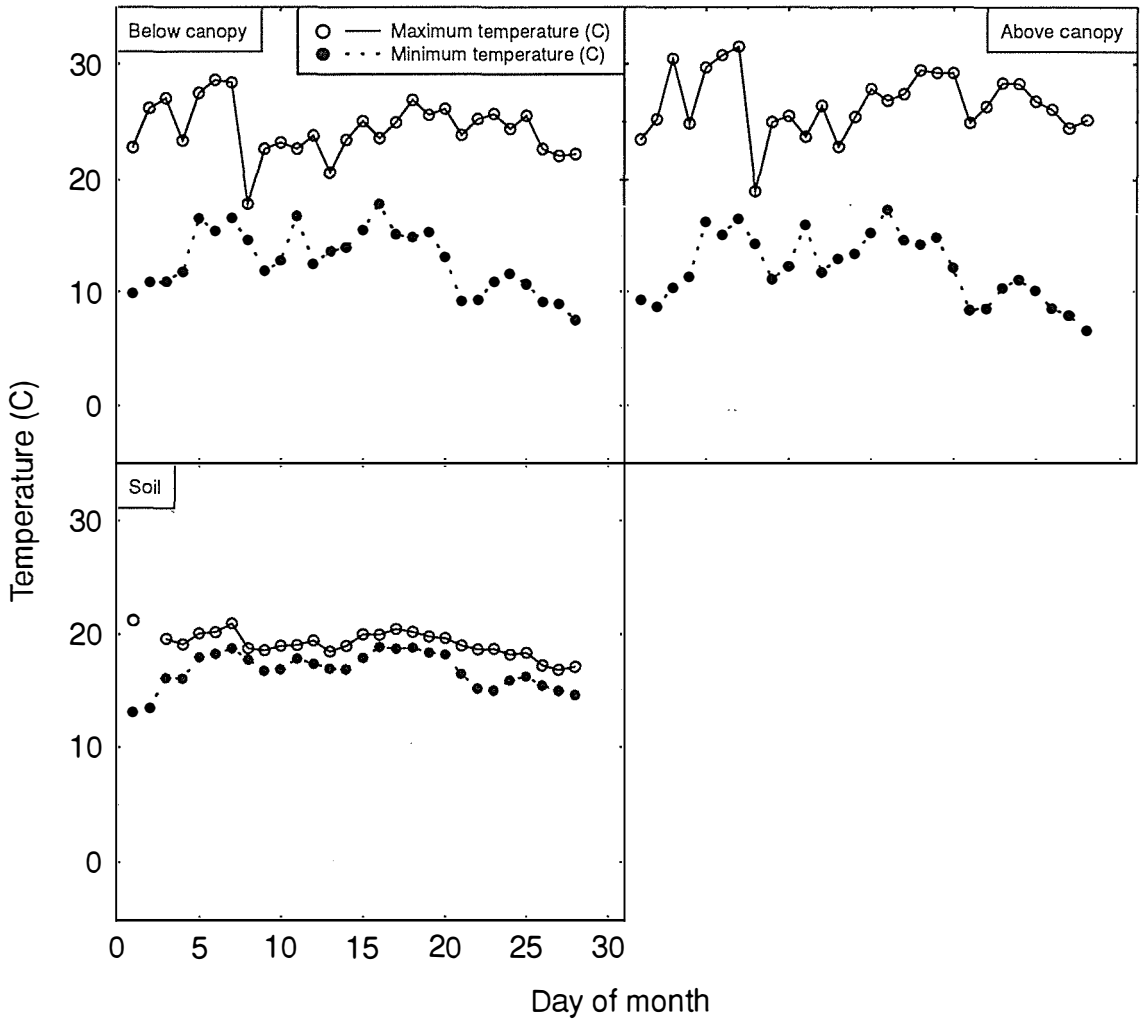
Leaf:fruit ratio treatment	Slope (N day^{-1})
1:1	-0.546
5:1	-0.620

A.3.10 Regression coefficients from fitted exponential curves for increase in soluble solids concentration of kiwifruit grown at two different leaf:fruit ratios. From Fig. 4.3. R^2 = 0.75.

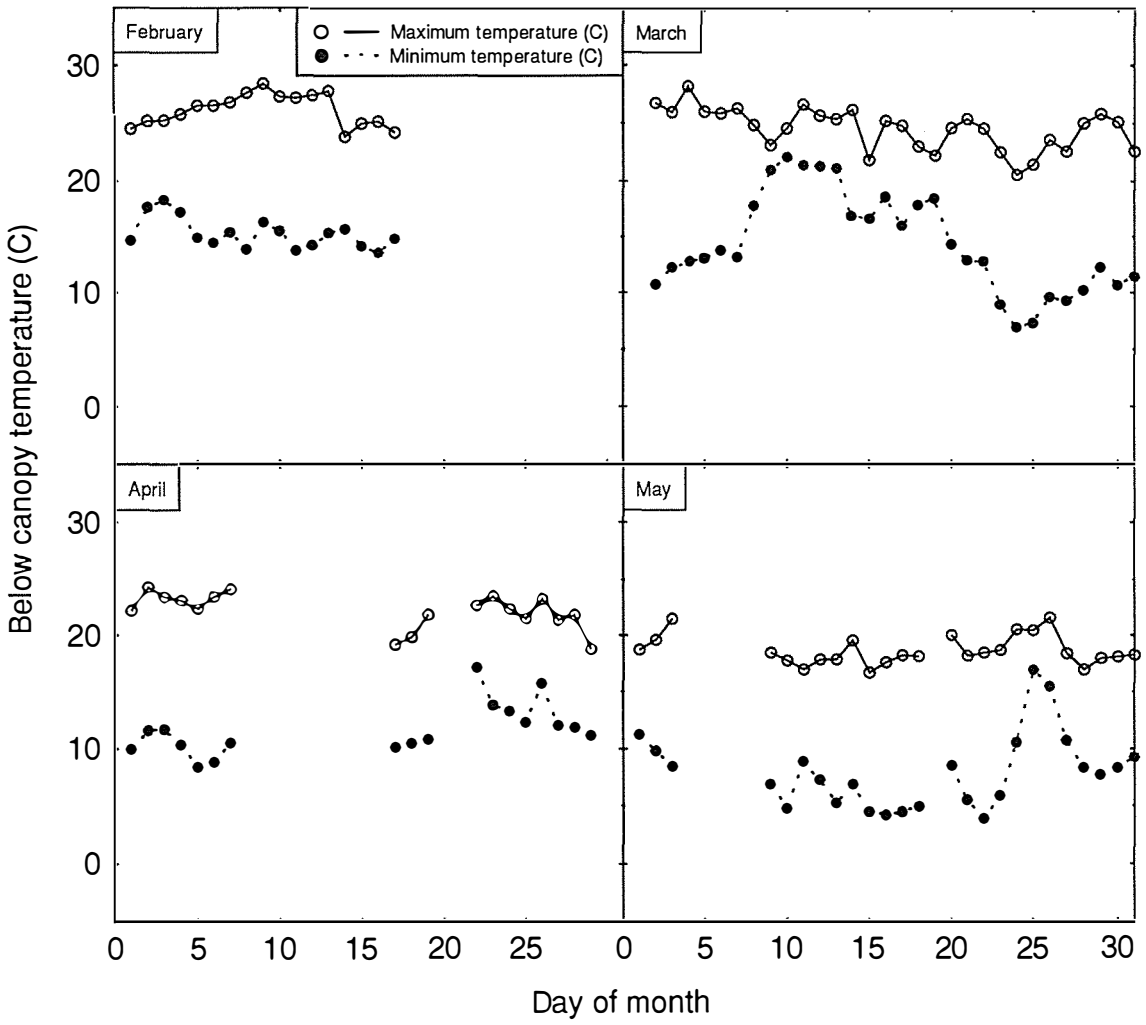
Leaf:fruit ratio treatment	Intercept (%)	Slope (% day^{-1})
1:1	3.56	0.055
5:1	3.71	0.158

APPENDIX 4

TEMPERATURE DATA FROM ORCHARD LOCATIONS AND SHELTERED SITE

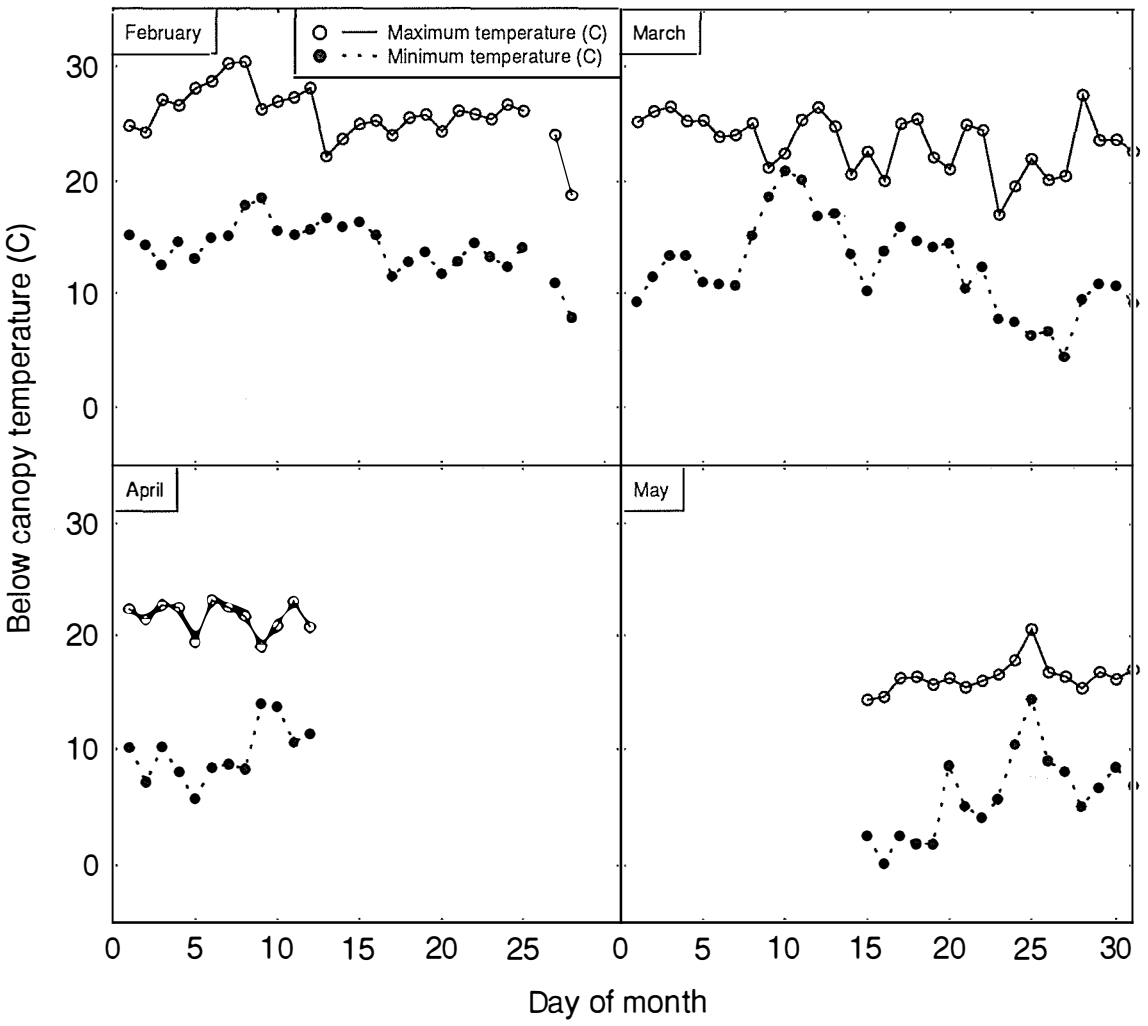


A.4.1 Maximum and minimum temperatures (C) measured below (1.3 m above ground) and above the canopy (2.3 m above ground) and soil temperature (200 mm depth) of kiwifruit grown at Riwaka in February 1990.



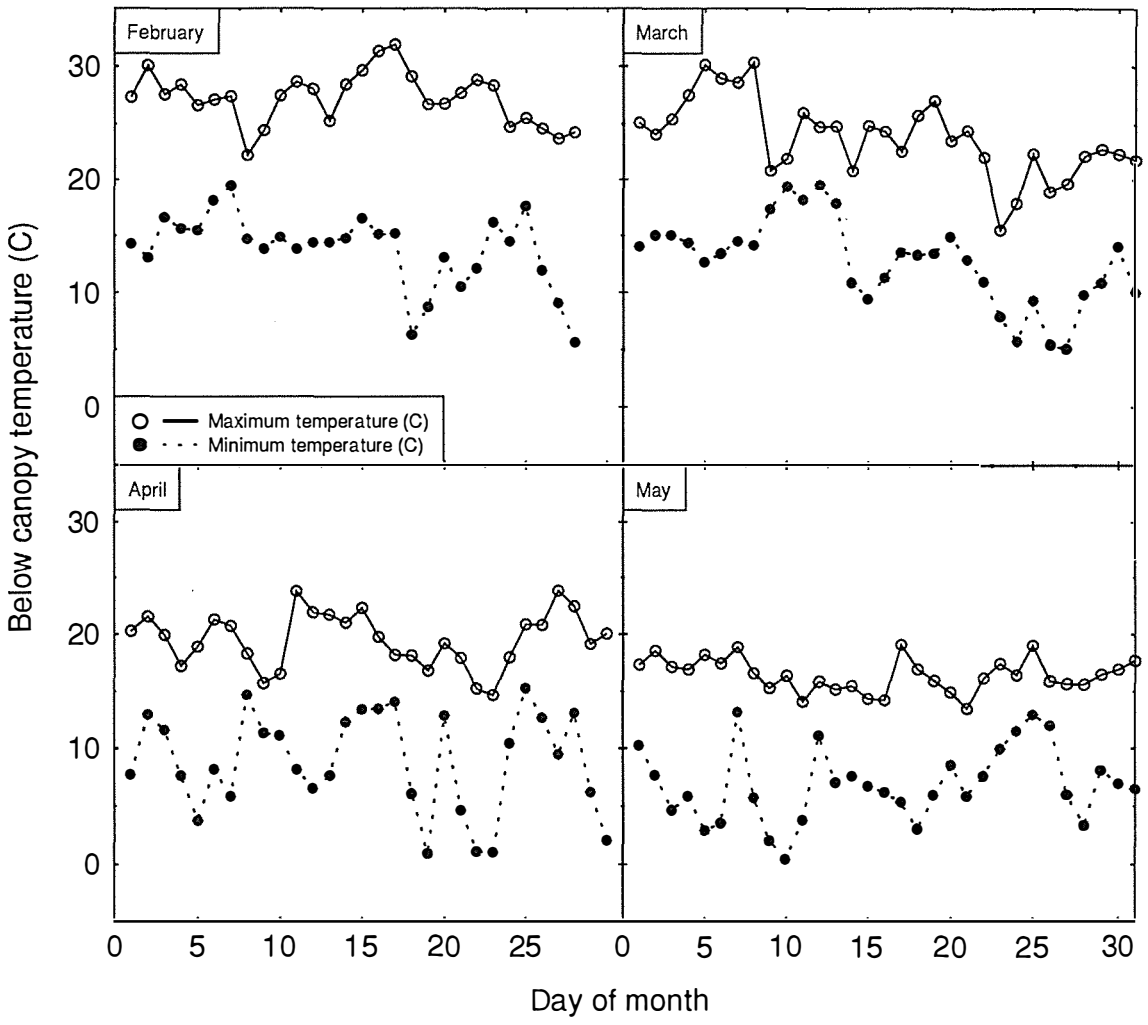
A.4.2 Maximum and minimum temperatures (C) in February, March, April and May measured below the canopy of kiwifruit grown at Kerikeri 1990.

Missing data: February 18-28, March 1-2, April 7-16 and 22-24, May 4-9 and 18-20.

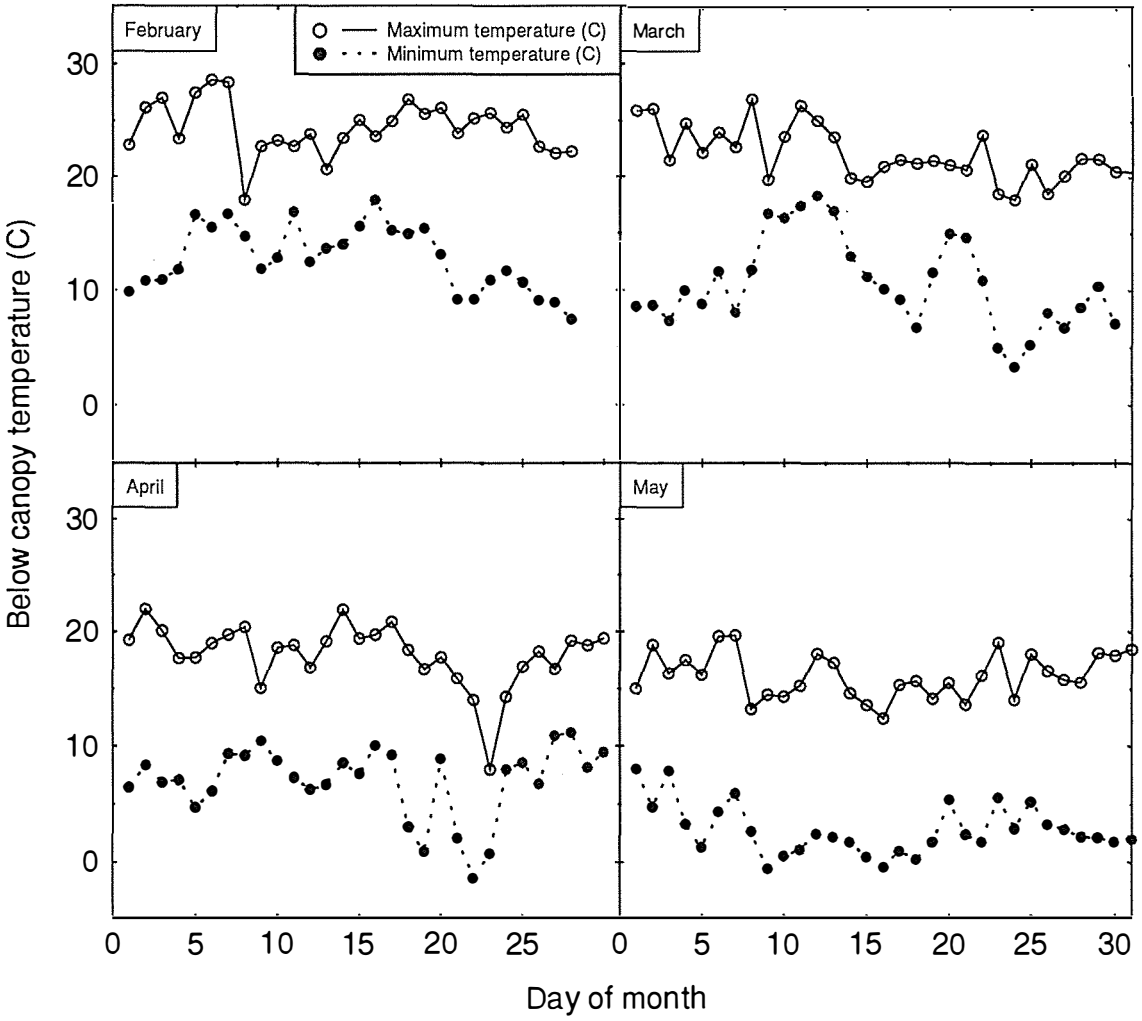


A.4.3 Maximum and minimum temperatures (C) in February, March, April and May measured below the canopy of kiwifruit grown at Te Puke 1990.

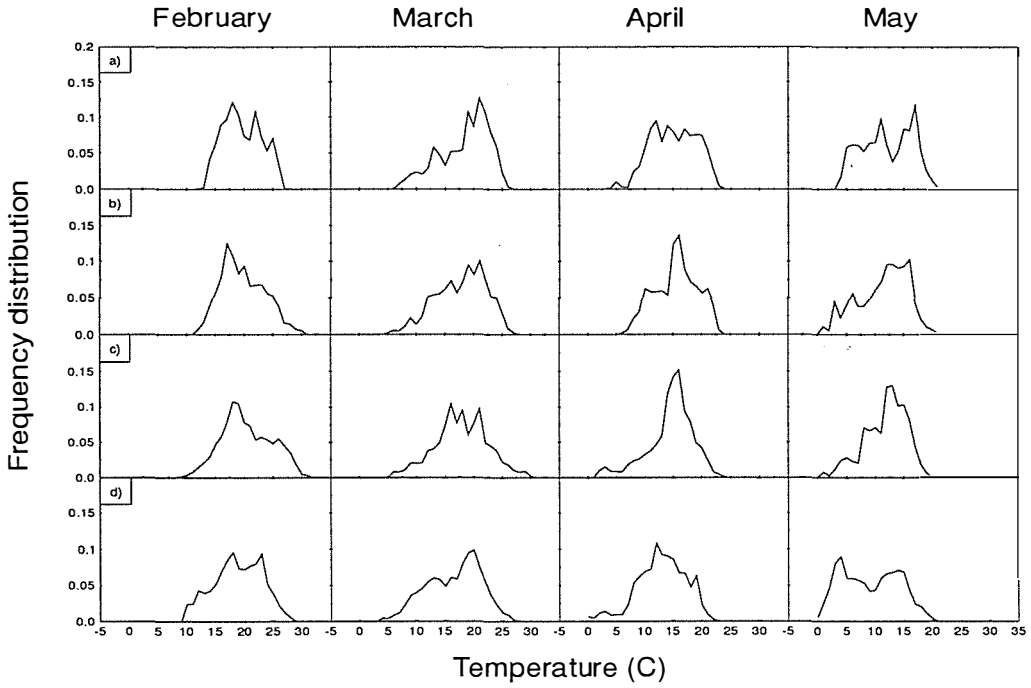
Missing data: February 26, April 13-30, May 1-15.



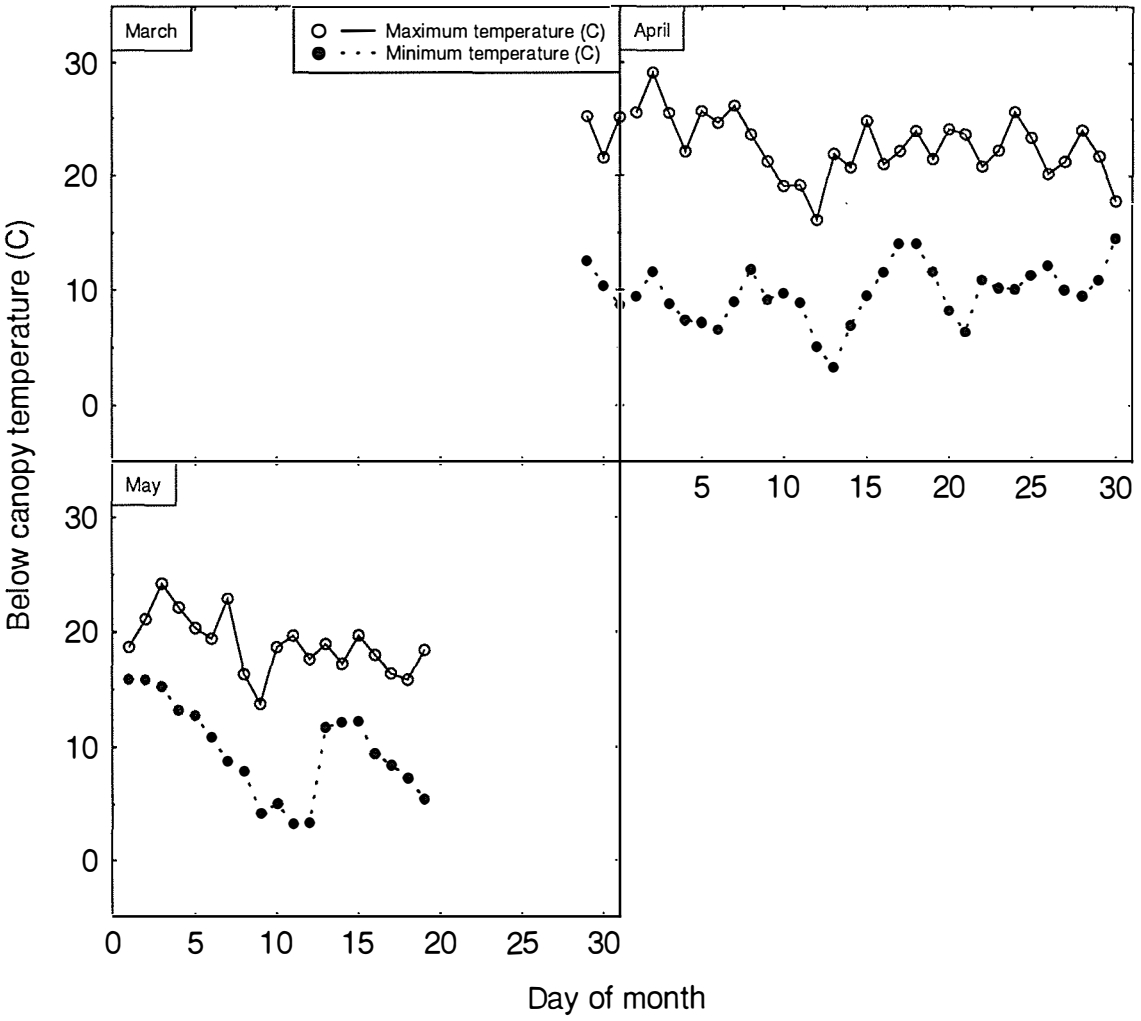
A.4.4 Maximum and minimum temperatures (C) in February, March, April and May measured below the canopy of kiwifruit grown at Palmerston North 1990.



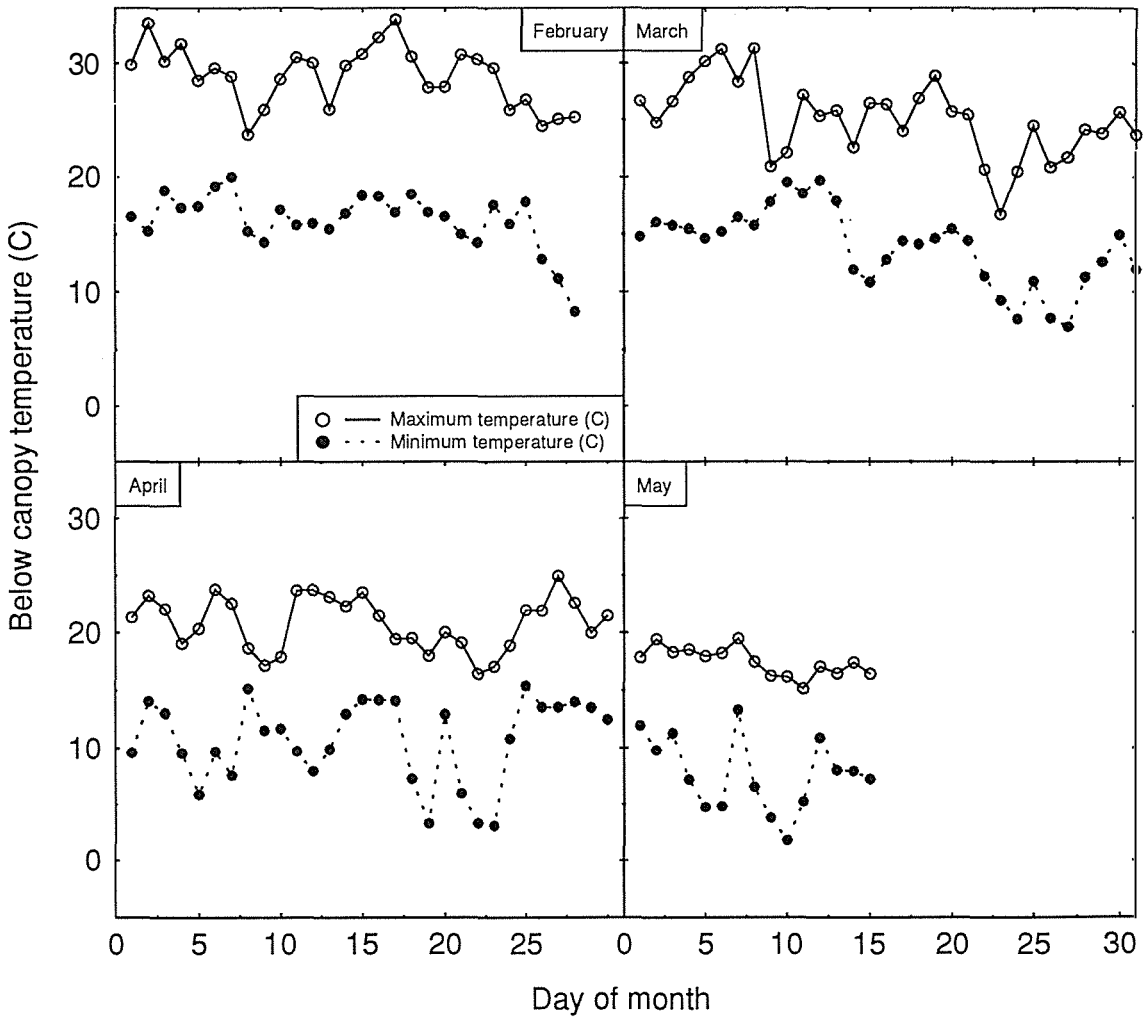
A.4.5 Maximum and minimum temperatures (C) in February, March, April and May measured below the canopy of kiwifruit grown at Riwaka 1990.



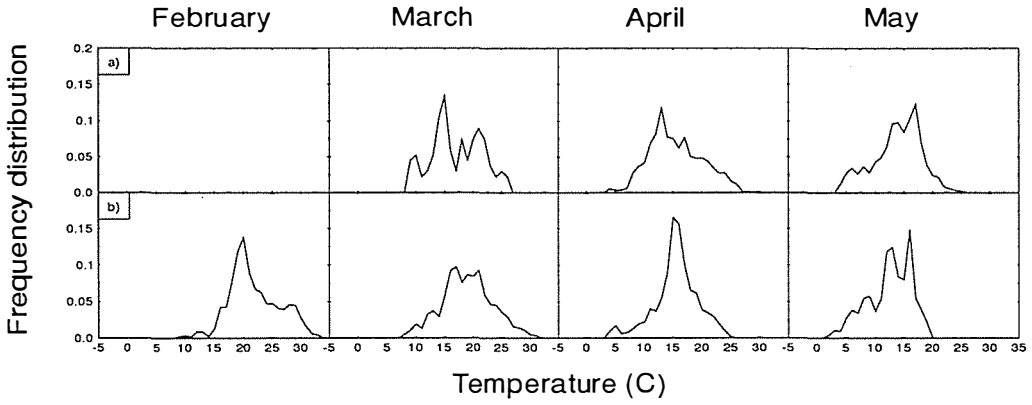
A.4.6 Frequency distribution of below canopy temperatures (C) in kiwifruit grown at a) Kerikeri, b) Te Puke, c) Palmerston North and d) Riwaka during February, March, April and May 1990.



A.4.7 Maximum and minimum temperatures (C) in February, March, April and May measured below the canopy of kiwifruit grown at the sheltered site in Palmerston North 1989.



A.4.8 Maximum and minimum temperatures (C) in February, March, April and May measured below the canopy of kiwifruit grown at the sheltered site in Palmerston North 1990.



A.4.9 Frequency distribution of below canopy temperatures (C) in kiwifruit grown in the sheltered site in a) 1989 and b) 1990, during February, March, April and May.

APPENDIX 5

MODELS FOR ACCUMULATION OF SOLUBLE SOLIDS CONCENTRATION

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A.5.1 Descriptions of accumulation of soluble solids concentration

In Chapters 2 and 3 the logistic equation

$$S = a + \frac{c}{1 + e^{-h(t - m)}} \qquad (1)$$

was used to describe accumulation of soluble solids (SSC) in fruit during maturation. Here, S is soluble solids concentration (SSC), a and $a+c$ are initial and final asymptotes, h is a rate constant, t is time and m gives the position of the point of inflection. Eq. (1) describes the entire time course of accumulation of SSC from an initial value through to a plateau at the end of ripening. Only the first part of this curve is required for the practical purpose of predicting permissible harvest dates. Alternative descriptions of changes in SSC are sought over the first part of accumulation curves - that is, up to or a little beyond the point of inflection on the curve in Fig. 5.1.

There are two obvious equations suited to this description. One is the exponential equation

$$S_j(t) = S^0 e^{h_j t}, \qquad (2)$$

where S^0 is the intercept at $t = 0$, h_j is a rate coefficient and the subscript j identifies a particular treatment. The other is an exponential equation with an additive component,

$$S_j(t) = S^b + (S^0 - S^b) e^{h_j t}, \qquad (3)$$

where S^0 is the intercept at $t = 0$ and S^b is the basal SSC (see below).

Sets of equations in the form of (2) or (3) can be fitted to experimental data under the assumption that SSC accumulation curves from a given year will share the same S^0 (eq. (2)), or S^0 and S^b (eq. (3)), but will have different h_j, according to temperature histories. This approach is illustrated in Section 5.3.1 using data from Experiments 1 and 2.

A.5.2 The two-component hypothesis for SSC accumulation

A.5.2.1 Exponential (eq. 2)

The hypothesis that SSC accumulation involves a basal, metabolic component which is present throughout fruit development and a 'maturation' component which is induced at some point during development was discussed in Chapter 6. The following is an algebraic description of this two-component hypothesis and set out its relationship with eq. (2).

If τ is introduced to measure time from a starting point such as fruit set and $\tau_i^m > 0$ to identify the point at which the maturation phase starts in the i th year, the two-component model can be written as

$$S_i(\tau) = \begin{cases} S_i^b(\tau), & \tau \le \tau_i^m \\ S_i^b(\tau) + S_i^m(\tau), & \tau > \tau_i^m \end{cases} \qquad (4)$$

where S_i^b and S_i^m are, respectively, the basal and maturation components. Eq. (4) provides a general summary of SSC accumulation. To make it useful, appropriate descriptions of each of the components and a method for determining when the maturity induction point occurs are required. A number of assumptions are required to accomplish these objectives.

The first of these concerns the basal component, S_i^b. This could vary in time during a growing season and between years. It shall be assumed, initially, that S_i^b is fixed within a season although not necessarily between years. This

assumption implies that the absolute amounts of soluble sugars and other compounds involved in basal metabolism increase in strict proportion to the absolute amount of juice as fruits grow (that is, the concentration does not change).

If S_i^b is fixed, attention can then shift to τ_i^m. Here, two cases arise, depending on the relative timing of the beginning of maturation and of experimental treatments.

Case 1: treatments begin before the beginning of maturation. If experimental treatments begin at τ_i^e and $\tau_i^e \leq \tau_i^m$, it is convenient to define a time variable, t, for the experimental period using

$$t = \tau - \tau_i^e. \tag{5}$$

If maturation starts after the beginning of experimental treatments it is possible that treatments might affect the timing of the maturity induction point. This is done by defining

$$t_{ij}^m = \tau - \tau_{ij}^m \tag{6}$$

where τ_{ij}^m and t_{ij}^m are maturity induction points in absolute and experimental time, respectively, and j identifies a specific treatment. Using eqs (5) and (6), eq. (4) can be modified to read

$$S_{ij}(t) = \begin{cases} S_i^b, & t \leq t_{ij}^m \\ S_i^b + S_{ij}^m(t), & t > t_{ij}^m \end{cases} \tag{7}$$

To proceed from eq. (7), a specific form for the maturation-component function, $S_{ij}^m(.)$ is required. One candidate is

$$S_{ij}^m(t) = \begin{cases} 0, & t \leq t_{ij}^m, \\ S_i^b[e^{h_{ij}(t - t_{ij}^m)} - 1], & t > t_{ij}^m, \end{cases} \tag{8}$$

so that S^m is zero before the maturation induction point and increases exponentially thereafter. Equation (8) implies that total SSC accumulation is described by

$$S_{ij}(t) = \begin{cases} S_i^b, & t \le t_{ij}^m, \\ S_i^b[e^{h_{ij}(t - t_{ij}^m)}], & t > t_{ij}^m. \end{cases} \quad (9)$$

Case 2: treatments begin after the beginning of maturation. If $\tau_i^e > \tau_i^m$, then t defined by eq. (5) can still be used as an experimental-time variable but eq. (4) becomes simply

$$S_{ij}(t) = S_i^b + S_{ij}^m(t). \quad (10)$$

Once again, a specific model for $S_{ij}^m(.)$ is required to proceed. If eq. (8) is adopted, changes in S^m must be accounted for before the beginning of the experimental period as well as after. Let

$$t_i^e = \tau_i^e - \tau_i^m \quad (11)$$

so that eq. (8) becomes

$$S_{ij}^m(t) = S_i^b[e^{\overline{h}_i t_i^e + h_{ij} t} - 1], \quad (12)$$

where $\overline{h}_i$ is a mean rate constant for the maturation period prior to the beginning of the experiment. If S_i^0 is defined

$$S_i^0 = S_i^b e^{\overline{h}_i t_i^e} \quad (13)$$

and then substitute (12) in (10) then

$$S_{ij}(t) = S_i^0 e^{h_{ij}t}, \qquad (14)$$

which is simply eq. (2) in a slightly modified form. Thus, the exponential equations used to describe SSC accumulation in Experiments 1 and 2 (Section 5.3.1) are compatible with the two-component hypothesis.

A.5.2.2 Exponential with additive component (eq. 3)

Equation (3) can also be related to the framework set out in Section A.5.2.1. Once again, the cases where treatments begin either before or after the beginning of the maturation phase must be considered.

Case 1: treatments begin before the beginning of maturation. In Section A.5.2.1 it was assumed that S^b_i was fixed throughout a season. In the absence of experimental data to confirm this assumption it could equally well be assumed that S^b_i varies in the season. One particular pattern of variation would be for S^b_i to assume two fixed values, say S^{b-}_i before the beginning of maturation and $S^{b+}_i < S^{b-}_i$ thereafter. Then eq. (7) becomes

$$S_{ij}(t) = \begin{cases} S_i^{b-}, & t \le t_{ij}^m \\ S_i^{b+} + S_{ij}^m(t), & t > t_{ij}^m \end{cases} \qquad (15)$$

The two fixed values for S^b_i need to be linked to each other and to the maturation component, S^m. If it is assumed that the latter increases exponentially, this can be accomplished by writing

$$S_{ij}^m(t) = \begin{cases} 0, & t < t_{ij}^m, \\ S_i^{b-} - S_i^{b+}, & t = t_{ij}^m, \\ (S_i^{b-} - S_i^{b+}) e^{h_{ij}(t - t_{ij}^m)}, & t > t_{ij}^m. \end{cases} \qquad (16)$$

The total SSC accumulation is then described by

$$S_{ij}(t) = \begin{cases} S_i^{b-}, & t \le t_{ij}^m, \\ S_i^{b+} + \left(S_i^{b-} - S_i^{b+}\right) e^{h_{ij}(t - t_{ij}^m)}, & t > t_{ij}^m. \end{cases} \quad (17)$$

Thus, the downwards jump in S^b at t_{ij}^m is matched by an upwards jump in S^m. Thereafter S^m increases exponentially and the total, S, increases as the sum of S^m and S^{b+}.

Case 2: treatments begin after the beginning of maturation. If eq. (11) is adopted to define the interval between the beginning of the maturation phase and the beginning of the experiment, eq. (17) can be modified to

$$S_{ij}(t) = S_i^{b+} + \left(S_i^{b-} - S_i^{b+}\right) e^{\bar{h}_i t_i^e + h_{ij} t} \quad (18)$$
$$S_i^{b+} + \left(S_i^{b-} - S_i^{b+}\right) e^{\bar{h}_i t_i^e} e^{h_{ij} t}.$$

If S_i^0 is defined

$$S_i^0 = S_i^{b+} + \left(S_i^{b-} - S_i^{b+}\right) e^{\bar{h}_i t_i^e}, \quad (19)$$

substitute this in eq. (18) and rearrange, then

$$S_{ij}(t) = S_i^{b+} + \left(S_i^0 - S_i^{b+}\right) e^{h_{ij} t}. \quad (20)$$

which is equivalent to eq. (3). Thus, eq. (3) is also compatible with the two-phase hypothesis for SSC accumulation.

A.5.3 Models for SSC accumulation

The equations used in Sections A.5.1 and A.5.2 merely describe observed SSC accumulation curves, or possible accumulation curves, under the two-phase hypothesis. They do not include any of the mechanisms and processes governing fruit growth and fruit maturation discussed in various chapters in this thesis.

Clearly, links between descriptive equations and models for processes are desirable.

If empirical descriptions of a process are to be justified, then there must be some underlying model for the process. This 'bottom-up' approach will often lead to a differential equation in which the rate of change of a variable describing the process is expressed as some function of other variables. Thus, for SSC accumulation a rate model might be developed in the form

$$\frac{dS}{dt} = f(I, E), \qquad (21)$$

where f is a function and I and E stand, respectively, for the sets of variables internal and external to fruit that affect SSC accumulation. Unfortunately, equations in the form of (21) are often of little value. For complicated processes such as SSC accumulation, both the nature of f and the variables that should be included in I and E are largely unknown. A simpler, more empirical 'top-down' approach to developing models for the process is required.

In the top-down approach, a rate model is constructed from the available data. The data in Chapter 2 consist of different temperature treatments and observations of changes in S that occurred under these treatments so we are restricted to development of models of the form

$$\frac{dS}{dt} = F(S, T). \qquad (22)$$

where S is SSC and T is temperature. Given (22), the first challenge is to find a suitable form for the function, F. Theory developed for analysis of biochemical systems (Voit et al. 1991) suggests the general model

$$\frac{dS}{dt} = g(T)\left[h S^{\alpha} - k S^{\beta}\right], \qquad (23)$$

where h, k, α and β are constants and $g(T)$ is a temperature-dependent rate function[1]. The model underlying eq. (1) is

$$\frac{dS^*}{dt} = g(T)\left[h S^* - (h/c)(S^*)^2\right] \qquad (24)$$

The equivalence between eqs. (24) and (23) are then $k = h/c$, $\alpha = 1$ and $\beta = 2$. The variable S in eq. (23) is treated as

$$S^* = S - S^b, \qquad (25)$$

where S^b is basal SSC. If c is infinite, eq. (24) becomes

$$\frac{dS}{dt} = g(T)\, h(S - S^b), \qquad (26)$$

after re-use of eq. (25). This is the model underlying eq. (3). In it, the rate of accumulation of SSC is equals to total SSC minus the basal level. If the rate of accumulation is proportional to total SSC the following equation is obtained

$$\frac{dS}{dt} = g(T)\, h S. \qquad (27)$$

This is the model underlying eq. (2).

Equations (26) and (27) are used as models for accumulation of SSC in this thesis. They are referred to as the *base + exponential* and the *exponential* models, respectively. A third variant of eq. (23) has also been investigated as a candidate for SSC accumulation. If k in eq. (23) is set equal to zero then

[1]It is possible to justify eq. (23) starting with rate laws for the various processes that are involved in SSC accumulation, such as sugar accumulation, starch synthesis and hydrolysis, water exchange and dry matter change.

$$\frac{dS}{dt} = g(T)\, h\, S^{\alpha}. \qquad (28)$$

This is referred to as the *power-law* model. It can be shown that this model is compatible with the two-component hypothesis for SSC accumulation using an analysis that parallels Section A.5.2.

A.5.4 A discrete-rate model for rate coefficients

Results discussed in Chapters 2 and 3 demonstrate the general thesis that high rates of SSC accumulation are associated with low mean temperatures and vice versa. Here a better model is required for the dependence of SSC accumulation on temperature. Equation (27) will be used to illustrate a discrete-rate approach to finding such a model.

Equation (27) models SSC accumulation as a process in which the rate of accumulation is proportional to the amount of SSC present and in which the proportionality relationship is temperature-dependent. This temperature dependence is embodied in the rate-coefficient function *g(T)*. The problem is to define the nature of this function using available experimental data.

Equation (27) can be integrated formally to give

$$S_j(t) = S^0 \exp\left(h \int_0^t g(T_j(s))\, ds \right) \qquad (29)$$

where $T_j(s)$ describes the time course of temperature in the j th treatment and s is a dummy variable of integration. This equation can be modified into a more tractable form using the 'discrete-rate' approach of McNaughton *et al.* (1985). In this approach, the temperature treatment to which plants are exposed during maturation is divided into discrete classes and the mean value of the rate-

coefficient function applying to each class is found. Temperature classes used for Experiments 1 and 2 are listed in Tables 5.1 and 5.2. Plants were exposed to fixed daily temperature cycles in each treatment so the fraction of time between harvests spent in the k th temperature class in the j th treatment can be specified as f_{jk} (Tables 5.1 and 5.2). Using g_k to stand for the mean rate coefficient over the k th temperature interval and the theory described on p. 582 of McNaughton *et al.* (1985), eq.(29) can be rewritten as

$$S_j(t) = S^0 \exp\left(h \, \Sigma_k \, g_k f_{jk} \, t \right). \qquad (30)$$

Thus, the integral in eq. (29) now appears as a linear combination of known fractions of time, the f_{jk}'s, and of unknown mean values for the rate-coefficient function, the g_k's. Values for the latter can be estimated by fitting equations in the form of (30) simultaneously to each of the treatments involved in an experiment.

The steps from eq. (27) to eq. (30) can be matched using eqs (26) (base + exponential model) and eq. (28) (power-law model) as starting points. For eq. (26) the equivalent of eq. (29) is

$$S_j(t) = S^{b+} + (S^0 - S^{b+}) \exp\left(h \, \Sigma_k \, g_k f_{jk} \, t \right) \qquad (31)$$

(cf. eq. (20)). For eq. (28) it is

$$S_j(t) = \left[\frac{1}{S^0} + (1 - \alpha)\left(h \, \Sigma_k \, g_k f_{jk} \, t \right) \right]^{\frac{1}{1 - \alpha}} \qquad (32)$$

Note that in these equations, and in eq. (30), the rate parameter, h, appears multiplicatively in front of the summation. This means the equations can be simplified by defining

$$g_k^* = h g_k. \qquad (33)$$

Parameter estimates obtained using eqs (30)-(32) modified in this fashion are illustrated and discussed in Section 5.3.1 using data from Experiments 1 and 2.

A.5.5 Empirical rate-coefficient functions

The advantage of the discrete-rate method is that it reveals patterns of responses to environmental factors and opens the way for selection of suitable continuous response functions that is, for the *g(T)* function in eq. (16). The pattern of discrete-rate coefficients in Fig. 5.5 suggests that a suitable rate-coefficient function should fall towards zero at about 24C. The data also suggest that the function should have either a plateau or a maximum value at around 10C. Since Fig. 5.5 suggests that there is a low-temperature cut-off in SSC accumulation, a suitable function should rise from zero to a maximum value at around 10C before falling again towards zero at higher temperatures.

A number of functions have this behaviour. The function used in this thesis is based loosely on an equation derived using rate-response theory (Johnson and Thornley 1985). The rate response of a catalysed chemical reaction where the rate coefficient for conversion of substrates into products follows an Arrhenius response curve to temperature and the catalyst becomes inactive at high temperatures can be described using

$$g(K) = \frac{A e^{-a/K}}{1 + B e^{-b/K}} \qquad (34)$$

where K is temperature in degrees Kelvin and A, a, B and b are constants. This equation describes a temperature response that starts from zero at $K = 0$, rises to a peak and then declines towards an asymptote of $A/(1+B)$.

Extremely large values are often required for A and B and this makes eq. (34) difficult to fit to data[2]. Analysis in this thesis has therefore been carried out using eq. (34) written in terms of degrees Celsius. With this scale for temperature, eq. (34) is forced to zero at 0C.

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[2]A reparameterisation that makes this job much easier was discovered too late to be used in this thesis.

A.5.6 Fitting the rate-coefficient function

Choice of a particular form for *g(T)* means that the sums in eqs (28), (31) and (32) can be replaced by integrals. Thus, eq. (28) can be written

$$S_j(t) = S^0 \exp\left(h \int_0^t \frac{A e^{-a/T_j(s)}}{1 + B e^{-b/T_j(s)}} \, ds \right) \tag{35}$$

with *s* as a dummy variable of integration. Unfortunately, this equation, and its equivalents for the base + exponential and power-law models, cannot be fitted directly to experimental data because the integral does not have an analytical solution. To proceed, schemes must be established for numerical solution of eqs (26)-(28) with eq. (34) appearing for *g(T)*.

The starting point for this scheme is the approximation that

$$\frac{S_t - S_{t-1}}{\Delta t} \approx \frac{dS}{dt}, \tag{36}$$

when Δt is small. This means that if *t* is measured in hours rather than days, as previously, the SSC value, S_t, at the end of an hour can be approximated in terms of the value at the beginning of an hour, S_{t-1}, by writing

$$S_t \approx S_{t-1} + \frac{dS}{dt} \Delta t. \tag{37}$$

The next step is to substitute eq. (36) in one of the candidate models for SSC accumulation. For example, in the case of the exponential model, eq. (27), can be written

$$S_t \approx S_{t-1} + S_{t-1} \left(h \frac{A e^{-a/\bar{T}}}{1 + B e^{-b/\bar{T}}} \right) \Delta t, \tag{38}$$

where $\bar{T}$ is the mean temperature over the hour between *t-1* and *t*.

Eq. (37), or its equivalents for the base + exponential and power-law models, provides the basis for a numerical integration scheme that can be used in conjunction with nonlinear least-squares estimation to find values for the parameters in eq. (33) from experimental data. Starting with an initial guess for S^0 and the mean temperature in a treatment over the first half-hour a value can be estimated at the end of the half-hour, say S_j^1. This value can then be used in conjunction with the mean temperature over the second half-hour to find S_j^2, and so on until the end of an experimental run. Differences between observed and estimated SSC accumulations are calculated whenever the counting index for hours matches a sampling time in days. Estimates of parameters can then be obtained by minimising these residuals over all harvests and all treatments.

This numerical scheme was set up within the DUD nonlinear routine of SAS and fitting was carried out on a combined data set made up of SSC measured in both Experiments 1 and 2. This combined analysis necessitated one further modification. It is apparent from Fig. 5.5 that discrete-rate coefficients vary between years, particularly when the exponential and power-law models are used for SSC accumulation. To accommodate this possibility in the analysis of the combined data set, h and A in eq. (38) were combined into a single year-dependent rate coefficient, (h_iA). This separation means that the fitted $g(T)$ is fixed across years and may therefore reflect a true underlying physiological response. Parameters and fitted functions obtained on this basis are discussed in Section 5.3.1.

A.5.7 Scaling the rate-coefficient function

The fitted rate-coefficient functions illustrated in Fig. 5.8 differ according to the model used for SSC accumulation. Similarities between the functions can be illustrated by rescaling. If g_{max} is the maximum value for a fitted function at the optimum temperature, then

$$(h_f A)\, g(T) = (h_f A)\, g_{max} \frac{g(T)}{g_{max}} = h_i^*\, g^*(T), \quad (39)$$

so that $g^*(T)$ is scaled to 1.0 at the optimum temperature and h_i^* is a new, year-dependent rate coefficient. This scaling is discussed further in Section 5.3.1.

A.5.8 Testing the models

Data on SSC accumulation in Experiments 3 and 4, in the sheltered site and in orchards are available for testing the models described in preceding sections. There are three possibilities:

i) Fitted models and parameters apply directly to alternative data sets.

ii) Fitted models apply, but new parameters are required either for g(T), or for the kinetics of SSC accumulation, or both.

iii) A totally different model is required.

Analyses of the efficacy of various combinations of fixed and fitted parameters were then carried out. These are discussed in Section 5.3.2. In all of these cases, curves through observed data were generated using modifications of the finite difference scheme described in Section A.5.6.

A.5.9 Use of maximum and minimum temperatures as input data

The integration method used in fitting and testing models, and outlined in Section A.5.7, is only feasible when temperature records are complete and frequent. It breaks down if there are gaps in records[3], as occurred at Kerikeri

[3]Gaps of a few hours in a day could be accommodated using a suitable interpolation scheme to bridge missing observations.

and Te Puke (Section 3.1.2), and cannot be used at all when temperature records are restricted to a few daily values, such as the maximum and minimum temperatures available from standard meteorological stations.

In many practical situations, temperature maxima and minima are the only available data. For this reason, considerable effort has been devoted to developing methods for using these data to generate synthetic temperature functions. Usually, some linear or curvilinear interpolation method is used for this purpose (Reicosky *et al.*, 1989) but methods based on temperature frequencies have also been developed (A.J. Hall and H.G. McPherson, HortResearch, pers. com.). Here, a third approach contains elements of both these methods.

This approach has three stages:

i) changing integration steps from hours to days,

ii) estimating temperature effects using linear interpolation between maximum and minimum temperatures plus a daily correction factor and

iii), estimating values for the correction factor from temperature maxima and minima.

These stages are illustrated for the base + exponential model, eq. (26). This can be integrated to give

$$S(t) = S^{b+} + (S^0 - S^{b+}) \exp\left(h^* \int_0^t g^*(T(s))\, ds \right) \qquad (40)$$

Here, eq. (39) has been used to replace $(h_i A).g(T)$ with $h^*.g^*(T)$. When t is an integer equal to d the integral in eq. (40) can be rewritten as

$$\int_0^t g^*(T(s))\, ds = \int_0^{d-1} g^*(T(s))\, ds + \int_{d-1}^{d} g^*(T(s))\, ds. \qquad (41)$$

where d counts numbers of days from the first SSC measurement. Back substitution into eq. (40) followed by some manipulation then leads to

$$S(d) = S^{b+} + (S(d-1) - S^{b+}) \exp\left(h^* \int_{d-1}^{d} g^*(T(s))\, ds \right) \qquad (42)$$

This accomplishes stage *(i)* of the new approach: using eq. (42) daily steps can be taken forward from the initial SSC, provided the integral can be evaluated.

Stage *(ii)* of the new approach involves a rather long argument so the result is given first. Under various assumptions, yet to be specified, eq. (42) can be simplified by rewriting the integral as

$$\int_{d-1}^{d} g^*(T(t))\, dt = k_d\, \overline{g^*(T)}, \qquad (43)$$

where $\overline{g^*(T)}$ is the mean value of g^* over the temperature range from T_d^n to T_d^x, the maximum and minimum temperatures on d th day, and k_d gives the ratio between the original integral and this mean on a specific day.

Eq. (43) is derived as follows. The temperature range in the d th day is divided into J_d classes of width

$$\Delta_d = \left(T_d^x - T_d^n \right) / J_d \qquad (44)$$

and the indicator function is defined

$$I_j(t) = \begin{cases} 0, & T(t) < T_j \\ 1, & T(t) \geq T_j \end{cases} \qquad (45)$$

Then the integral in eq. (43) becomes

$$\int_{d-1}^{d} g^*(T(t))\,dt = \sum_{j=1}^{J_d} \left\{ \int_{d-1}^{d} \left[l_{j-1}(t) - l_j(t) \right] g^*(T(t))\,dt \right\} \tag{46}$$

If the total amount of time spent in the jth temperature class on the dth day is

$$f_{dj} = \int_{d-1}^{d} \left[l_{j-1}(t) - l_j(t) \right] dt, \tag{47}$$

and the temporal-mean temperature in this period is

$$\overline{g_{dj}} = \left(1/f_{dj}\right) \int_{d-1}^{d} \left[l_{j-1}(t) - l_j(t) \right] g^*(T(t))\,dt, \tag{48}$$

then eq. (46) becomes

$$\int_{d-1}^{d} g^*(T(t))\,dt = \sum_j \overline{g_{dj}}\, f_{dj}. \tag{49}$$

The temperature-based mean of g^* in the jth interval is now defined by

$$g_j = \left(1/\Delta_d\right) \int_{T_{j-1}}^{T_j} g^*(T)\,dT. \tag{50}$$

Equation (49) can be written as

$$\int_{d-1}^{d} g^*(T(t))\,dt = \sum_j g_j f_{dj} - \sum_j \left(\overline{g_{dj}} - g_j\right) f_{dj}. \tag{51}$$

Since the fractions of time spent in each temperature class, the f_{dj} in eq. (51), are unknown let

$$\delta = 1/J_d \tag{52}$$

be the fraction of a day that would be spent in each temperature class if change between T_d^n and T_d^x were linear. Equation (51) can be written as

$$\int_{d-1}^{d} g^*(T(t))\,dt = \delta \sum_j g_j - \sum_j (f_{dj} - \delta)\, g_j - \sum_j (\overline{g_{dj}} - g_j)\, f_{dj}. \tag{53}$$

Now it follows from (44), (50) and (52) that

$$\begin{aligned} \delta \sum_j g_j &= (\delta / \Delta_d) \left[\int_{T_d^n}^{T_1} g^*(T)\,dT + \ldots + \int_{T_{J_d-1}}^{T_d^x} g^*(T)\,dT \right] \\ &= \frac{1}{T_d^x - T_d^n} \int_{T_d^n}^{T_d^x} g^*(T)\,dT \\ &\equiv \overline{g^*(T)}. \end{aligned} \tag{54}$$

so that the first term on the right-hand side of eq. (53) is simply the mean value of g^* over the temperature range from T_d^n to T_d^x. Thus, eq. (53) can be rewritten as

$$\int_{d-1}^{d} g^*(T(t))\,dt = \overline{g^*(T)} \left[1 - \frac{\sum_j (f_{dj} - \delta)\, g_j}{\overline{g^*(T)}} - \frac{\sum_j (\overline{g_{dj}} - g_j)\, f_{dj}}{\overline{g^*(T)}} \right] \tag{55}$$

(*i*) (*ii*)

Setting k_d equal to the bracketed term on the right-hand side of eq. (55), then eq. (43) is derived. It follows that eq. (42) can be written as

$$S(d) = S^{b+} + (S(d-1) - S^{b+}) \exp\left(h^* k_d\, \overline{g^*(T)} \right) \tag{56}$$

This completes stage *(ii)*.

The net effect of the steps to eq. (56) is that the integral based on the unknown daily time course of temperature is replaced by a temperature-based average, calculated from daily maxima and minima, and by a new daily term, k_d. This is a gain provided the latter can be evaluated.

It is clear from eq. (55) that k_d acts as a daily correction factor that accounts for the error that arises when a temperature-based mean is used in place of the daily integral. The are two sources for this error. Term *(i)* in eq. (55) shows the relative error that arises from using a linear time course for daily temperature in place of the true time course. Term *(ii)* gives the relative error caused by use of the temperature-based mean of $g^*()$ in place of the true temporal mean. Clearly, the temperature-based mean will closely approximate the true daily integral only when both of these terms are small. But a more pertinent question is - what range of values does k_d take and can these be estimated empirically? This, the third stage of the new approach, is examined in Section 5.3.2.

A.5.10 References

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