Understanding the flesh browning disorder of 'Cripps Pink' apples

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Declaration of originality

The text of this thesis contains no material that has been accepted as part of the requirements for any degree or diploma in any university, or any material previously published or written unless reference to this material is made.

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James, H., G. Brown, E. Mitcham, D. Tanner, S. Tustin, I. Wilkinson, A. Zanella, and J. Jobling. 2005. Flesh browning in Pink Lady™ apples: research results have helped to change market specifications for blush colour which is an added bonus for growers. Proceedings of the International Conference Postharvest Unlimited Downunder 2004. Acta Horticulturae. 687, 175-180.

James, H., J. Jobling, D. Tanner, S. Tustin, and I. Wilkinson. 2005. Climatic conditions during growth relate to risk of Pink Lady™ apples developing flesh browning during storage. Proceedings of the 9th International Controlled Atmosphere Research Conference. In Press.

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Abstract

The Flesh Browning (FB) disorder of 'Cripps Pink' apples presents a significant threat to the established market identity of the 'Cripps Pink' apple in Australian and export markets. Climatic conditions during fruit growth and development predispose 'Cripps Pink' apples to developing the FB disorder during storage. The FB disorder can be classified into two distinct disorders based on their physiological and structural differences and by seasonal climatic conditions. The diffuse type of FB (DFB) is a chilling injury, occurring in districts or seasons accumulating less than 1100 growing degree days (GDD) above 10^{o}C between full bloom and harvest. In these climatic conditions, 'Cripps Pink' apples have delayed postharvest ethylene production. Diffuse FB effects fruit cortex tissue and is characterised as cellular collapse. Storing fruit at 3^{o}C can reduce the incidence of DFB. The radial type of FB (RFB) is primarily a senescent disorder, occurring in districts or seasons accumulating greater than 1400 GDD above 10^{o}C between full bloom and harvest. In these climatic conditions, postharvest ethylene production is not delayed. Radial FB affects the cells adjacent to the vascular tissue of the fruit and is characterised by damaged cell walls. Storing fruit at 1^{o}C can reduce the incidence of RFB. Harvest maturity and the level of CO_2 in the storage atmosphere are additive influences on the development of RFB. Seasons or districts accumulating more than 1700 GDD have a very low risk for developing RFB. Seasonal climatic conditions can provide a guide for predicting the risk of developing RFB and DFB during storage.

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Abbreviations

1-MCP	1-Methylcyclopropene
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	analysis of variance
AVG	aminoethoxyvinylglycine hydrochloride
B	boron
BBD	braeburn browning disorder
BG	background
BH	brown heart
CA	controlled atmosphere
Ca	calcium
CB	core browning
CI	chilling injury
CO_2	carbon dioxide
Cu	copper
DAFB	days after full bloom
DBH	days before harvest
DFB	diffuse flesh browning
DNA	dioxyribonucleic acid
DPA	Diphenylamine
EC	ethylene climacteric
FB	flesh browning
Fe	iron
FF	flesh firmness
GDD	growing degree days
IB	internal browning
IEC	internal ethylene concentration
K	potassium
LSD	least significant difference
LTB	low temperature breakdown
Mg	magnesium
Mn	manganese
O_2	oxygen
P	probability
P'	phosphorus
RDI	regulated deficit irrigation
REML	residual maximum likelihood
RFB	radial flesh browning
RNA	ribonucleic acid
S	sulphur
SAM	S-adenosylmethionine
SEM	scanning electron microscopy
SG	skin greasiness

SPI	starch pattern index
TCA	tricarboxylic acid
TSS	total soluble solids
Zn	zinc
TCSA	trunk cross sectional area

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General Introduction

1.1 The ‘Cripps Pink’ cultivar

The ‘Cripps Pink’ apple cultivar was developed in the Western Australian apple breeding program and was released for commercial evaluation in 1986 (Cripps et al., 1993). The West Australian apple breeding program began in 1972 with a program that produced 700-1200 seedlings a year, the majority of which came from the cross of the ‘Golden Delicious’ cultivar with the ‘Lady Williams’ cultivar (Nicholas and Vermey, 1998). This cross aimed to combine the sweet flavour of ‘Golden Delicious’ with the firm, long storing qualities of the ‘Lady Williams’ cultivar (Cripps et al., 1993). One of the end results of this program was the development of the ‘Cripps Pink’ cultivar. The ‘Cripps Pink’ cultivar has since become a major variety around the world and is regarded as a cultivar with premium qualities. It has a distinctive colour and flavour and does not have the tendency to go mealy during long term storage.

The ‘Cripps Pink’ apple is medium to large in size (70 to 75 mm average diameter) and oblong-conical in shape, with areas of green skin and a solid pink-red blush (Cripps et al., 1993). The flesh of the fruit is white, dense and moderately juicy (Cripps et al., 1993). Sensory analysis has indicated that the ‘Cripps Pink’ apple had the highest level of acceptability of several late season cultivars (Corrigan et al., 1997). As a result, consumers indicated that they would buy ‘Cripps Pink’ apples more frequently than other apple cultivars and would be willing to pay a price premium (Corrigan et al., 1997).

The ‘Cripps Pink’ apple continues to be recognised as a new cultivar. As a late season cultivar, the ‘Cripps Pink’ apple has the market advantage of fitting into a production gap, extending the harvest season. New apple cultivars, such as ‘Cripps Pink’ with distinct qualities are rapidly expanding their share of the current market, at the expense of the more traditional cultivars (O'Rourke, 2003).

In order to establish a strong position among the new apple cultivars that have become available, the ‘Cripps Pink’ apple has been extensively marketed in export

markets under the name Pink Lady™. The widespread marketing has resulted in a unique market identity with the Pink Lady™ apple being one of the first apple varieties to be branded in the same way that food companies brand processed products. The Pink Lady™ trademark is owned by Apple and Pear Australia Ltd and a royalty of $US1 per carton of apples covers licensing and marketing costs associated with the trademark branding and development. As a result of this marketing, the Pink Lady™ apple has become the most popular apple in supermarkets throughout the United Kingdom and attracts a price of up to 4 times that of other cultivars and accounts for approximately one third of the $30 million value of Australian exported apples (Studdert, 2002; Wilkinson, 2000). The growth of the popularity of the Pink Lady™ apple in the United Kingdom has been helped by substantial promotion and advertising. In the United Kingdom, the Pink Lady™ apple has been advertised in magazines such as Marie Clair and Cosmopolitan in order to attract young female consumers who are thought to be more likely to associate with the branding of the apple as an 'upmarket' product (Gapper, 2004). However, concerns have also been raised that the apple is becoming so popular that it will lose this upmarket niche and become "just another new variety" (Gapper, 2004). Maintaining the quality of the cultivar is therefore essential to ensure ongoing consumer satisfaction and loyalty to the Pink Lady™ brand.

This unique market identity was tainted in the year 2000 when the quality of exported Pink Lady™ apples was found to suffer due to the fruit being harvested late (Wilkinson, 2000). Shipments arriving in the United Kingdom following 5 weeks of air storage and a 6 week sea-freight voyage were found to be below the acceptable limits for flesh firmness and had also developed a greasy coating on the skin of the fruit prompting stricter quality guidelines to be established (Wilkinson, 2000). In September of the same year, the first occurrence of the flesh browning disorder was also recorded (Brown et al., 2003). Flesh browning was first recognised in Tasmania in fruit that had been stored in controlled atmosphere storage and the disorder was soon reported throughout other growing regions of Australia and around the world (Brown et al., 2003). In the year 2003, 35 containers of Pink Lady™ apples that had been exported from Australia to the United Kingdom had been rejected due to the presence of the flesh browning disorder (Anon., 2004), representing a large economic loss to Australian apple growers and the potential loss of the reputation of the Pink Lady™ brand.

1.2 The flesh browning disorder of 'Cripps Pink' apples

The flesh browning disorder was found to be sporadic in nature, with symptoms not being present in every season or in every district. Being such an unpredictable and intermittent disorder, it rapidly undermined the established confidence in the cultivar and eroded the market advantage that had been developed.

The flesh browning disorder was defined as three separate disorders (Figure 1.1), diffuse flesh browning, radial flesh browning and CO_2 injury based on the visual expression of symptoms (Jobling et al., 2004). These symptoms were observed in 'Cripps Pink' apples grown in different regions internationally. No clear cause of either of the flesh browning disorders was easily identified; however anecdotal reports suggested a range of factors that might be involved including fruit maturity and nutrition, storage conditions and climatic conditions.

Figure 1.1 The flesh browning disorder of 'Cripps Pink' apples. Left: diffuse flesh browning (DFB), centre: radial flesh browning (RFB), right: CO_2 injury. Images adapted from Jobling and James (2004).

In order to determine the pre and postharvest factors responsible for predisposing Pink Lady™ apples to developing the flesh browning disorder during storage, in 2002 a research project entitled 'Understanding the flesh browning disorder of Pink Lady™ apples' was established. The project was funded by Horticulture Australia (project number HAL AP02009) and involved collaboration between the University of Sydney (Australia), Sydney Postharvest Laboratory (Australia), Food Science Australia (Australia), the Victorian Institute of Horticultural Development (Australia) the University of California (United States of America), HortResearch (New Zealand) and the Laimburg Agricultural Research Centre (Italy). The collaborative project examined a wide range of pre and postharvest factors for their potential involvement in the development of the flesh browning disorder. Although this was a collaborative project, the research presented in this thesis is wholly the work of the candidate and

the use of results or conclusions from other groups within the project are duly referenced.

When the international project began, the three expressions of flesh browning of 'Cripps Pink' apples had not been defined based on physiological factors and were thought of as different expressions of the one disorder. Research by Jobling et al. (2004) found that fruit maturity at harvest was a critical factor predisposing fruit to developing the disorder during storage. Research by DeCastro and Mitcham (2004) found that the level of CO_2 in the storage atmosphere was a significant factor resulting in the development of injury. Research by Zanella (2004) showed that the storage temperature was also a significant factor leading to the development of the disorder. However, in that research, the type of flesh browning present had not been defined as being the radial, diffuse or CO_2 Injury type.

1.3 Thesis outline

The objectives of the research presented in this thesis were to investigate the expressions of flesh browning of 'Cripps Pink' apples in Australian growing regions and to establish the physiological causes of each disorder to determine if they had similar or contrasting mechanisms of injury. This research also had an additional outcome of producing recommendations for the commercial storage of 'Cripps Pink' apples to minimise or prevent the development of the flesh browning disorder.

Chapter 2 covers the influences of climatic conditions during fruit growth on the processes of maturation and ripening of 'Cripps Pink' apples. This chapter examines a range of maturity indicators and their relationship to ethylene production in order to determine the most appropriate maturity index for the determination of harvest maturity of 'Cripps Pink' apples.

Chapter 3 examines the structure and physiology of the different expressions of flesh browning of 'Cripps Pink' apples. Through a process of examination of scanning electron microscopy and of the distribution of affected tissue within the fruit, the RFB and DFB disorders are shown to be structurally different disorders. An analysis of fruit mineral nutrition in this chapter further distinguishes the causes of each disorder.

The fourth chapter determines the influence of postharvest storage conditions on the incidence and development of RFB and DFB during storage. The influences of fruit

maturity at harvest, storage time, the composition of the storage atmosphere and the storage temperature are examined in order to determine the storage conditions that promote or exacerbate symptoms of flesh browning and in contrast, the storage conditions that reduce the development of the disorder. The effects of modified storage conditions on the quality of 'Cripps Pink' apples during long term storage are also considered.

The fifth chapter examines the influences of seasonal climatic conditions on the development of RFB and DFB during storage. The accumulation of growing degree days>10^{o}C during specific climatic periods of fruit growth and development are examined to determine the relationship between climatic conditions and the development of the flesh browning disorder. The potential for using climatic conditions for the prediction of the development of flesh browning during storage are discussed.

In the final chapter, the RFB and DFB disorders of 'Cripps Pink' apples are classified based on physiology and storage behaviour. Pre and postharvest factors that increase the risk of developing each disorder are evaluated and recommendations for the prevention of RFB and DFB are given.

1.4 References

Anon. 2004. Internal browning problem overcome, HortLink Vol. Spring, pp. 8.

Brown, G., L. Schimanski, and D. Jannings. 2003. Investigating internal browning of Tasmanian 'Pink Lady' apples. Acta Horticulturae. 628: 161-166.

Corrigan, V.K., P.L. Hurst, and G. Boulton. 1997. Sensory characteristics and consumer acceptability of 'Pink Lady' and other late-season apple cultivars. New Zealand Journal of Crop and Horticultural Science. 25: 375-383.

Cripps, J.E.L., L.A. Rishards, and A.M. Mairata. 1993. 'Pink Lady' apple. HortScience. 28: 1057.

De Castro, E.and B. Mitcham. 2004. Controlled atmosphere induced internal browning in Californian Pink Lady^{TM} apples, p. 26-32. In: J. Jobling and H. James (eds.). Final Report HAL AP02009: Understanding the Flesh Browning disorder of Pink Lady^{TM} apples.

Gapper, J. 2004. Pink Lady in a brand new fight, The Australian, Sydney.

Jobling, J., G. Brown, E. Mitcham, D. Tanner, S. Tustin, I. Wilkinson, and A. Zanella. 2004. Flesh browning of Pink Lady^{TM} apples: why do symptoms occur?

Results from an international collaborative study. Acta Horticulturae. 682: 851-858.

Jobling, J.and H. James. 2004. Final Report HAL AP02009: understanding the flesh browning disorder of Pink Lady™ apples.

Nicholas, P.and B. Vermey. 1998. The national apple breeding program in Western Australia. Agriculture Western Australia Farmnote. 48/98.

O'Rourke, D. 2003. World production, trade, consumption and economic outlook for apples. In: D. Ferree and I. Warrington (eds.). Apples: botany, production and uses. CABI Publishing, UK, USA.

Studdert, J. 2002. A Pink Lady rides to the rescue, The Australian, Sydney.

Wilkinson, I. 2000. Pink Lady apples - maintaining export quality, IHD Media Release, IHD Knoxfield, Knoxfield.

Zanella, A. 2004. Results from the Agricultural Research Centre, Laimburg, Italy, p. 41-124. In: J. Jobling and H. James (eds.). Final report HAL AP02009: understanding the flesh browning disorder of Pink Lady™ apples.

2

The determination of maturity and the physiology of ripening of 'Cripps Pink' apples grown in contrasting climatic conditions

2.1 Abstract

Harvest maturity and postharvest ripening are important considerations in the optimal long term storage of 'Cripps Pink' apples. The prediction and determination of optimal harvest maturity is based on a number of orchard, seasonal and physical fruit properties. Apples are a climacteric fruit, producing ethylene during ripening. Ethylene is responsible for the initiation of climacteric ripening in apples, however not all processes of ripening are maintained by ethylene. The most commonly used commercial indicator of apple maturity is the starch pattern index (SPI), this index estimates the amount of starch that has been degraded in the flesh of the fruit and this can be correlated to the progression of ripening in some apple cultivars. The SPI is a ripening process that is initiated by ethylene but may not be dependent on ethylene for regulation following initiation. This work has used the concept of 'greenlife'. The greenlife is defined as the number of days from harvest taken to reach a climacteric level of ethylene production. In this work, the length of greenlife gave an indication of the stage of ripening of the fruit and the level of synchronisation between ethylene dependent and independent ripening processors of the fruit. The greenlife was found to vary between 'Cripps Pink' apples grown in Batlow (New South Wales) and those grown in the Huon Valley (Tasmania). Fruit from the Huon Valley had a longer period of greenlife than fruit grown in Batlow, when fruit harvested at the same SPI were compared. The Huon Valley has a much cooler climate than Batlow and it is likely that seasonal climatic conditions are responsible for delaying the production of climacteric ethylene production in 'Cripps Pink' apples grown in the Huon Valley.

2.2 Introduction

The processes of both maturation and ripening can have substantial effects on the quality and storage potential of many horticultural products. The terms maturity and ripening are frequently interchanged but refer to different stages of physiological

development. The processes of fruit ripening are complex and involve a cascade of physical and chemical changes. Maturity, on the other hand is a point during the ripening process that corresponds to either a commercial or physiological stage of development. In apples, both ripening and maturity can have a considerable influence on the quality of the fruit as well as the storage potential and the occurrence of many storage disorders.

2.2.1 Fruit ripening

Fruit ripening refers to a physiological stage during which the development of the fruit has reached completion and the process of senescence has begun. Fruit ripening is a complex event in the physiological development of a fruit. It is a genetically programmed event and is the product of a number of physical and chemical changes, resulting in changes in colour, texture, flavour and aroma (White, 2002). During the ripening of an apple, the major changes that occur include the loss of firmness, the degradation of starch, an increase in acidity, the synthesis of oils and waxes, the production of esters and alcohols, the degradation of chlorophyll and an increase in the rate of respiration and ethylene production (Little and Holmes, 2000).

Ethylene, along with abscisic acid, gibberellins, auxins, and cytokinins, is a hormone produced by plants for regulating growth and development. Ethylene is widely called the “ripening hormone” due to the influence that this compound has on ripening the processes in many fruit. Some of the effects of ethylene were established early on in the investigations into fruit ripening. It was in 1935 that Crocker established that ethylene was the plant hormone responsible for fruit ripening (Crocker et al., 1935) and the presence of ethylene was soon demonstrated to speed up the ripening of many fruits including apples (Kidd and West, 1932; Kidd and West, 1936; Smith et al., 1969).

2.2.1.1 Ethylene and fruit ripening

Fruit can be divided into two distinct classifications based on their response to ethylene. Fruit that do not show a characteristic rise in ethylene production during ripening are termed “non-climacteric”. Fruit that produce a burst of ethylene during ripening in conjunction with an increase in the rate of respiration are termed “climacteric”. Climacteric fruit, such as apples and bananas, produce autocatalytic ethylene during ripening. Climacteric fruit often soften significantly during ripening

and continue to sweeten following harvest. Some of the biochemical changes which occur during the ripening of climacteric fruit include increases in cell wall degradation, pigment accumulation and most notably, an increase in the enzymes responsible for the biosynthesis of ethylene (Roberts and Hooley, 1988). Many climacteric products can be induced to ripen through the application of low concentrations of exogenous ethylene (Jerie et al., 1978). For products such as bananas and avocadoes, this process of ripening is used on a commercial scale which results in practical benefits for the supply chain (Wills et al., 1998). Interestingly, ethylene has been shown to be the product responsible for the initiation of ripening in apples on or off the tree and during storage (Dilley and Dilley, 1985). Although it is widely established that ethylene is the dominant trigger for ripening in climacteric fruit, it has been suggested that both ethylene dependent and independent gene regulation pathways coexist and coordinate the ripening processes in both climacteric and non-climacteric fruit (Alexander and Grierson, 2002).

It is thought that there are two systems of ethylene production operating in ripening fruit. System 1 ethylene is involved in the regulation of senescence and exists at very low concentrations in both climacteric and non-climacteric fruit (McMurchie et al., 1972). In climacteric fruit, system 1 ethylene triggers system 2 ethylene during the process of ripening (McMurchie et al., 1972). It is system 2 ethylene that is responsible for the large increase in ethylene production observed during the ripening of climacteric fruit (McMurchie et al., 1972). Non-climacteric fruit have not been found to have an active system 2 as part of their ethylene biosynthesis metabolism (McMurchie et al., 1972; Wills et al., 1998).

Ethylene has been demonstrated as being derived from methionine via a pathway that includes intermediates S-adenosyl-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC), both of which have been targeted in attempts to limit the biosynthesis of ethylene (Bufler, 1984; Bufler, 1986). The conversion of SAM to ACC by the ACC synthase enzyme has been shown to be the rate limiting step in the production of ethylene (Fluhr and Mattoo, 1996; Xin-Jian and Jiarui, 2000). Both ACC synthase and ACC oxidase have been examined in order to establish methods for controlling ethylene production (Fluhr and Mattoo, 1996; Xin-Jian and Jiarui, 2000).

Ethylene production of apples can be inhibited in the field, or following harvest through the application of different treatments. ReTain® (Valent Biosciences), is a commercial formulation of a aminoethoxyvinylglycine hydrochloride (AVG), a plant

growth regulator that competitively inhibits ACC production and consequently inhibits ethylene biosynthesis (Greene, 2003; Halder-Doll and Bangerth, 1987). ReTain® is applied in the field up to 6 weeks prior to harvest and has been found to successfully suppress ethylene production, delay the ethylene climacteric and maintain apple flesh firmness though the storage period (Brackman and Waclawovsky, 2001; Jobling et al., 2005; Park et al., 1999).

Rather than inhibiting the biosynthesis of ethylene, SmartFresh™ (Agrofresh Inc.), a commercial formulation of 1-Methylcyclopropene (1-MCP), inhibits the perception of ethylene (Watkins, 2006). Similarly to the other classes of plant hormones, ethylene binds to specific receptors to form a complex, which is then responsible for the triggering of the proceeding ripening processes (Hiwasa et al., 2003; Tassoni et al., 2006; Watkins, 2006). 1-MCP is thought to interact with the ethylene receptors and consequently prevent ethylene dependent responses from proceeding (Sisler and Serek, 2003; Watkins, 2006). 1-MCP is applied following harvest and has been found to successfully inhibit the ripening of apples resulting in an extension of storage life through the maintenance of quality characteristics including firmness and colour retention (DeEll et al., 2005; Lafer, 2003; Watkins, 2006).

The increase in the biosynthesis of ethylene at the onset of ripening of apples is regulated by a number of genes (Lara and Vendrell, 2000). Throughout the ripening process, the expression of many genes has been shown to be initiated or up-regulated (Alexander and Grierson, 2002). Interestingly, it has been demonstrated through the use of transgenic plants that not all the genes identified as being involved in ripening are regulated by ethylene (Alexander and Grierson, 2002). This further confirms the hypothesis that not all ripening processes are initiated or controlled by ethylene.

Research has shown that many fruit increase in sensitivity to ethylene as their development progresses towards senescence (Wills et al., 1998). Early on in the development of fruit, the concentration of exogenous ethylene required to trigger ripening is high however this decreases as the fruit development progresses (Wills et al., 1998). In addition to its role in initiating ripening, it has also been demonstrated that ethylene has a vital role in maintaining the ripening process (Hiwasa et al., 2003). For example, following the initiation of ripening in pears, firmness begins to decline. However a subsequent treatment with 1-MCP to inhibit the action of ethylene was found to arrest the progression of softening indicating that ethylene is required

not only for initiation, but also for regulation of softening of pears (Hiwasa et al., 2003).

The biosynthesis and action of ethylene are influenced by a number of both pre- and postharvest conditions. Particularly, climatic conditions during fruit growth and development have been found to influence an apple's sensitivity to ethylene as well as the capacity to produce ethylene. Tromp (1999) found that low night temperatures during the six weeks prior to harvest resulted in a higher rate of ethylene production in apples, an advanced climacteric and an accelerated rate of starch degradation. These results highlight the importance of climatic conditions on the fruits physiological development.

Postharvest conditions have also been found to significantly influence the fruits sensitivity to and capacity to produce ethylene. There is often a delicate balance in the postharvest management of ethylene to reduce the sensitivity of the product without stimulating the production of ethylene through a stress response (Kays, 1991). The sensitivity to ethylene can be reduced through storage at low temperatures, however low temperature stress has also been found to initiate ethylene production in apples (Jobling and McGlasson, 1995a; Kays, 1991). These results show that the optimal storage temperature of apples is cultivar specific. Other stresses, such as surface injuries and microbial infections have also been found to initiate ethylene production via the wound response (Bhowmik and Matsui, 2004; Bouquin et al., 1997; Kato et al., 2002; Yokotani et al., 2004). Another storage tool that can be used to reduce the sensitivity of fruits to ethylene is controlled atmosphere (CA) storage (Beaudry, 1999). Increasing the concentration of CO_2 and reducing the concentration of O_2 in the storage atmosphere has been shown to reduce the sensitivity of apples to ethylene (Beaudry, 1999; Gorny and Kader, 1996).

2.2.2 Fruit maturity

During fruit development, there are several overlapping phases. For example, the maturation phase of fruit development overlaps somewhat with the ripening phase. Maturation is a relatively arbitrary term, but is generally used to refer to the stage of development that meets the needs of consumers. In apples this is characterised by the accumulation of products, such as sugars, manufactured through photosynthesis that make the fruit appealing to the consumer. However, harvest maturity is not necessarily the same thing as utilisation maturity. For example, apples may be

harvested at a maturity that is suitable for storage which will be different to the maturity that is suitable for immediate consumption. The stage of fruit maturity is an important parameter of fruit quality and the prediction of an optimal harvest date is of great economic importance for fruit production.

The optimal harvest maturity of apples is generally defined as the maturity that will result in the minimal development of disorders and the maximum storage quality for the intended period of storage time under specific storage conditions. Harvest is an extremely critical time in the apple production process and it is often difficult to harvest the entire crop at the right time for optimal quality and storage. The correlation between harvest maturity and storage quality in apples has been thoroughly investigated (Beaudry et al., 1993; Blankenship et al., 1997; Fellman et al., 2002; Jobling and McGlasson, 1995b; Watkins et al., 2002). By definition, optimum harvest maturity involves a balance between market acceptability and storage potential. For maximum storage potential, it is widely accepted that apples are harvested in a pre-climacteric state, before the process of ripening has begun. At this stage of development the fruit is said to be mature, yet the production of the ripening hormone ethylene has not yet commenced. However for optimal marketability, the fruit must have begun to ripen and achieved the required balance of colour, aroma, sweetness, acidity, crispness and juiciness. Achieving this balance between storage potential and marketability is the commercial challenge for the postharvest management of apples.

2.2.2.1 Prediction of maturity

As successful postharvest management requires that the fruit is harvested at the correct physiological stage of development, various methods have been developed in order to predict and determine the maturity of the product.

2.2.2.1.1 Average harvest date

Historically, one of the most common methods used for the prediction of apple maturity is the use of long-term average harvest dates (Little and Holmes, 2000). These recommendations were often provided by Australian State Departments of Agriculture and were useful in a time when less emphasis was placed on consumer satisfaction and when consumer demand for a year round supply of apples didn’t

exist. In today's market, where there is an expectation to supply quality apples to the consumer for twelve months of the year, this method is rarely the sole method used.

2.2.2.1.2 Days from full bloom

Unlike the long-term average harvest date, the number of days after full bloom to harvest (DAFB) for a given variety of apple is less variable between seasons and districts (Little and Holmes, 2000). Data relating to the DAFB have been collected for over eighty years in some Australian regions and can be useful for predicting harvest maturity for some cultivars in some districts (Blanpied and Little, 1991; Little and Holmes, 2000). However, this method still has some limitations that primarily relate to the accurate assessment of the date of full bloom and the incorrect assumption that climatic conditions will not alter the DAFB. In order to overcome these limitations, modern DAFB models take into account elevation, growing region and seasonal temperatures (Little and Holmes, 2000). Inconsistency in the definition of 'full bloom' has resulted in variable reports of DAFB. In the UK, full bloom is defined as 50% of open blossoms, whereas in Australia it is the date at which 60-80% of blossoms have opened (Little and Holmes, 2000). Such inconsistencies are not uncommon in methods used for the prediction and determination of apple maturity and as a result work on maturity prediction continues to be done in many research laboratories.

2.2.2.2 Determination of quality and maturity

While the prediction of apple harvest maturity often relies on climactic and historical data, the measurement of apple maturity relies on a range of physical and chemical tests for which indices have been developed. Multiple studies have been conducted on the correlation between harvest maturity indices and the storage performance of many apple cultivars (Beaudry, 1995; Blankenship et al., 1997; Blanpied and Little, 1991; Drake and Eisele, 1997; Little and Holmes, 2000; Plotto et al., 1995; Zude-Sasse et al., 2001). Similarly to the prediction of apple harvest maturity, the measurement of apple maturity is often limited in sensitivity and accuracy and consequently it is strongly recommended that a range of maturity tests are carried out. Due to the variability between individual fruit the reliability of any one of these measures of apple maturity depends strongly on the method of sampling fruit within a tree and within the orchard.

2.2.2.2.1 Total soluble solids

Total soluble solids (TSS) is a quality component of apples that has also been used to measure fruit maturity. Total soluble solids is a measure of the soluble compounds (such as carbohydrates, salts and acids) in the cell which increase during ripening, primarily from the conversion of starch to sugar (Little and Holmes, 2000; Watkins, 2003). During apple maturation, sugars become the primary component of the soluble solids and consequently the TSS gives a measurement of the sweetness of the fruit. Because of this, TSS is often used as a quality component and a minimum TSS is often required for export markets. However, the concentration of acids and the ratio of TSS to acid is also an important aspect of the perception of flavour. The 'Cripps Pink' apple was first described as having a TSS between 12.5% and 13.5% (Cripps et al., 1993). However, for the export of 'Cripps Pink' apples under the Pink Lady™ name, a minimum TSS of 13% and an average of 15% is required (Hurndall and Fourie, 2003). Drake et al. (2002) found that 'Cripps Pink' apples harvested at an early maturity had a TSS of 13.3%, while those harvested at a late maturity had a TSS of 14.4%.

2.2.2.2.2 Flesh firmness

Flesh firmness (FF) is another quality measurement of apples that has also been used to determine optimal storage maturity. Flesh firmness in apples decreases during maturation as a result of the thinning of cell walls and of the action of pectinase enzymes during fruit ripening (Kays, 1991). Similarly to the limitations of TSS, FF at optimal maturity can vary considerably between seasons and apple cultivars (Little and Holmes, 2000; Watkins, 2003). Flesh firmness has successfully been used to measure the harvest maturity of 'Delicious' apples when used in combination with TSS however the variation reported for other cultivars limits the success of this measure for other cultivars (Little and Holmes, 2000). Similarly to TSS, FF is a useful measure of consumer acceptance of apples as textural qualities of apples are often reported by consumers to be amongst the top requirements for acceptability (Harker et al., 2003). Although it must be pointed out that penetrometer measurements of FF do not correlate well to consumer perceptions of crispness, as such penetrometer readings should only be used as a quality guide (Harker et al., 2003). The 'Cripps Pink' apple was described as a 'crisp and crunchy' apple with a firmness of 83N at harvest (Cripps et al., 1993). For the export of 'Cripps Pink' apples under the Pink Lady™ name, the fruit are required to have a minimum FF of 66.7N

and an average FF of 68.6N (Hurndall and Fourie, 2003). Drake et al. (2002) found that ‘Cripps Pink’ apples harvested at an early maturity had a FF of 94.2N, while those harvested at a late maturity had a FF of 90.0N.

2.2.2.2.3 Skin colour

Change in both the red blush colour and the background (BG) colour of the skin of apples are another important quality characteristic and the change in the skin colour can be used in some cultivars to determine the maturity of the fruit. In apples, the blush colour develops during ripening and is used to grade the fruit as being suitable for harvest, storage and marketing (Lau, 1985; Watkins, 2003). As well as the development of blush colour, the change in BG colour can be a used as a maturity guide (Huybrechts et al., 2003; Watkins et al., 1993). This measure is particularly successful in blushed varieties of apple where the green BG colour changes to yellow as the fruit matures. Visual changes in BG colour have been found to be relevant maturity indicators for ‘Gala’, ‘Braeburn’ and ‘Fuji’ apple cultivars (Little and Holmes, 2000). The BG colour of apples is determined by the concentrations of green pigments (chlorophyll) and yellow pigments (carotenoids) in the skin of the fruit (Kays, 1991; Little and Holmes, 2000). During the process of maturation, the chlorophyll breaks down and reveals more of the carotenoids resulting in the colour change from green to yellow (Kays, 1991; Little and Holmes, 2000). The change in colour can be quantified through the extraction and measurement of chlorophyll, through the use of colorimeters or more commonly through the comparison with standard colour charts (Lau, 1985; Little and Holmes, 2000). This method is less suitable for highly blushed cultivars of apple, such as ‘Red Delicious’ or apples with no red blush at all as the change in colour is not significant enough for the development of a suitable guide (Little and Holmes, 2000). For the export of ‘Cripps Pink’ apples under the Pink Lady™ name, the fruit are required to have a pale green BG colour (Hurndall and Fourie, 2003).

2.2.2.2.4 Starch content

The starch-iodine test has been well established as the most practical method for the determination of harvest maturity in apples. Starch begins to accumulate in a developing apple fruit 3 to 4 weeks after full bloom, following the period of cell division (Little and Holmes, 2000; Magein and Leurquin, 2000; Smith et al., 1979). Over the following 2 months, starch accumulates to a maximum value and

subsequently declines during maturation (Dilley and Dilley, 1985). Starch is ultimately hydrolised into soluble sugars metabolized by the cell in respiration (Dilley and Dilley, 1985; Kays, 1991). The process of starch conversion starts in the core area of the fruit and then proceeds outwards into the cortex area of the fruit in a pattern that is often variety specific (Little and Holmes, 2000). The starch-iodine test estimates the amount of starch that has been converted to sugar by dipping a cut surface of the fruit into an iodine solution. In the presence of starch, the iodine stains the flesh of the fruit a blue-black colour. The pattern can then be compared to a rating scale known as the starch pattern index (SPI). While the SPI is widely used for the commercial determination of apple harvest maturity, it also has several limitations.

For example, SPI scales have been developed independently in several countries and no single standard scale is used. The rate of degradation of starch has also been shown to vary greatly between apple cultivars (Plotto et al., 1995). These factors have resulted in the development of cultivar specific SPI charts, leading to further inconsistencies in reported results. In the United States, a 1-6 scale is commonly used whereas in Europe a 1-10 scale has been adopted and in Canada a 1-9 scale is common. In Australia, two indexes are commonly used, a 1-6 scale and a 0-10 scale. Compounding the inconsistencies, Australia also uses 2 different patterns for the 1-6 SPI scale, the radial type and the concentric type. The radial type SPI is used for 'Jonathan', 'Gala', 'Golden Delicious', Pink Lady™, 'Sundowner', 'Granny Smith' and 'Lady Williams' cultivars (Little and Holmes, 2000). On the other hand, the concentric type SPI is used for 'Red Delicious', 'Braeburn' and 'Fuji' cultivars (Little and Holmes, 2000). Unfortunately, there is little correlation between intermediate stages on any of the scales making comparisons between different studies difficult.

Despite common awareness, starch content is not an unequivocal benchmark for the determination of maturity in apples. Due to the subjective nature of the method, errors of up to 60% have been reported for different inspectors of the same samples (Peirs et al., 2002). Compounding the subjectivity is the non-linear scale which is used in many of the widely used SPI scales (Peirs et al., 2002). Despite these limitations, the SPI is one of the major indicators of fruit maturity used internationally in commercial apple production (Drake et al., 2002). The test is cheap and fast and provides an indication of the total starch content and a useful, although limited, indication of apple maturity.

An increase in SPI has been linked to in increase in ethylene production (Plotto et al., 1995), however starch hydrolysis is not directly an ethylene dependent process (Dilley and Dilley, 1985). An increase in ethylene production can increase the rate of respiration, which consequently increases the rate of starch degradation, however ethylene production and starch hydrolysis are processes that are independent of each other (Dilley and Dilley, 1985). For example, apples can be ripened with the application of ethylene and show no significant change in SPI and conversely can show a depletion of starch while still in a pre-climacteric state (Dilley and Dilley, 1985; Watkins et al., 1993). The relationship between ethylene production and the SPI is also dependent on seasonal conditions and has been observed to vary erratically on a seasonal and cultivar basis (Fellman et al., 2003; Plotto et al., 1995). These results indicate that while the SPI is a useful tool for determining the maturity of the fruit, it does not directly or consistently relate to the physiological stage of ripening.

2.2.2.2.5 Respiration rate and rate of ethylene production

Both the respiration and ethylene production rates can accurately determine the physiological stage of development of the fruit and in turn accurately correlate to storage potential. As previously discussed, apples are a climacteric fruit and as such produce elevated levels of ethylene and CO_2 during fruit ripening. In practice, the measurement of ethylene is preferred to the measurement of respiration as the delay between detectable and autocatalytic ethylene allows for the accurate determination of an optimal harvest date (Plotto et al., 1995; Smith et al., 1969). Kidd and West (1932; 1936) demonstrated that the longest storage life of apples is achieved when they are harvested before the climacteric rise in ethylene production. As was found with other determiners of apple maturity, the rate of ethylene production has been shown to be cultivar specific (Graell et al., 1993; Jobling et al., 1993; Walsh and Altman, 1993) meaning that ethylene production rates cannot be generalised across multiple apple cultivars. Importantly, the rate of ethylene production has also been shown to vary considerably between seasons and orchards (Graell et al., 1993). Despite the clear benefits of using ethylene for the determination of the climacteric stage of maturity, the method is not practical for growers and requires a substantial investment in specialised equipment. Consequently, the commercial use of this method is generally only conducted by consulting services and large packing companies.

2.2.2.2.6 Limitations in the determination of maturity

As each of the measures of maturity suffers from a range of limitations, it is common practice to employ a combination of maturity measures in order to overcome the limitations of each individual method. The Streif index utilises a combination of SPI, TSS and FF while other successful models only use TSS and FF (DeLong et al., 1999; Little and Holmes, 2000). By combining several of the more practical but less accurate methods, a more satisfactory determination of apple maturity can be achieved in most circumstances.

The aim of combining several maturity methods is to overcome the lack of precision and accuracy that exists. Maturity indicators such as the SPI have a relatively low sensitivity and consequently require a high number of samples in order to overcome the variability between fruit and provide an accurate and precise measure. Homogeneity in stored fruit is an important consideration in terms of consistent outturn after removal from storage. Variability in maturity can be the result of the sampling procedure. The between fruit variability is often the result of a range of environmental factors during the growth and development of the fruit. These environmental factors can relate to both the climatic conditions and to orchard management practices. By using more targeted sampling techniques or increasing the sample size used for commercial maturity assessments, such variability may be reduced.

2.2.2.3 Variability in maturity

A number of orchard factors have been shown to influence the maturity of certain apple cultivars.

2.2.2.3.1 Rootstock

Dwarfing rootstocks are commonly used in most commercial apple orchards. Rootstocks can have several effects on the tree and fruit physiology, however these effects are interactive and multifaceted (Webster and Wertheim, 2003). Rootstocks can impart several characteristics to the scion in addition to vigour control, such as cropping and fruit mineral nutrition characteristics (Webster and Wertheim, 2003). The impact of the rootstock on fruit mineral nutrition is discussed in Chapter 3.

Barden and Marini (1992) have shown that rootstocks can have a significant effect on the maturity of 'Delicious' apples. Interestingly, this study found that different rootstocks had variable effects on different measures of maturity further suggesting that not all ripening processors are controlled by the same mechanism. Background colour was found to be most advanced on the dwarf M.26 rootstock and least advanced on the highly dwarfing M.27 rootstock (Barden and Marini, 1992). However, the SPI was found to be lowest on MAC9 rootstocks and highest on MAC24. This suggests that BG colour and SPI have differing mechanisms of regulation. Overall, it was found that M.27, MAC9 and M.9 rootstocks had a more advanced maturity than the other rootstocks assessed, although the maturity indices were found to vary significantly from season to season (Barden and Marini, 1992). There is a need for further research into the consistency of rootstock effects among seasons, cultivars and climates. Cripps et al. (1993) recommended that 'Cripps Pink' trees were grown on dwarfing rootstocks, MM.106, MM.104 and MM.109 were recommended as suitable choices.

2.2.2.3.2 Within tree variation

Apple maturity varies depending on the position of the fruit on the tree (Little and Holmes, 2000; Tomala, 1999; Volz et al., 1995), this within tree variation can help to explain the lack of uniformity in the storage performance of some harvests. It is proposed that the position of the fruit on the tree can be related to the mineral composition of the fruit, this in turn can influence maturity and storage potential (Little and Holmes, 2000). Tree factors such as aspect, shading, fruit/leaf ratio and the distance from rootstock, although often poorly understood, have all been found to influence apple maturity (Little and Holmes, 2000; Tomala, 1999; Wilson, 1998). More specifically, measurements of the internal ethylene concentration in 'Hi Early Delicious' grown on a central leader training system in Washington State (United States of America) have shown the influence of fruit position on maturity (Little and Holmes, 2000). The internal ethylene concentration was found to be lower in apples from the upper branches than in fruit from the lower branches, maturity was also found to decrease along individual branches and smaller fruit and those with less colouration were often more mature than the larger, more coloured apples (Little and Holmes, 2000). Brookfield et al. (1993) showed that multiple harvests of 'Royal Gala' apples significantly reduced the variability in maturity within each harvest and resulted in fruit that were firmer, greener and less greasy following storage. Further

work is required to determine the physiological basis for many of the tree and fruit factors.

2.2.2.3.3 Crop load

Aside from the position of the fruit on the tree, the tree crop load has also been found to influence fruit maturity. Results from several cultivars indicate that fruit maturity was significantly more advanced on low cropping trees compared to high cropping trees (Ferguson and Watkins, 1992; Wunsche et al., 2000) although contradicting these results are those of Volz et al. (1993), who found no significant effect of crop load on fruit maturity. Elgar et al. (1999) showed that fruit from low cropping trees have a higher level of ethylene production without a significant difference in SPI, although Volz et al. (1993) showed a significantly higher SPI in fruit from trees with a high crop load. While the effect of crop load on maturity remains relatively unclear, it is well established that fruit from light cropping trees are more susceptible to storage disorders than are fruit from medium or high cropping trees, this is thought to primarily be due to the fact that fruit from light cropping trees have a lower concentration of calcium (Ferguson and Watkins, 1992; Little and Holmes, 2000; Tough et al., 1996; Volz et al., 1993). The influence of calcium on cell structure and the development of storage disorders of apples is discussed in Chapter 3.

2.2.2.3.4 Irrigation

Further to the influence of tree and crop load, the method and timing of irrigation has also been shown to affect apple maturity. Ebel et al. (1993) found that regulated deficit irrigation (RDI), a method of irrigation used to control vegetative growth, effected both the timing of the ethylene climacteric and the degradation of starch. The study, carried out on 'Delicious' trees in Washington State (United States of America), found that apples harvested from trees subjected to RDI reached a climacteric level of ethylene production sooner than those not subjected to RDI (Ebel et al., 1993). The study also found that fruit harvested from trees subjected to RDI had a delayed degradation of starch (Ebel et al., 1993). This study further indicates that starch degradation is independent of climacteric ethylene production. Withholding irrigation during fruit growth can also affect apple maturity. Kilili et al. (1996) showed that withholding irrigation, particularly in the late season, resulted in a significantly advanced maturity.

2.2.2.3.5 Orchard location

While several factors within the orchard have been shown to significantly affect apple maturity, the effect of the orchard location has also been found to have a strong influence. Location effects are often the result of climatic effects such as growing degree days and diurnal temperature variation. High temperatures from 4 to 6 weeks following full bloom have been found to accelerate ripening to a greater degree than temperatures later in the season (Tromp, 1999). For example, 'Elstar' apples grown at 16^{o}C for the 6 weeks following full bloom showed a lower SPI at harvest than fruit grown at 22^{o}C for the same period (Tromp, 1997). The influence of climatic conditions on apple development and storage potential are discussed in Chapter 5.

2.2.2.4 Maturity and storage disorders of apples

Fruit maturity at harvest can be closely related to the occurrence of several commercially significant storage disorders. The impact of harvest maturity on the development of storage disorders is discussed in Chapter 4.

As the 'Cripps Pink' apple is a relatively new cultivar, the influences of orchard, season and climate have not previously been established. As both ripening and maturity have a significant effect on a number of different storage disorders in other apple cultivars, it is hypothesized that these factors will contribute to the development of the flesh browning disorder of 'Cripps Pink' apples. The variability in ripening and maturity as a result of pre and postharvest conditions may also help to explain some of the observed variability in the incidence of the flesh browning disorder of 'Cripps Pink' apples.

2.3 Aims

- To determine the optimal harvest maturity indicator for optimal long term storage of 'Cripps Pink' apples.
- To establish if seasonal climatic conditions influence the timing of maturity.
- To establish if seasonal climatic conditions influence the progression of ripening.

2.4 Materials and methods

2.4.1 Fruit sources

2.4.1.1 Batlow (New South Wales)

In the 2004, 2005 and 2006 seasons, 'Cripps Pink' apples were harvested from seven year old trees on a commercial orchard in Batlow (35°31'S 148°09'E). The trees were grown and managed using current commercial practices. The trees were on M9 rootstocks, it is unfortunate that due to commercial restraints the trees in Batlow were grown on different rootstocks to those grown in the Huon Valley. Tree rootstocks can influence tree and fruit physiology (Chapter 3) and this was taken into consideration in the results. Apples were harvested at weekly intervals three times during the commercial apple season.

2.4.1.2 The Huon Valley (Tasmania)

In the 2004 and 2005 seasons, 'Cripps Pink' apples were harvested from eight year old trees on a commercial orchard in the Huon Valley (43°16'S 146°92'E). The trees were grown and managed using current commercial practices. The trees were on MM106 rootstocks with M9 interstems for vigour control. Apples were harvested at weekly intervals up to 4 times during the commercial apple season. In 2006, fruit from the Huon Valley were not available.

2.4.2 Fruit numbers

In the 2004 season 60 fruit per harvest per location were obtained, 30 for the assessment of maturity and 30 for the greenlife experiment. In Batlow, fruit were harvested from 5 orchard blocks (12 fruit per block). In Batlow, the same methodology was followed for the 2005 and 2006 seasons, however the number of fruit for the greenlife experiment was reduced to 10 apples for the 2005 season in the Huon Valley as no block variation was observed.
Thirty fruit per harvest per location were assessed for maturity on arrival at Sydney Postharvest Laboratory, Sydney, New South Wales, by measuring FF, BG colour, internal ethylene concentration (IEC) and SPI.

2.4.3 Maturity and quality

2.4.3.1 Internal ethylene concentration

Internal ethylene concentration was measured using a 0.5 ml gas sample extracted from the core space of each apple. The sample was analysed using a gas chromatograph. The gas chromatograph (Shimadzu GC-17A) had a flame ionization

detector, GS-Q column (J & W Scientific) 30m in length with an internal diameter of 0.53mm and had helium as the carrier gas. The column temperature was 80^{o}C, the injector was 130^{o}C and the detector was 200^{o}C.

2.4.3.2 Flesh firmness

Flesh firmness was measured using a drill-press mounted Effegi penetrometer fitted with an 11 mm tip. Two measurements were taken for each fruit; from the blushed and unblushed sides of the fruit equator after the skin was removed.

2.4.3.3 Background colour

The fruit background colour was assessed using a Ctifl (Centre Technique Interprofessionnel des Fruits et Légumes, France) Pink Lady™ colour chart (2=green to 7=yellow).

2.4.3.4 Starch pattern index

The starch pattern index (SPI) was assessed by cutting the fruit equatorially and dipping the cut surface of the apple in a solution of potassium iodide (15g.L^{-1} potassium iodide, 6g.L^{-1} iodine). The degree of staining was rated using the Ctifl 10 point SPI scale (Centre Technique Interprofessionnel des Fruits et Légumes, France). A score of 1 indicated no starch clearing, a score of 10 indicated complete starch degradation.

2.4.4 Ripening evaluation

The remaining 30 fruit per harvest per maturity were stored at 20^{o}C and assessed biweekly for the rate of ethylene production. Ethylene production was determined by sealing individual fruit in 600ml gastight containers for 1hr. A 0.5ml headspace sample was then extracted and analysed for ethylene concentration using the gas chromatograph and conditions outlined previously, in the 2004 season a second headspace sample (1.0ml) was also extracted and analysed for the carbon dioxide concentration. Fruit were assessed over time until they reached the climacteric phase of ethylene production, defined as 1μL.kg^{-1}hr^{-1}. The number of days from harvest until the fruit reached a rate of ethylene production of 1μL.kg^{-1}hr^{-1} was defined as the greenlife of the fruit.

2.4.5 Statistical analysis

For IEC and FF an analysis of variance (ANOVA) was performed and least significant differences (5%) calculated using the general analysis of variance procedure in GenStat statistical software (9th edition, version 9.1.0.147, Lawes Agricultural Trust, supplied by VSN International Ltd). Internal ethylene concentration was transformed using a log transformation to normalise the data.

As the SPI and skin BG colour were score data they were analysed using an ordinal logistic regression (GenStat statistical software). Statistical significance was determined from the reference treatment (Harvest 1).

Correlations between days of greenlife, BG colour, FF, SPI and IEC were determined using Genstat statistical software, the significance of correlations was determined using Pearson's R table. Significant correlations between the days of greenlife and SPI, FF, BG colour or IEC were analysed using the general linear model procedure, a stepwise regression was carried out to determine the optimal lineal model (Genstat statistical software). To determine if seasonal regressions had significantly different slopes, a simple linear regression with groups was also completed (Genstat statistical software).

As the rate of ethylene production over time at 20°C were repeated measures completed on the same fruit, an analysis of variance was determined using the residual maximum likelihood (REML), linear mixed model procedure and least significant differences (5%) calculated (Genstat statistical software).

The results for each experiment within a season have been presented on figures with the same axis scale to allow for simple comparison of the data.

2.5 Results

2.5.1 Maturity

2.5.1.1 Batlow

In Batlow the measures of quality and maturity (BG colour, SPI, FF and IEC) were found to vary between successive harvests within a season in 2004 (Table 2.1), 2005 (Table 2.2) and 2006 (Table 2.3).

The BG colour index increased significantly as the colour changed from green to yellow between successive harvests in 2004 (P<0.001), 2005 (P<0.01) and 2006 (P<0.001), indicating advancing maturity between successive harvests.

The SPI indicates the degree of starch staining in the flesh of the fruit. A low level of starch staining indicates a more mature apple. The SPI increased significantly from harvest 1 with each successive harvest in both the 2004 (P<0.001) and 2006 (P<0.001). However there was no significant change in the SPI between harvest dates in 2005 (Table 2.2).

Flesh firmness decreased significantly (P<0.001) with successive harvests in the 2005 (Table 2.2) and 2006 seasons (Table 2.3). In the 2004 (Table 2.1) season there was no significant reduction in FF between successive harvests.

The IEC was also found to increase significantly between successive harvests in all seasons. The IEC had increased to post-climacteric levels by the third harvest in both the 2004 and 2006 seasons, however in the 2005 season, the IEC of the fruit remained pre-climacteric at the final harvest (Table 2.2).

Table 2.1 Effect of harvest date on quality and maturity characteristics of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2004

Harvest No.	Harvest date	Greenlife (days)	BG colour (2-7)	SPI (1-10)	FF (N)	IEC ($\mu L.L^{-1}$)
1	7/04/2004	13.8	3.1	1.4	123.6	0.287
2	15/04/2004	9.1	3.6	3.1	120.6	0.099
3	21/04/2004	6.2	3.9	4.8	120.6	1.158
LSD					0.29	0.127
Significance			***	***	ns	***

***Significant at P< 0.001

Flesh firmness (FF) and internal ethylene concentration (IEC) analysed by ANOVA and LSD (5%) calculated

Background (BG) colour and starch pattern index (SPI) analysed by ordinal logistic regression, significance determined from reference treatment (Harvest 1)

Table 2.2 Effect of harvest date on quality and maturity characteristics of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2005

Harvest No.	Harvest date	Greenlife (days)	BG colour (2-7)	SPI (1-10)	FF (N)	IEC ($\mu L.L^{-1}$)
1	21/04/2005	11.3	3.9	4.3	94.1	0.052
2	28/04/2005	4.7	4.6	5.4	93.2	0.150
3	4/05/2005	3.2	4.2	5.4	90.2	0.174
LSD					0.5	0.098
Significance			**	ns	***	**

,*Significant at P<0.01 or 0.001 respectively
Flesh firmness (FF) and internal ethylene concentration (IEC) analysed by ANOVA and LSD (5%) calculated
Background (BG) colour and starch pattern index (SPI) analysed by ordinal logistic regression, significance determined from reference treatment (Harvest 1)

Table 2.3 Effect of harvest date on quality and maturity characteristics of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2006

Harvest No.	Harvest date	Greenlife (days)	BG colour (2-7)	SPI (1-10)	FF (N)	IEC ($\mu L.L^{-1}$)
1	30/03/2006	16.6	3.2	1	114.7	0.016
2	10/04/2006	10.5	4	3.5	107.9	0.016
3	21/04/2006	5.2	4.1	5.5	97.1	4.325
LSD					0.399	0.654
Significance			***	***	***	**

,*Significant at P<0.01 or 0.001 respectively
Flesh firmness (FF) and internal ethylene concentration (IEC) analysed by ANOVA and LSD (5%) calculated
Background (BG) colour and starch pattern index (SPI) analysed by ordinal logistic regression, significance determined from reference treatment (Harvest 1)

2.5.1.1 The Huon Valley

In the Huon Valley, the maturity measurements also varied between successive harvests in the 2004 (Table 2.4) and 2005 (Table 2.5) seasons. In a contrast to Batlow, the fruit from the Huon Valley did not reach a climacteric level of ethylene production by the final harvest in either season. However, the IEC was found to increase significantly in both the 2004 ($P<0.01$) and 2005 seasons ($P<0.001$).

Despite the delayed production of ethylene, fruit from the Huon Valley showed significant decreases in FF and increases in the BG colour and SPI between successive harvests in both seasons.

The differences in the seasonal effects on fruit maturity and ripening for apples from Batlow and the Huon Valley are an example of how postharvest fruit physiology and quality is affected by preharvest factors.

Table 2.4 Effect of harvest date on quality and maturity characteristics of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in 2004

Harvest No.	Harvest date	Greenlife (days)	BG colour (2-7)	SPI (1-10)	FF (N)	IEC ($\mu L.L^{-1}$)
1	1/04/2004	17.2	4.1	1.8	127.5	0.53
2	7/04/2004	15.4	4.2	4.3	126.5	0.19
3	14/04/2004	9.8	4.8	5.6	123.6	0.21
4	26/04/2004	4.6	4.8	8.1	107.9	0.28
LSD					0.27	0.20
Significance			**	**	***	**

,*Significant at P<0.01 or 0.001 respectively
Flesh firmness (FF) and internal ethylene concentration (IEC) analysed by ANOVA and LSD (5%) calculated
Background (BG) colour and starch pattern index (SPI) analysed by ordinal logistic regression, significance determined from reference treatment (Harvest 1)

Table 2.5 Effect of harvest date on quality and maturity characteristics of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in 2005

Harvest No.	Harvest date	Greenlife (days)	BG colour (2-7)	SPI (1-10)	FF (N)	IEC ($\mu L.L^{-1}$)
1	31/03/2005	26.8	3	2.6	96.1	0.369
2	6/04/2005	14.2	3.3	7.9	96.1	0.256
3	13/04/2005	14.2	3.6	8.7	89.2	0.344
LSD					0.43	2.31
Significance			***	***	***	***

***Significant at P<0.001
Flesh firmness (FF) and internal ethylene concentration (IEC) analysed by ANOVA and LSD (5%) calculated
Background colour and SPI analysed by ordinal logistic regression, significance determined from reference treatment (Harvest 1)

2.5.2 Ripening

2.5.2.1 Respiration rate

The respiration rate of fruit grown in Batlow (Figure 2.1) and the Huon Valley (Figure 2.2) increased with increasing days from harvest, stored at 20^{o}C. With each successive harvest, the fruit had a higher respiration rate indicating further advanced maturity.

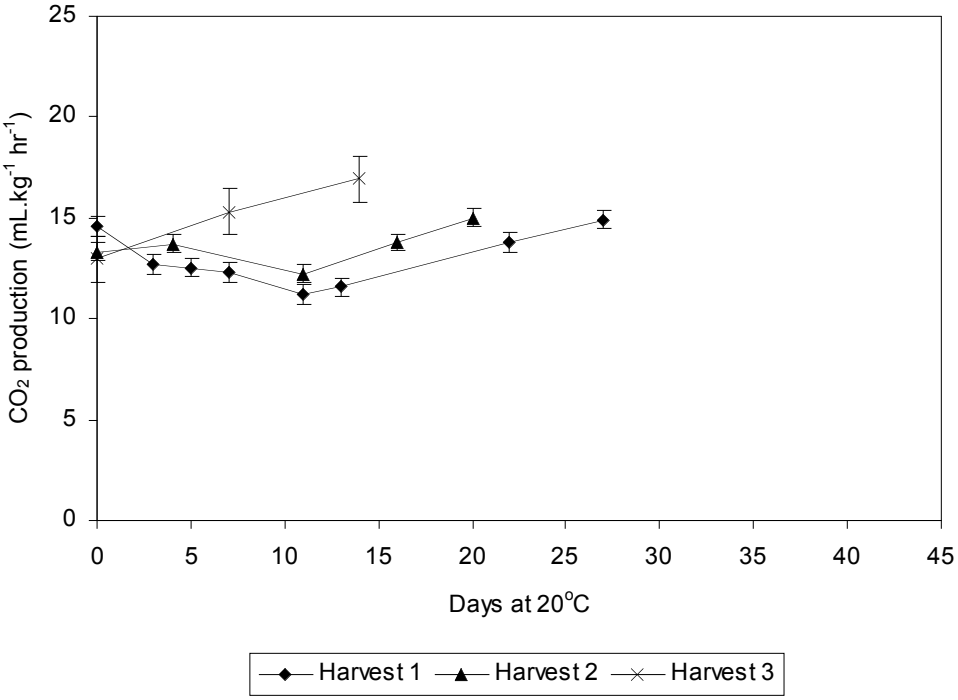

Figure 2.1 Respiration rate (mL.kg^{-1}.hr^{-1}) of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2004. Harvest 1 (07/04/2004) starch pattern index: 1.4, Harvest 2 (15/04/2004) starch pattern index: 3.1, Harvest 3 (21/04/2004) starch pattern index 4.8. Bars represent standard error.

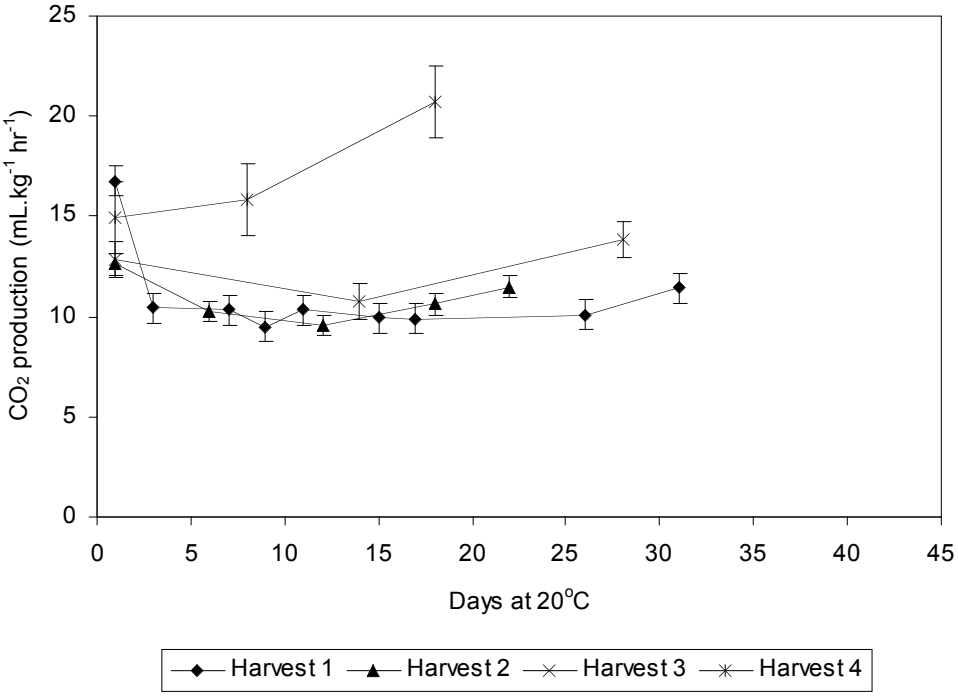

Figure 2.2 Respiration rate (mL.kg^{-1}.hr^{-1}) of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in 2004. Harvest 1 (01/04/2004) starch pattern index: 1.8, Harvest 2 (07/04/2004) starch pattern index: 4.3, Harvest 3 (14/04/2004) starch pattern index 5.6, Harvest 4 (26/04/2004) starch pattern index 8.1 Bars represent standard error.

2.5.2.2 Rate of ethylene production

The rate of ethylene production increased with increasing days from harvest and stored at 20°C for fruit from all harvests from Batlow and the Huon Valley in all seasons (Figures 2.3-2.7). 'Cripps Pink' apples grown in Batlow (Figures 2.3-2.5) had similar rates of ethylene production between the seasons. In contrast, fruit from the Huon Valley in 2004 (Figure 2.6) took an extended period of time before any rise in ethylene production was observed in comparison to the 2005 season (Figure 2.7). Fruit harvested before the commercial harvest maturity (Harvest 3) were pre-climacteric in all cases and took between 9 and 40 days to reach an ethylene production rate of 1μL.kg^{-1} hr^{-1}, or to enter the climacteric stage of ripening (EC). The number of days taken to reach the EC was defined as the greenlife of the fruit. With each successive harvest, the number of days of greenlife was reduced as fruit reached the EC more quickly.

2.5.2.2.1 Batlow

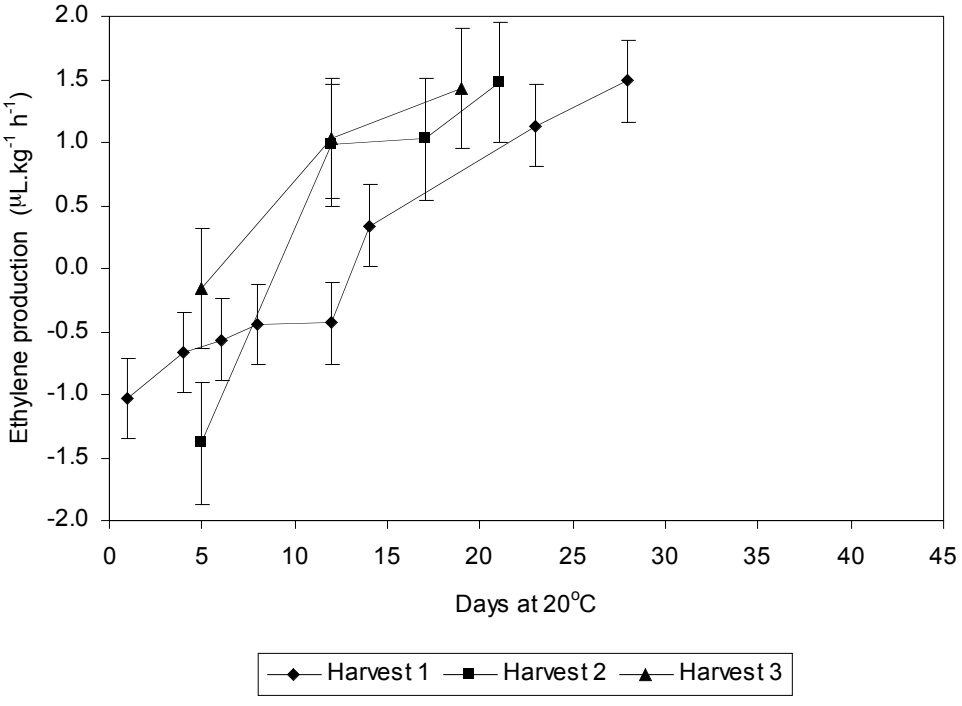

Figure 2.3 Ethylene production at 20°C of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2004. Harvest 1 (07/04/2004) starch pattern index: 1.4, Harvest 2 (15/04/2004) starch pattern index: 3.1, Harvest 3 (21/04/2004) starch pattern index 4.8. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 1.31

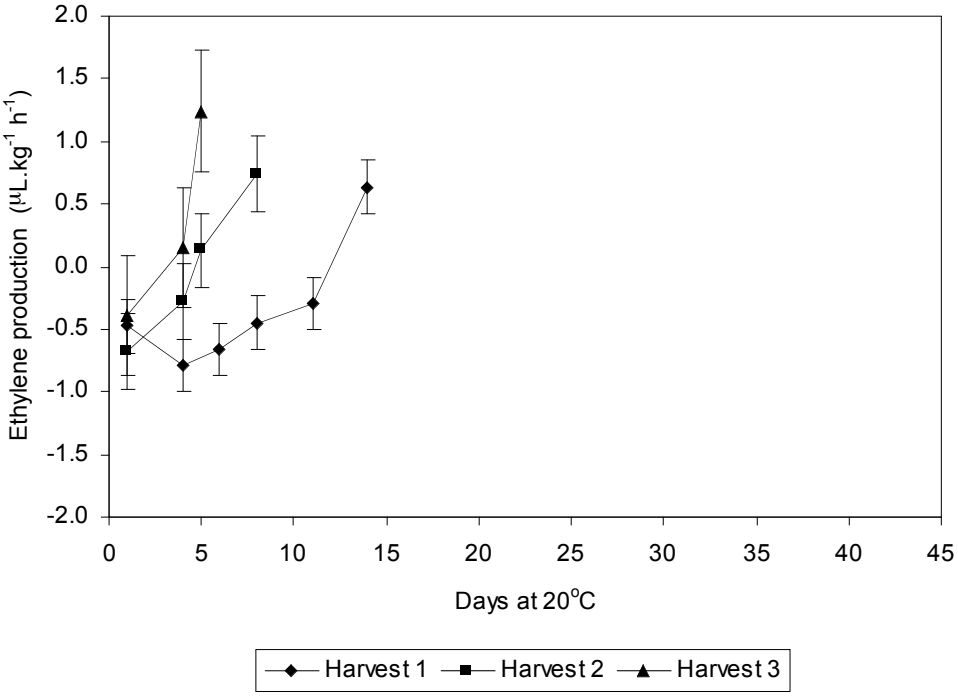

Figure 2.4 Ethylene production at 20°C of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2005. Harvest 1 (21/04/2005) starch pattern index: 4.3, Harvest 2 (28/04/2005) starch pattern index: 5.4, Harvest 3 (04/05/2005) starch pattern index 5.4. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 0.86

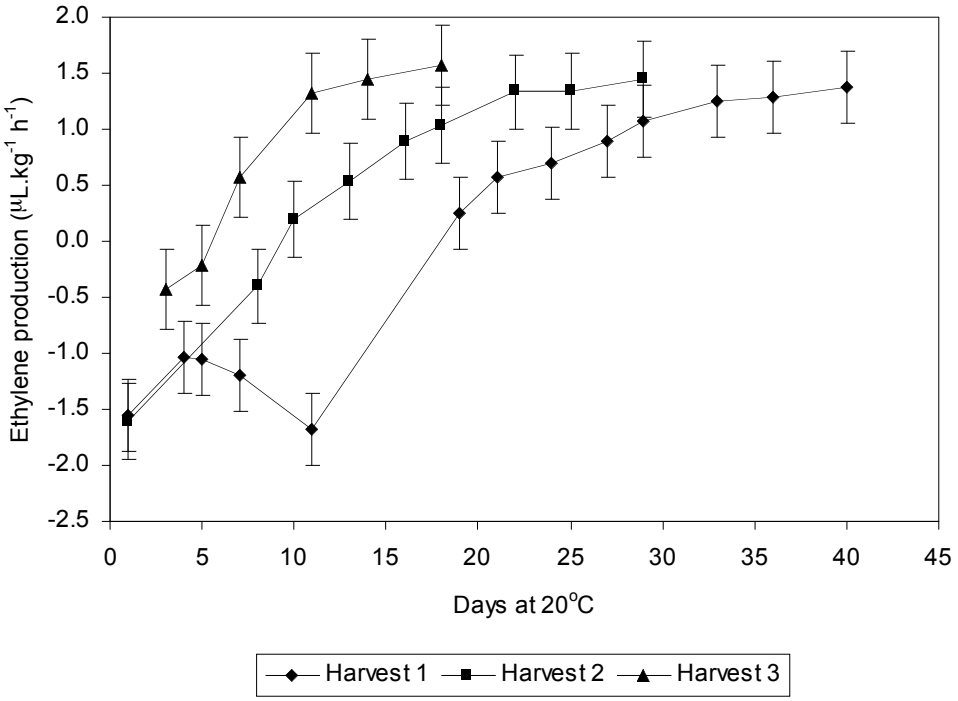

Figure 2.5 Ethylene production at 20°C of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2006. Harvest 1 (30/03/2006) starch pattern index: 1.0, Harvest 2 (10/04/2006) starch pattern index: 3.5, Harvest 3 (21/04/2006) starch pattern index 5.5. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 0.95

2.5.2.2.2 The Huon Valley

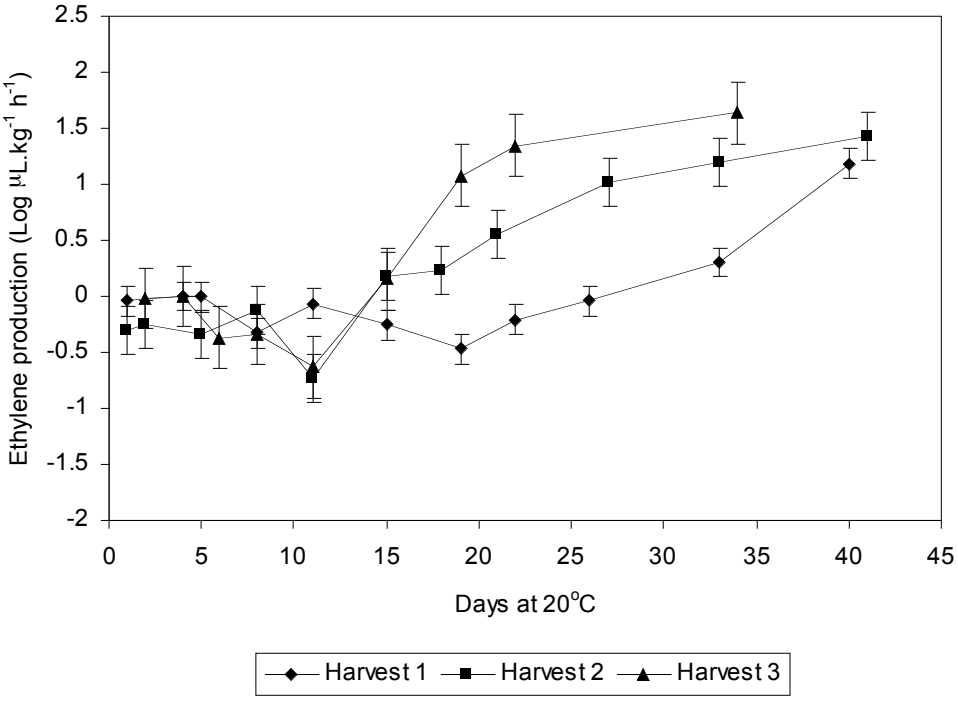

Figure 2.6 Ethylene production at 20°C of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in 2004. Harvest 1 (01/04/2004) starch pattern index: 1.8, Harvest 2 (07/04/2004) starch pattern index: 4.3, Harvest 3 (14/04/2004) starch pattern index 5.6, Harvest 4 (26/04/2004) starch pattern index 8.1. Bars represent standard errors, for comparison between harvests and times, LSD (5%): 0.94

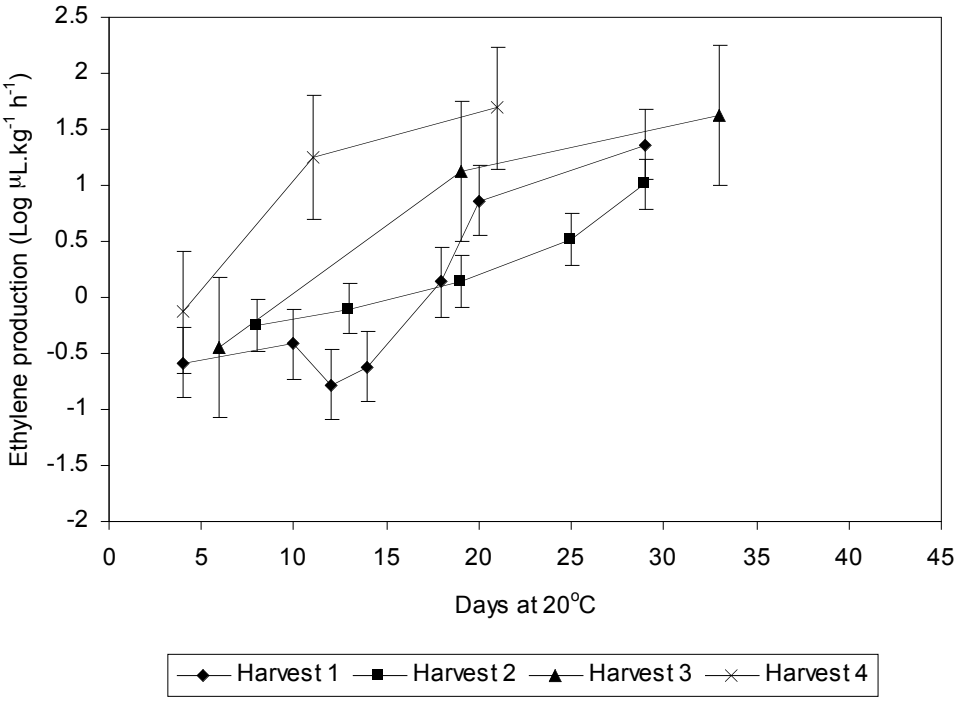

Figure 2.7 Ethylene production at 20°C of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in 2005. Harvest 1 (31/03/2005) starch pattern index: 2.6, Harvest 2 (06/04/2005) starch pattern index: 7.9, Harvest 3 (13/04/2005) starch pattern index 8.7. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 0.60

2.5.3 Greenlife

Regression analysis was used to quantify the relationship between maturity at harvest and the days of greenlife. The dependent variable was days of greenlife and the independent variables were maturity and quality measures including SPI, BG colour, FF and IEC (Table 2.6). When data from all seasons from Batlow and the Huon Valley are combined, the regression analysis was significant (P<0.001) however it accounted for only 42.9% of the variation. When the days of greenlife were plotted against the SPI at harvest it was found that fruit with a high SPI at harvest had a lower number of days of greenlife (Figure 2.8).

Table 2.6 Regression equations, significance and % variation accounted for, for regression analysis of days of greenlife and maturity at harvest of 'Cripps Pink' apples grown in Batlow (New South Wales) and the Huon Valley (Tasmania) in the 2004, 2005 and 2006 seasons.

District	Season	Regression equation	Significance	% Variation accounted for
Batlow + Huon Valley	2004 + 2005 + 2006	greenlife = -1.69xSPI + 16.57	P<0.001	32.9
Batlow + Huon Valley	2004 + 2005 + 2006	greenlife = -0.874xSPI - 4.72xBG colour + 31.48	P<0.001	42.9
Huon Valley	2004 + 2005	greenlife = -1.609xSPI + 23.54	ns	31.8
Huon Valley	2004 + 2005	greenlife = -1.368xSPI - 7.18xBG colour + 50.58	P=0.002	94.1
Huon Valley	2004	greenlife = -2.092xSPI + 22.07	P=0.036	89.3
Huon Valley	2005	greenlife = -2.166xSPI + 32.24	ns	97.1
Batlow	2004 + 2005 + 2006	greenlife = -2.474xSPI + 18.41	P<0.001	67.8
Batlow	2004 + 2005 + 2006	greenlife = -2.771xSPI - 0.552xFF + 25.56	P<0.001	68.3
Batlow	2004	greenlife = -2.274xSPI + 16.81	P<0.001	69.5
Batlow	2005	greenlife = -5.826xSPI + 35.53	P<0.001	83.6
Batlow	2005	greenlife = -5.257xSPI - 7.73xIEC + 33.66	P<0.001	84.2
Batlow	2006	greenlife = -2.47xSPI + 19.02	P<0.001	64.5

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Regression analysis with SPI as the response variate are presented for each season and district and for districts and seasons combined, if a stepwise regression indicated a second response variate improved the model, the model with 2 response variates is also presented.

The number of days of greenlife was found to vary considerably between the 2 districts; fruit sourced from Batlow took fewer days to reach the EC than fruit from the Huon Valley (Figure 2.9). When data from all seasons was combined (Figure 2.9), the average greenlife for fruit grown in Batlow and harvested at an SPI of 3.5 (the recommended optimal SPI for long term storage) was 10 days whereas the seasonal average for fruit grown in the Huon Valley and harvested at a SPI of 3.5 was 23 days.

When the analysis was repeated for each district independently (Figure 2.9), to remove the variability associated with the district, the linear correlation coefficient between days of greenlife and the SPI increased (Batlow R^2=0.678, Huon Valley R^2=0.657). When the analysis was repeated again for each season within a district, to remove the variability associated with seasonal factors, the best fit of the data was observed. For example, the highest correlation between greenlife and SPI in Batlow (Figure 2.10) occurred in the 2005 season (R^2=0.921). In the Huon Valley (Figure 2.11), the highest correlation was found for the 2004 season (R^2=0.964).

In both Batlow and the Huon Valley, the number of days of greenlife also varied between seasons. At the commercial harvest (SPI of 3.5), fruit from Batlow had a greenlife of between 9 and 15 days depending on the season (Figure 2.10), whereas fruit from the Huon Valley had a greenlife of between 15 and 25 days (Figure 2.11). In both districts, fruit from the 2004 season showed the shortest greenlife, while fruit from the 2005 season had the longest period of greenlife. In Batlow, the 2005 season was the coolest of the 3 growing seasons assessed (Chapter 5, Table 5.2)

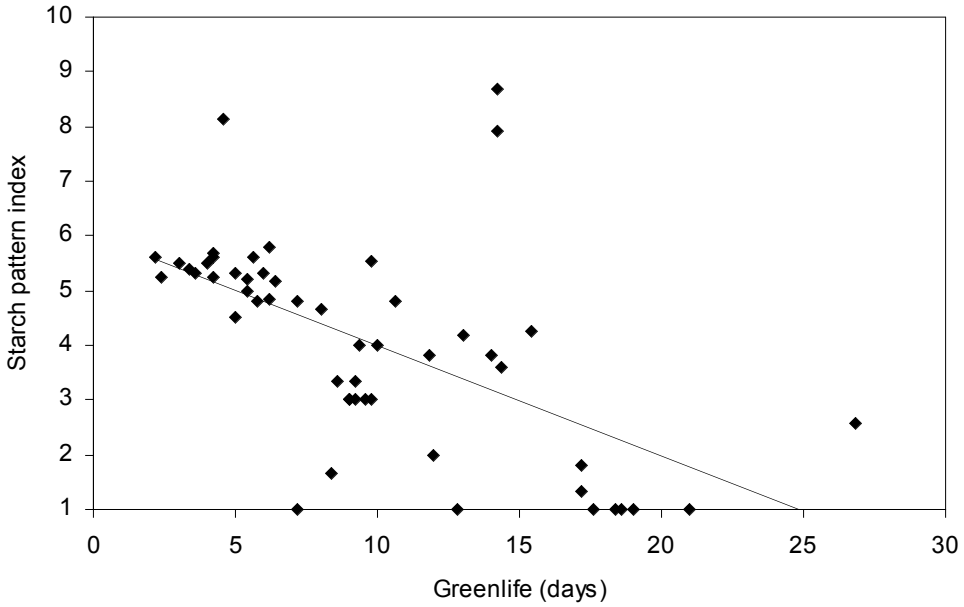

Figure 2.8 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from Batlow (New South Wales), 2004, 2005 and 2006 seasons and the Huon Valley (Tasmania) 2004 and 2005 seasons combined. R^2 = 0.585, P<0.001

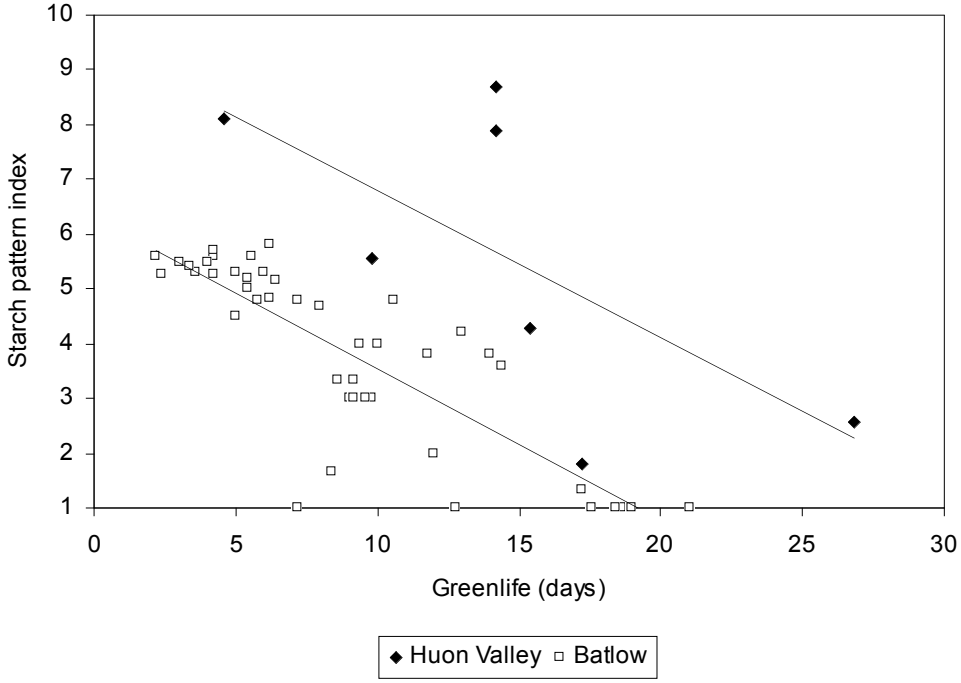

Figure 2.9 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from Batlow (New South Wales), 2004, 2005 and 2006 seasons combined and the Huon Valley (Tasmania) 2004 and 2005 seasons combined. Batlow R^2=0.683, P<0.001, Huon Valley R^2=0.657, ns

2.5.3.1 Batlow

In Batlow, the SPI was significantly correlated with the days of greenlife in each season (P<0.001, in all seasons and for seasonal data combined) (Tables 2.7-2.10). In Batlow, greenlife was also found to be significantly correlated with other maturity indicators, such as BG colour, FF and IEC in some seasons, however the SPI was found to be the best single predictor of greenlife consistently in all seasons and when data from multiple seasons was combined (Table 2.7).

Using a regression analysis, the SPI accounted for between 64.5 and 83.6% of the variation observed in the greenlife, depending on the season. The regression was only slightly improved with the addition of a second indicator of maturity in the 2005 season or when combining the data from all seasons. In the 2005 season, a stepwise regression analysis indicated a small improvement in the relationship with the addition of IEC to the SPI in the regression model (83.6% for SPI alone, 84.2% for SPI and IEC).

When combining data from the three seasons, a slightly stronger regression included the average FF level in addition to the SPI (67.8% for SPI alone, 68.3% for SPI and FF).

In Batlow, the slope of the regression line was found to vary between seasons. In the 2005 season, the slope of the regression line was significantly different from the 2004 and 2006 seasons (P=0.002). The slope of the regression line in 2005 was reduced as a result of an extended period of greenlife in Batlow for that season. Consequently, there was a larger change in the days of greenlife with each unit change in the SPI in 2005 than was observed in the other seasons.

Table 2.7 Correlation matrix of maturity and quality at harvest and days of greenlife for 'Cripps Pink' apples grown in Batlow (New South Wales) with data from the 2004, 2005 and 2006 seasons combined

	BG colour	FF	IEC	SPI	Greenlife
BG colour	1	-0.622 ****	0.113 n/s	0.839 ****	-0.676 ****
FF		1	-0.097 n/s	-0.663 ****	0.465 ****
IEC			1	0.215 n/s	-0.119 n/s
SPI				1	-0.828 ****
Greenlife					1

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at $20^{o}C$ until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Significance: **** P<0.001

Table 2.8 Correlation matrix of maturity and quality at harvest and days of greenlife for 'Cripps Pink' apples grown in Batlow (New South Wales) with data from the 2004 season

	Background	Firmness	IEC	SPI	Greenlife
BG colour	1	-0.207 n/s	0.598 ***	0.841 ****	-0.695 ****
FF		1	-0.191 n/s	-0.438 n/s	0.318 n/s
IEC			1	0.747 ****	-0.545 **
SPI				1	-0.846 ****
Greenlife					1

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Significance: ****,***,** represent P<0.001, <0.02, <0.05 respectively

Table 2.9 Correlation matrix of maturity and quality at harvest and days of greenlife for 'Cripps Pink' apples grown in Batlow (New South Wales) with data from the 2005 season

	BG colour	FF	IEC	SPI	Greenlife
BG colour	1	0.25 n/s	0.663 ****	0.556 **	-0.558 **
FF		1	-0.082 n/s	-0.26 n/s	0.233 n/s
IEC			1	0.562 **	-0.627 ***
SPI				1	-0.921 ****
Greenlife					1

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Significance: ****,***,** represent P<0.001, <0.02, <0.05 respectively

Table 2.10 Correlation matrix of maturity and quality at harvest and days of greenlife for 'Cripps Pink' apples grown in Batlow (New South Wales) with data from the 2006 season

	BG colour	FF	IEC	SPI	Greenlife
BG colour	1	-0.649 ****	0.271 n/s	0.86 ****	-0.659 ****
FF		1	-0.418 n/s	-0.896 ****	0.799 ****
IEC			1	0.374 n/s	-0.236 n/s
SPI				1	-0.819 ****
Greenlife					1

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Significance: **** P<0.001

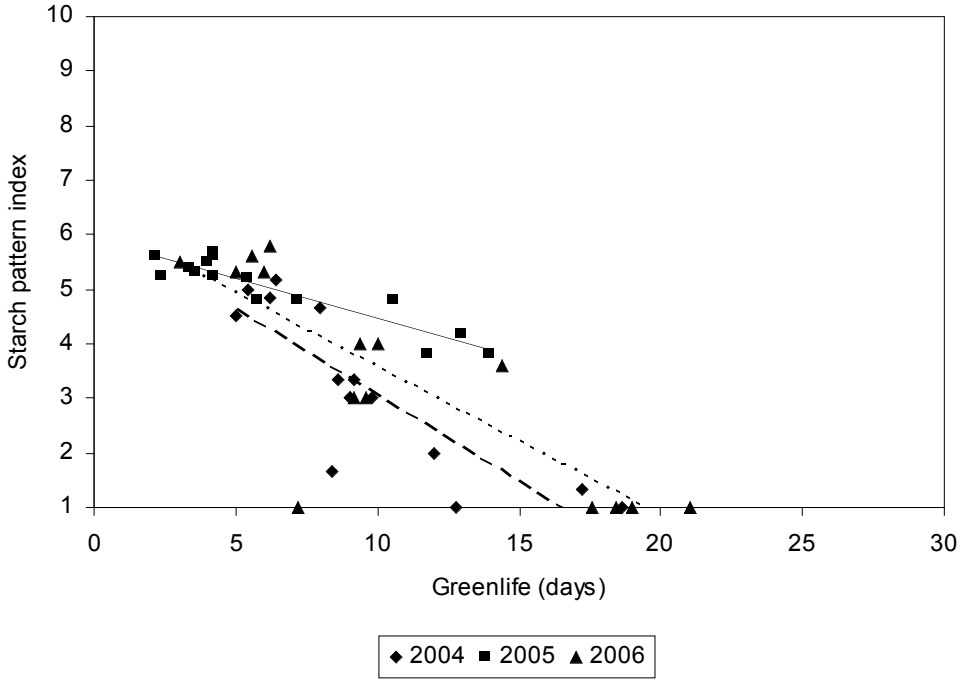

Figure 2.10 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from Batlow (New South Wales), 2004, 2005 and 2006 seasons. 2004 R^2=0.695, P<0.001. 2005 R^2=0.842, P<0.001. 2006 R^2=0.645, P<0.001.

2.5.3.2 The Huon Valley

In the Huon Valley, the SPI was also significantly correlated with the days of greenlife (Tables 2.11-2.12). The SPI accounted for 89.3% and 97.1% of the variation in greenlife in the 2004 and 2005 seasons respectively (Table 2.6). However, the regression analysis in the 2005 season was not significant, despite the high variation that was accounted for. In 2005 and when data from both seasons was combined, the SPI was the only maturity indicator which had a significant correlation with greenlife. However, the lower number of replicates used in the experiments in the Huon Valley meant that the correlation had to be very high in order to be significant.

In the Huon Valley the regression relationship was improved with the addition of a second term in the 2004 season. The addition of the IEC to the regression model increased the variation accounted for to 91.2%.

When the seasonal data from 2004 and 2005 was combined for the Huon Valley, there was a poor fit of the SPI to the greenlife, the regression was not significant and only 31.8% of the variation was accounted for. This indicates that the greenlife varied substantially between these two seasons in this district. The regression with seasonal data combined was improved with the addition of a second term to the regression model. By adding BG colour to the regression model, the model was improved from accounting for 31.8% of the variation in greenlife to accounting for 94.1% (Table 2.6).

In contrast to Batlow, the slope of the regression line did not change significantly between seasons in the Huon Valley. However the position of the line did vary considerably resulting in a vastly different greenlife for the same SPI between seasons (~24 days difference between 2004 and 2005 for SPI of 3.5). This indicates that there are large seasonal variations in greenlife in the Huon Valley.

Table 2.11 Correlation matrix of maturity and quality at harvest and days of greenlife for 'Cripps Pink' apples grown in the Huon Valley (Tasmania) with data from the 2004 and 2005 seasons combined

	BG colour	FF	IEC	SPI	Greenlife
BG colour	1	0.666 n/s	-0.29 n/s	0.134 n/s	-0.809 **
FF		1	-0.028 n/s	-0.545 n/s	-0.215 n/s
IEC			1	-0.478 n/s	0.413 n/s
SPI				1	-0.657 n/s
Greenlife					1

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at 20^{o}C until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Significance: ** P<0.05

Table 2.12 Correlation matrix of maturity and quality at harvest and days of greenlife for 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in the 2004 season

	BG colour	FF	IEC	SPI	Greenlife
BG colour	1	-0.729 n/s	-0.5 n/s	0.883 n/s	-0.94 *
FF		1	0.191 n/s	-0.885 n/s	0.916 *
IEC			1	-0.609 n/s	0.424 n/s
SPI				1	-0.964 **
Greenlife					1

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at 20^{o}C until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Significance: **,* represent P<0.05, <0.1 respectively

Table 2.13 Correlation matrix of maturity and quality at harvest and days of greenlife for 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in the 2005 season

	BG colour	FF	IEC	SPI	Greenlife
BG colour	1	-0.855 n/s	-0.245 n/s	0.933 n/s	-0.882 n/s
FF		1	-0.292 n/s	-0.611 n/s	0.511 n/s
IEC			1	-0.579 n/s	0.672 n/s
SPI				1	-0.993 *
Greenlife					1

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples
Flesh firmness (FF): N
Internal ethylene concentration (IEC): $\mu L.L^{-1}$
Starch pattern index (SPI): Ctifl 10 point scale
Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu L.kg^{-1}\ hr^{-1}$
Significance: * P<0.1

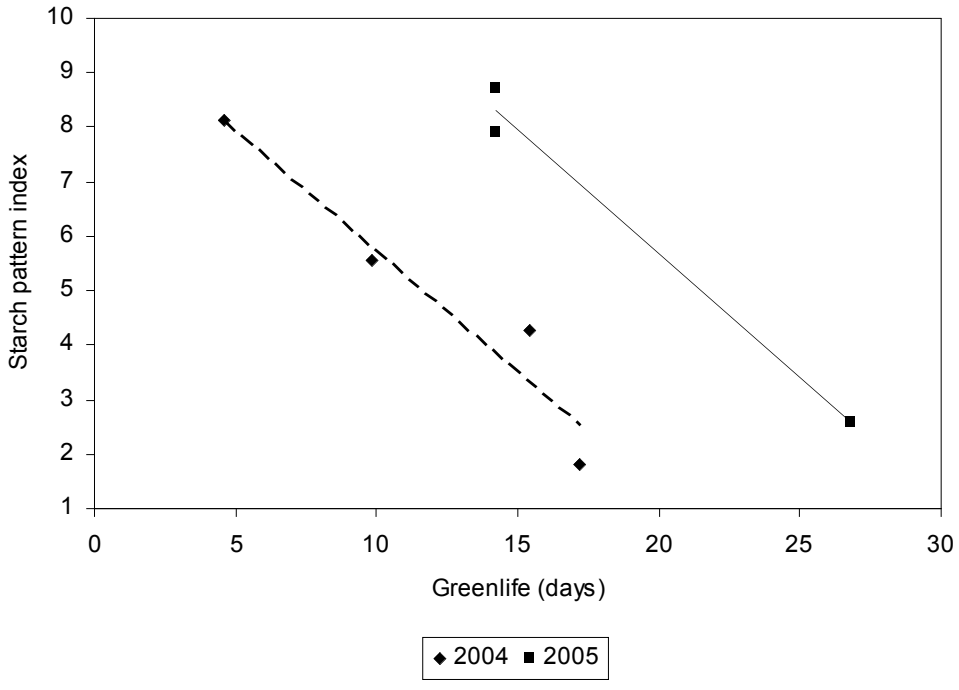

Figure 2.11 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from the Huon Valley (Tasmania), 2004 and 2005 seasons. 2004 R^2=0.0.964, P=0.036. 2005 R^2=0.993, ns

2.6 Discussion

The aim of these experiments was to understand the relationship between the physiological ripening of 'Cripps Pink' apples and commercially used indicators of apple maturity and quality. Maturity is a term used to describe the stage of development of the fruit at harvest. Maturity is described as the capacity of the fruit to ripen once removed from the tree. The term ripening is used to describe the physiological processes of respiration and ethylene production of fruit postharvest. Using this definition, ripening relates to the physiological changes that occur following harvest. In this work, geenlife is the term used to describe the number of days from harvest until the beginning of the climacteric rise in ethylene production and relates to the time taken for the fruit to ripen. The term 'green-life' has been used to describe the period of time taken for the skin of banana fruit to change from green to yellow, a process dependent on production of ethylene (Peacock, 1972). In this research, the greenlife is similarly defined as the period of time from harvest until the level of ethylene production reaches a climacteric level.

This work investigated the relationships between commercial maturity and quality indicators and the days of greenlife for 'Cripps Pink' apples grown in two districts over 3 seasons in order to establish the best predictor of harvest maturity for this cultivar. This work relates to the postharvest changes in quality and maturity indicators and how these are influenced by preharvest climatic conditions.

The influences of both ripening and maturity at harvest on fruit quality and the storage potential of apples has been examined extensively (Brookfield and Watkins, 1993; DeEll et al., 2000; Jobling and McGlasson, 1995b; Jobling et al., 1993; Lau, 1998; Lau and Lane, 1998; Little and Holmes, 2000; Watkins et al., 2002). However, as the 'Cripps Pink' cultivar is relatively new, little information is available on how these factors will influence the storage behaviour of this apple and how these may relate to the development of the flesh browning disorder.

Apples are a climacteric fruit, as indicated by the characteristic rise in ethylene and CO_2 production during ripening (Blanpied and Little, 1991; Little and Holmes, 2000; Watkins et al., 2002; White, 2002). In this work, the analysis of postharvest ethylene production over time illustrates that the less mature the fruit is at harvest, the longer the fruit will take to reach a climacteric level of ethylene production (Figures 2.3-2.7). This result is supported by similar studies relating ethylene production to maturity in

other apple cultivars (Blanpied and Little, 1991; De Castro et al., 2007) and indicates that the 'Cripps Pink' apple follows the established pattern of apple ripening.

Climacteric fruit also undergo a number of physical and chemical changes throughout ripening, including changes in colour, texture, flavour and aroma (White, 2002). Several of these changes that are associated with the ethylene climacteric have become useful benchmarks for the determination of apple maturity and are used for determining the harvest date in the commercial apple orchard (Little and Holmes, 2000).

However, not all ripening processes are regulated by ethylene (Alexander and Grierson, 2002). Recent research has shown that ethylene does not simply switch on and control all of the changes in fruit quality that are associated with climacteric ripening (Alexander and Grierson, 2002; Hiwasa et al., 2003). For some elements of ripening ethylene is only the trigger to initiate changes while for others ethylene is also required for controlling the rate of change. In particular, starch degradation (the most commonly used indicator of apple maturity) has been shown to be initiated by ethylene but the rate of degradation is not regulated by ethylene, once initiated starch degradation does not require the presence of ethylene for it to continue to progress (Dilley and Dilley, 1985). However, the loss of firmness and changes in BG colour are ethylene dependent processes (Hiwasa et al., 2003; Picton et al., 1995). These changes are not only initiated by ethylene, but are also maintained by ethylene (Hiwasa et al., 2003; Picton et al., 1995).

The current hypothesis is that the processes responsible for the changes associated with climacteric fruit ripening can be segregated into those that are independent of ethylene, or dependent on ethylene for their regulation (Alexander and Grierson, 2002). However, despite contrasting modes of regulation, most research to date indicates that all processes are initiated by ethylene (Alexander and Grierson, 2002; Hiwasa et al., 2003; Picton et al., 1995).

The ripening of apples is understood to be initiated by low concentrations of ethylene, in the range of $0.1 \mu l.kg^{-1}hr^{-1}$ (Knee, 1985; Wills et al., 1998). However the climacteric phase of autocatalytic production of ethylene, responsible for the maintenance of ethylene dependent ripening processes, commences at the higher concentration of $1 \mu l.kg^{-1}hr^{-1}$ of ethylene (Blanpied and Little, 1991). In these experiments, greenlife

was determined as the number of days to an ethylene production rate of $1\mu l.kg^{-1}hr^{-1}$ as such, the term 'greenlife' indicates the degree to which the processes of ripening have been initiated and the ethylene dependent processes have been maintained.

In this study, the SPI was found to be the best predictor of greenlife. However, the variation in the relationship between SPI and greenlife between districts and between seasons indicates that this measure of maturity is influenced by other seasonal factors. This supports the work that shows that starch degradation is initiated by ethylene but not regulated by it. Starch begins to break down once the fruit change from a predominantly growth phase to a growth and ripening phase. Ethylene sensitivity also changes at this time (Little and Holmes, 2000).

The greatest variation in greenlife was found between the different growing districts, Batlow (New South Wales) and the Huon Valley (Tasmania) (Figure 2.9). When harvesting at a SPI of 3.5 (recommended maturity for long-term storage), fruit from Batlow had an average greenlife of 10 days while fruit from the Huon Valley had an average greenlife of 23 days. This shows that fruit harvested at the same SPI can take vastly different amounts of time to reach the EC. This suggests that seasonal growing conditions influence the physiology of the fruit at harvest.

It has been established that climatic conditions during fruit growth and development can influence the rate of apple ripening in the field (Little and Holmes, 2000; Tromp, 1997; Tromp, 1999). Warm temperatures during ripening can increase the rate of starch degradation observed and conversely, cool temperatures can slow the rate of degradation (Little and Holmes, 2000). However, this does not explain the seasonal and district variation in greenlife observed in this experiment. This experiment was carried out postharvest with the fruit being held at a constant temperature (20^oC). This work indicates that the preharvest climatic conditions have influenced the postharvest physiology of the fruit.

These two districts have contrasting climatic conditions during fruit growth and development. Batlow has a much warmer climate than the Huon Valley (Chapter 5, Tables 5.2-5.3) and it has been shown that the climate effects several areas of the physiological development of the fruit (Little and Holmes, 2000; Martin, 1954; Palmer et al., 2003; Sharples, 1975). Similar studies completed in Hawke's Bay (New Zealand), Nelson (New Zealand), Laimburg (Italy) and California (USA) support the

finding that ‘Cripps Pink’ apples grown in cool climates have a longer period of greenlife than those grown in warm climates (James et al., 2005).

Temperatures during fruit growth and development have been shown to influence apple fruit ripening. Such climatic effects can help to explain the contrasting periods of postharvest greenlife observed in Batlow and the Huon Valley. Interestingly, climatic conditions during the 6 weeks prior to harvest have been shown to have little or no effect on apple (Tromp, 1999) or pear (Lombard et al., 1971) ripening with regard to ethylene production, starch degradation, loss of FF or changes in BG colour. However, climatic conditions during the 6 week period following full bloom have been found to significantly alter apple ripening (Tromp, 1997).

In apple physiological development, the first 6 weeks following full bloom are characterized primarily by cell division (Denne, 1963). The period of cell division usually varies between 30 and 50 days from full bloom, depending on the cultivar (Denne, 1963). Tromp (1997) found that cool temperatures (16^{o}C) during this period can delay the onset of ripening in ‘Elstar’ apples compared to those grown at 22^{o}C. There is no doubt that apple fruit ripening has a component of genetic control with ethylene being responsible for turning on genes for the transcription and translation of enzymes responsible for some of the biochemical changes associated with fruit ripening (Huybrechts et al., 2003). Climatic conditions during this period of cell division may therefore influence the genetic potential of the fruit to ripen within a period of time. The theory that not all ripening process are maintained by ethylene (Alexander and Grierson, 2002) also indicates that climatic conditions during the first 6 weeks from full bloom may independently alter the processes of apple ripening resulting in a lack of synchronization between ethylene dependent and independent processes.

Climatic conditions, hypothesized to be the accumulation of growing degree days (GDD), during different phases of apple development might also be associated with the switch between growth, maturation and ripening of apples. As such, the accumulation of GDD may provide the switch for the genes responsible for ripening in apples. Recent research has shown that climatic conditions during growth can influence the development and accumulation of polyamines in plant cells (Couee et al., 2004). Polyamines are thought to be involved in the control or moderation of senescence (de Dios et al., 2006; Tassoni et al., 2006). This research indicates that climatic conditions during growth could have a physiological link to ripening. It is

therefore likely that the differences in greenlife reported here are due to the different climatic conditions within the 2 regions and the subsequent impact on the genetic regulation of ripening.

The time taken to reach the EC is also of commercial importance. For optimal long-term storage, it is essential that apples are harvested and placed under ideal storage conditions before the EC. Placing post-climacteric apples into long-term storage can result in the loss of quality and a poor outturn (Chapter 4). As the commercial establishment of long-term storage conditions can take up to 15 days, it is valuable to know how long the greenlife of the fruit will be at harvest. However, the use of rapid CA technology can reduce the amount of time taken to establish CA conditions (Little and Holmes, 2000; Watkins, 2003). This technology ensures that CA conditions were established prior to the end of greenlife of fruit harvested at a SPI of 3.5 in both Batlow (New South Wales) and the Huon Valley (Tasmania).

In this study, the greenlife was correlated also to a number of commercially used measures of apple maturity and quality including the SPI, FF and BG colour.

The accurate assessment of fruit maturity is vital for the postharvest management of apples. Despite the fact that the SPI has a reputation for lacking sensitivity for the determination of maturity in apples (Magein and Leurquin, 2000; Peirs et al., 2002), it has proven to be the best available predictor of greenlife in ‘Cripps Pink’ apples grown in Batlow and in the Huon Valley. However, due to the significant variation observed between districts and seasons the influence of climatic conditions should also be taken into account when using the SPI for the determination of maturity in ‘Cripps Pink’ apples. Despite the climatic variation in SPI observed in this study, the SPI is suitable for the determination of harvest maturity in ‘Cripps Pink’ apples. In all cases, ‘Cripps Pink’ apples harvested at an SPI of 3.5 were pre-climacteric and as such, suited to long-term storage

Currently, the measurement of ethylene production is required in tandem with the SPI for the determination of greenlife. With further seasons of research, ripening synchronization and greenlife may be determined through the combined measurement of SPI, BG colour and FF providing a practical solution to this problem. It is also possible that the SPI scale could be calibrated based on climatic conditions so that the GDD combined with the SPI could be used for the accurate assessment

of maturity and storage potential. For example in cooler seasons, harvest could be delayed to allow for greater colour and flavour development.

Although the rate of starch degradation is not regulated by ethylene, in this work, the SPI had a stronger correlation with greenlife than changes in BG colour and loss of FF, which are both ethylene dependent processes. Both FF and BG colour are processes that are dependent on ethylene to initiate the changes associated with ripening, as well as for regulating the changes throughout the ripening process (Hiwasa et al., 2003). The application of ethylene inhibitors during climacteric ripening has been found to arrest the progress of changes in firmness and background colour (Hiwasa et al., 2003) illustrating the dependence of such processes on ethylene production for their regulation throughout ripening.

At no time was the IEC found to be significantly correlated to greenlife. This is not unexpected however due to the fact that the measurement of IEC is highly sensitive resulting in wide variation between samples reducing the likelihood of a significant correlation. Future work could study this in more detail using greater numbers of fruit in order more clearly model the relationship between IEC and greenlife.

It is possible that the degree to which greenlife is correlated to both ethylene independent and ethylene dependent processes indicates the degree to which these processes are synchronised. In the event where all ripening processes are synchronised, ripening would be initiated by ethylene and all processes of ripening would be maintained over time. As a result, all ripening processes should be equally correlated with greenlife.

In a situation where the processes are not synchronised, ripening would be initiated and starch (ethylene independent) would continue to degrade over time. There would however be a delay before the ethylene production required for the initiation of ripening increased to the level required for the regulation of ethylene dependent ripening changes such as FF and BG colour. Consequently these changes would not be maintained in synchronisation with changes in the SPI. Such a delay in the switch between ripening initiation and regulation concentrations of ethylene is hypothesised to be the cause of the de-synchronised ripening in ‘Cripps Pink’ apples grown in cool climates (Figure 2.12).

The degree of ripening synchronisation varied between the three seasons in Batlow, the variation in ripening synchronisation was found to be the same as the variation that was observed in the incidence of FB observed between the three seasons. In 'Cripps Pink' apples grown in Batlow, only the 2006 season had equally significant correlations between FF, BG colour and SPI to greenlife (Table 2.10). This suggests that only the 2006 season showed synchronisation between ethylene dependent and independent ripening processes. In the 2004 season, BG colour was equally correlated to the SPI with greenlife, but FF was not, perhaps suggesting partial synchronisation (Table 2.8). In the 2005 season, neither BG colour nor FF were equally correlated with the SPI (Table 2.9) indicating a lack of synchronisation in that season. The pattern of seasonal temperature was also found to follow the same trend, 2006 was the warmest season, 2005 was the coolest season and 2004 was intermediate between the other seasons (Chapter 5 table 5.2).

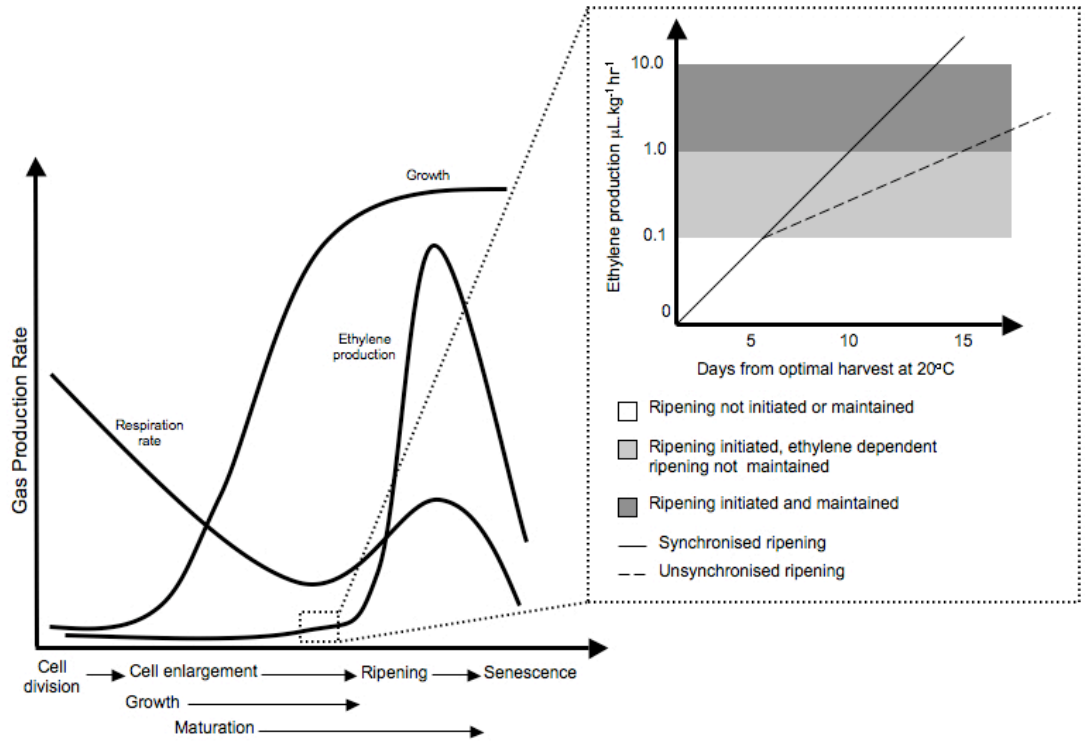

Figure 2.12 Relationship between fruit growth and development, respiration rate and ethylene production (adapted from Little and Holmes, 2000). Inset graph of days from optimal harvest at 20°C (greenlife) indicating hypothesised patterns of synchronised and unsynchronised ripening based on data collected from fruit grown in Batlow (New South Wales) and the Huon Valley (Tasmania).

The degree of synchronisation may in turn indicate the susceptibility of the fruit to developing disorders. The incidence of RFB was lowest in the 2006 season (10.00%, Chapter 5, Table 5.2), the season with the highest degree of ripening synchronization. The incidence of RFB was highest in the 2005 season (57.50%,

Chapter 5, Table 5.2), the season with no synchronisation. The 2004 season had an intermediate incidence of RFB (27.78%, Chapter 5, Table 5.2) and this season showed partial ripening synchronisation. Climatic variation between the seasons in both districts is hypothesised to be the cause of this variation in ripening.

It is possible that in cool climates (hypothesized to be those accumulating less than 1500 GDD>10^{o}C between full bloom and harvest), there is a delay between the initiation and regulation of ripening. Cool climates such as the Huon Valley or cool seasons, such as the 2005 season in Batlow, show extended periods of greenlife and are indicative of unsynchronised ripening. The potential consequences of unsynchronised ripening are unclear, although they may relate to the development of senescent or maturity related disorders during storage. Further work, collecting data over more seasons would be needed to determine the long term significance of the relationship between climate, greenlife and storage disorders.

2.7 Summary

- Maturity
  - ⇒ IEC, rate of ethylene production and CO_2 production, SPI, FF and BG colour progressed with successive harvests.
  - ⇒ The SPI was found to be the best indicator of maturity of those assessed, however this may be improved by adding in a seasonal temperature factor.
- Ripening
  - ⇒ The number of days taken to reach a climacteric level of ethylene production (1μL.kg^{-1} hr^{-1}) is the greenlife of the fruit.
  - ⇒ The greenlife was longer in fruit grown in the Huon Valley than for fruit grown in Batlow of comparable harvest SPI.
  - ⇒ Cool climatic conditions during the early stages of fruit development are hypothesised to extend the period of greenlife.

2.8 References

Alexander, L.and D. Grierson. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. Journal of Experimental Botany. 53: 2039-2055.

Barden, J.A.and M.E. Marini. 1992. Maturity and quality of 'Delicious' apples as influenced by rootstock. Journal of the American Society of Horticultural Science. 117: 574-550.

Beaudry, R. 1995. Michigan State University's apple maturity program: an extension tool to aid harvest and storage decisions. Tree Fruit Postharvest Journal. 6: 5-12.

Beaudry, R., P. Schwallier, and M. Lennington. 1993. Apple maturity prediction: an extension tool to aid fruit storage decisions. HortTechnology. 3: 233-239.

Beaudry, R.M. 1999. Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. Postharvest Biology and Technology. 15: 293-303.

Bhowmik, P.K.and T. Matsui. 2004. Changes in the activity and expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase, ACC oxidase, and phenylalanine ammonia-lyase in asparagus spears in response to wound-induced ethylene synthesis. Hortscience. 39: 1074-1078.

Blankenship, S.M., M. Parker, and C.R. Unrath. 1997. Use of maturity indices for predicting poststorage firmness of 'Fuji' apples. HortScience. 32: 909-910.

Blanpied, G.D.and C.R. Little. 1991. Relationship among bloom dates, ethylene climacteric initiation dates, and maturity-related storage disorders of Jonathan apples grown in Australia. Postharvest Biology and Technology. 1: 3-10.

Bouquin, T., E. Lasserre, J. Pradier, J.C. Pech, and C. Balague. 1997. Wound and ethylene induction of the ACC oxidase melon gene CM-ACO1 occurs via two direct and independent transduction pathways. Plant Molecular Biology. 35: 1029-1035.

Brackman, A.and A.J. Waclawovsky. 2001. Responses of 'Gala' apples to pre-harvest treatment with AVG and low ethylene CA storage. Acta Horticulturae. 553: 155-157.

Brookfield, J.G.and C.B. Watkins. 1993. Fruit maturity and quality of selectively picked 'Royal Gala' apples. New Zealand Journal of Crop and Horticultural Science. 21: 183-187.

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

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

Couee, I., I. Hummel, C. Sulmon, G. Gouesbet, and A. Amrani. 2004. Involvement of polyamines in root development. Plant Cell, Tissue and Organ Culture. 76: 1-10.

Cripps, J.E.L., L.A. Rishards, and A.M. Mairata. 1993. 'Pink Lady' apple. HortScience. 28: 1057.

Crocker, W., A.E. Hitchcock, and P.W. Zimmerman. 1935. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. 7: 231-248.

De Castro, E., W.V. Biasi, and E. Mitcham. 2007. Quality of Pink Lady apples in relation to maturity at harvest, prestorage treatments and controlled atmosphere during storage. HortScience. 42: 605-610.

de Dios, P., A.J. Matilla, and M. Gallardo. 2006. Flowers fertilization and fruit development prompt changes in free polyamines and ethylene in damson plum (Prunus insititia L.). Journal of Plant Physiology. 163: 86-97.

DeEll, J.R., S. Khanizadeh, and D.P. Murr. 2000. Preharvest and postharvest factors influencing apple fruit firmness. New England Fruit Meetings. 106: 43-46.

DeEll, J.R., D.P. Murr, R. Mueller, L. Wiley, and M.D. Porteous. 2005. Influence of 1-methylcyclopropene (1-MCP), diphenylamine (DPA), and CO_2 concentration during storage on 'Empire' apple quality. Postharvest Biology and Technology. 38: 1-8.

DeLong, J.M., R.K. Prange, and P.A. Harrison. 1999. Using the Streif Index as a final harvest window for controlled-atmosphere storage of apples. Hortscience. 34: 1251-1255.

Denne, M. 1963. Fruit development and some tree factors affecting it. New Zealand Journal of Botany. 1: 265-294.

Dilley, C.L.and D.R. Dilley. 1985. New technology for analyzing ethylene and determining the onset of the ethylene climacteric of apples. 4th National Controlled Atmosphere Research Conference. p. 353-362.

Drake, S.R.and T.A. Eisele. 1997. Quality of 'Gala' apples as influenced by harvest maturity, storage atmosphere and concomitant storage with 'Bartlett' pears. Journal of Food Quality. 20: 41-51.

Drake, S.R., D.C. Elfving, and T.A. Eisele. 2002. Harvest maturity and storage affect quality of 'Cripps Pink' (Pink Lady^{R}) apples. Horttechnology. 12: 388-391.

Ebel, R.C., E.L. Proebsting, and M.E. Patterson. 1993. Regulated deficit irrigation may alter apple maturity, quality, and storage life. HortScience. 28: 141-143.

Elgar, H.J., C.B. Watkins, and N. Lallu. 1999. Harvest data and crop load effects on carbon dioxide-related storage injury of 'Braeburn' apple. HortScience. 34: 305-309.

Fellman, J.K., D.R. Rudell, D.S. Mattinson, and J.P. Mattheis. 2002. Relationship of harvest maturity to flavour regeneration after CA storage of 'Delicious' apples. Postharvest Biology and Technology. 27: 39-51.

Fellman, J.K., D.R. Rudell, D.S. Mattinson, and J.P. Mattheis. 2003. Harvest maturity relationship to flavor regeneration after CA-storage of 'Delicious' apples. 8th International Controlled Atmosphere Conference. p. 541-554.

Ferguson, I.B.and C.B. Watkins. 1992. Crop load affects mineral concentrations and incidence of bitter pit in 'Cox's Orange Pippin' apple fruit. Journal of the American Society of Horticultural Science. 117: 373-376.

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

Gorny, J.R.and A.A. Kader. 1996. Regulation of ethylene biosynthesis in climacteric apple fruit by elevated CO_2 and reduced O_2 atmospheres. Postharvest Biology and Technology. 9: 311-323.

Graell, J., I. Recasens, J. Salas, and M. Vendrell. 1993. Variability of internal ethylene concentration as a parameter of maturity in apples. Acta Horticulturae. 326: 277-281.

Greene, D.W. 2003. Endogenous Hormones and Bioregulator Use on Apples, p. 267-302. In: D. Ferree and I. Warrington (eds.). Apples. Botany, Production and Uses. CABI Publishing.

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

Harker, F.R., A. Gunson, and S. Jaeger. 2003. The case for fruit quality: an interpretive review of consumer attitudes and preferences for apples. Postharvest Biology and Technology. 28: 333-347.

Hiwasa, K., Y. Kinugasa, S. Amano, A. Hashimoto, R. Nakano, A. Inaba, and Y. Kubo. 2003. Ethylene is required for both the initiation and progression of softening in pear (*Pyrus communis* L.) fruit. Journal of Experimental Botany. 54: 771-779.

Hurndall, R.and J. Fourie. 2003. The South African Pink Lady™ handbook. South African Pink Lady Association.

Huybrechts, C., T. Deckers, and R. Valcke. 2003. Predicting fruit quality and maturity of apples by fluorescence imaging: effect of ethylene and AVG. p. 243-247.

James, H., G. Brown, E. Mitcham, D. Tanner, S. Tustin, I. Wilkinson, A. Zanella, and J. Jobling. 2005. Flesh browning in Pink Lady™ apples: maturity at harvest is

critical but how accurately can it be measured? Acta Horticulturae. 694: 399-403.

Jerie, P.H., M.A. Hall, and M. Zeroni. 1978. Aspects of the role of ethylene in fruit ripening. Acta Horticulturae. 80: 325-332.

Jobling, J., H. James, M. Forbes-Smith, S. Morris, and J. Golding. 2005. Assessing the effect of a new growth regulator on the storage life of Pink Lady apples. Horticulture Australia Ltd, Sydney.

Jobling, J.and W.B. McGlasson. 1995a. Chilling at 0-Degrees-C in Air Induces Ethylene Production in Fuji and Lady-Williams Apples. Australian Journal of Experimental Agriculture. 35: 651-655.

Jobling, J.J.and W.B. McGlasson. 1995b. A comparison of ethylene production, maturity and controlled atmosphere storage life of Gala, Fuji and Lady Williams apples (*Malus domestica*, Borkh.). Postharvest Biology and Technology. 6: 209-218.

Jobling, J.J., W.B. McGlasson, P. Miller, and J. Hourigan. 1993. Harvest maturity and quality of new apple cultivars. Acta Horticulturae. 343: 53-55.

Kato, M., T. Kamo, R. Wang, F. Nishikawa, H. Hyodo, Y. Ikoma, M. Sugiura, and M. Yano. 2002. Wound-induced ethylene synthesis in stem tissue of harvested broccoli and its effect on senescence and ethylene synthesis in broccoli florets. Postharvest Biology and Technology. 24: 69-78.

Kays, S. 1991. Postharvest physiology of perishable plant products. Thomas Nelson Australia, Melbourne.

Kidd, F.and C. West. 1932. Effects of ethylene and of apple vapours on the ripening of fruits, p. 55-58. Reports of the Food Investigation Board, London.

Kidd, F.and C. West. 1936. Gas storage of fruit IV. Cox's Orange Pippin apples. J. Pomol. Hortic. Sci. 14: 276–294.

Kilili, A.W., M.H. Behboudian, and T.M. Mills. 1996. Postharvest performance of 'Braeburn' apples in relation to withholding of irrigation at different stages of the growing season. Journal of Horticultural Science. 71: 693-701.

Knee, M. 1985. Metabolism of 1-aminocyclopropane-1-carboxylic acid during apple fruit-development. Journal of Experimental Botany. 36: 670-678.

Lafer, G. 2003. Effects of 1-MCP treatments on fruit quality and storability of different apple varieties. Postharvest Unlimited. p. 65-69.

Lara, I.and M. Vendrell. 2000. Changes in abscisic acid levels, ethylene biosynthesis, and protein patterns during fruit maturation of 'Granny Smith' apples. Journal of the American Society of Horticultural Science. 125: 183-189.

Lau, O.L. 1985. Harvest indices for BC apples. British Columbia Orchardist. 7: 1A-20A.

Lau, O.L. 1998. Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. Postharvest Biology and Technology. 14: 131-141.

Lau, O.L.and W.D. Lane. 1998. Harvest indices, storability, and poststorage refrigeration requirement of 'Sunrise' apple. HortScience. 33: 302-304.

Little, C.R.and R. Holmes. 2000. Storage technology for apples and pears. Department of Natural Resources and Economics, Victoria.

Lombard, P.B., C.B. Cordy, and E. Hansen. 1971. Relation of post-bloom temperatures to Bartlett pear maturation. Journal of the American Society for Horticultural Science. 96: 799-&.

Magein, H.and D. Leurquin. 2000. Changes in amylose, amylopectin and total starch content in Jonagold apple fruit during growth and maturation. Acta Horticulturae. 517: 487-491.

Martin, D. 1954. Variation between apple fruits and its relation to keeping quality 3. between-season variation in relation to seasonal climate. Australian Journal of Agricultural Research. 5: 392-421.

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

Palmer, J.W., J.P. Prive, and D.S. Tustin. 2003. Temperature, p. 217-236. In: D. Ferree and I. Warrington (eds.). Apples. Botany, production and uses. CABI Publishing.

Park, M., H. Kweon, I. Kang, and J. Byun. 1999. Effects of AVG application on harvest time extension and storability improvement in 'Tsugaru' apples. Journal of the Korean Society for Horticultural Science. 40: 577-580.

Peacock, B.C. 1972. Role of ethylene in the initiation of fruit ripening. Queensland Journal of Agricultural and Animal Scences. 29: 137-145.

Peirs, A., N. Scheerlinck, A.B. Perez, P. Jancsok, and B.M. Nicolai. 2002. Uncertainty analysis and modelling of the starch index during apple fruit maturation. Postharvest Biology and Technology. 26: 199-207.

Picton, S., J. Gray, and D. Grierson. 1995. Ethylene genes and fruit ripening. In: P. Davis (ed.). Plant hormones physiology, biochemistry and molecular biology. Kluwer Academic Publishers, the Netherlands.

Plotto, A., A.N. Azarenko, J.P. Mattheis, and M.R. McDaniel. 1995. 'Gala,' 'Braeburn,' and 'Fuji' Apples: maturity indices and quality after storage. Fruit Varieties Journal. 49: 133-142.

Roberts, J.and R. Hooley. 1988. Plant growth regulators. Blackie and Son Ltd, London.

Sharples, R. 1975. Influence of climate on the storage quality of pome fruits. In: H.C. Pereira (ed.). Climate and the orchard. Commonwealth Agricultural Bureaux, England.

Sisler, E.C.and M. Serek. 2003. Compounds interacting with the ethylene receptor in plants. Plant Biology. 5: 473-480.

Smith, R.B., E.C. Lougheed, and E.W. Franklin. 1969. Ethylene production as an index of maturity for apple fruits. Canadian Journal of Plant Science. 49: 805-807.

Smith, R.B., E.C. Lougheed, E.W. Franklin, and I. McMillan. 1979. The starch iodine test for determining stage of maturation in apples. Canadian Journal of Plant Science. 59: 725-735.

Tassoni, A., C.B. Watkins, and P.J. Davies. 2006. Inhibition of the ethylene response by 1-MCP in tomato suggests that polyamines are not involved in delaying, but may moderate the rate of ripening of over-ripening. Journal of Experimental Botany. 57: 3313-3325.

Tomala, K. 1999. Orchard factors affecting fruit storage quality and prediction of harvest date of apples. Acta Horticulturae. 485: 373-382.

Tough, H.J., D.G. Park, K.J. Crutchley, F.B. Bartholomew, and G. Craig. 1996. Effect of crop loan on mineral status, maturity and quality of 'Braeburn' (*Malus domestica* Borkh.) apple fruit. Postharvest 1996. p. 53-57.

Tromp, J. 1997. Maturity of apple cv. Elstar as affected by temperature during a six-week period following bloom. Journal of Horticultural Science. 72: 811-819.

Tromp, J. 1999. Maturity of apple cvs 'Cox's Orange Pippin' and 'Elstar' as affected by mid-season and late-season temperature. Gartenbauwissenschaft. 64: 145-151.

Volz, R.K., I.B. Ferguson, J.H. Bowen, and C.B. Watkins. 1993. Crop load effects on fruit mineral nutrition, maturity, fruiting and tree growth of 'Cox's Orange Pippin' apple. Journal of Horticultural Science. 68: 127-137.

Volz, R.K., J.W. Palmer, and H.M. Gibbs. 1995. Within-tree variability in fruit quality and maturity for 'Royal Gala' apple. Acta Horticulturae. 379: 67-74.

Walsh, C.S.and S.A. Altman. 1993. Measurement of the ethylene evolution rate of apples during maturation, and its correlation with some commonly-used maturity indices. Acta Horticulturae. 343: 51-52.

Watkins, C.B. 2003. Principles and practices of postharvest handling and stress, p. 585-615. In: D. Ferree and I. Warrington (eds.). Apples. Botany, production and uses. CABI Publishing.

Watkins, C.B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances. 24: 389-409.

Watkins, C.B., P.L. Brookfield, and F.R. Harker. 1993. Development of maturity indices for the 'Fuji' apple cultivar in relation to watercore incidence. Acta Horticulturae. 326: 267-275.

Watkins, C.B., E. Kupferman, and D.A. Rosenberger. 2002. Apple. Postharvest quality management guidelines. In: K.C. Gross, Y. Wang, and M. Saltveit (eds.). The commercial storage of fruits, vegetables and florist and nursery crops. USDA.

Webster, A.D.and S.J. Wertheim. 2003. Apple Rootstocks, p. 91-124. In: D. Ferree and I. Warrington (eds.). Apples. Botany, Production and Uses. CABI Publishing.

White, P. 2002. Recent advances in fruit development and ripening: an overview. Journal of Experimental Botany. 53: 1995-2000.

Wills, R., B. McGlasson, D. Graham, and D. Joyce. 1998. Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals. University of New South Wales Press Ltd, Sydney.

Wilson, P.J. 1998. Variation in maturity traits within individual apples of cultivar Jonagold. Journal of Horticultural Science and Biotechnology. 73: 598-600.

Wunsche, J.N., J.W. Palmer, and D.H. Greer. 2000. Effects of crop load on fruiting and gas-exchange characteristics of 'Braeburn'/M.26 apple trees at full canopy. Journal of the American Society of Horticultural Science. 125: 93-99.

Xin-Jian, C.and F. Jiarui. 2000. Ethylene regulation of fruit ripening: molecular aspects. Plant Growth Regulation. 30: 193-200.

Yokotani, N., S. Tamura, R. Nakano, A. Inaba, W.B. McGlasson, and Y. Kubo. 2004. Comparison of ethylene- and wound-induced responses in fruit of wild-type, rin and nor tomatoes. Postharvest Biology and Technology. 32: 247-252.

Zude-Sasse, M., B. Herold, M. Geyer, and S. Huyskens-Keil. 2001. Influence of maturity stage on physical properties in apple. Acta Horticulturae. 553: 109-110.

3

The determination of the structure and physiology of flesh browning of 'Cripps Pink' apples and the influence of mineral nutrition on the development of the disorder

3.1 Abstract

The flesh browning disorder of 'Cripps Pink' apples was categorised into three separate disorders based on the visual description of symptoms. Radial flesh browning (RFB) was identified by browning of the vascular tissue, in contrast diffuse flesh browning (DFB) was identified as browning of the cortex tissue. Carbon dioxide injury of 'Cripps Pink' apples was identified by the formation of pits and cavities in the flesh of the fruit. The area of affected tissue within the fruit had a different distribution for the RFB and DFB disorders. The area of tissue affected by RFB was highest at the stem end of the fruit decreasing towards the calyx end. In contrast the area of tissue affected by DFB was highest at the stem and calyx ends of the fruit and lowest in the middle section. Examination by scanning electron microscope (SEM) revealed that RFB and DFB were structurally unique. The RFB disorder was associated with fractured cell walls whereas the DFB disorder was associated with the collapse of cells. Analysis of mineral nutrition, fruit density and tree crop load showed inconsistent relationships to the development of RFB and DFB of 'Cripps Pink' apples.

3.2 Introduction

Postharvest storage disorders of apples are often characterised by browning of the flesh of the fruit. Flesh browning in apples is the result of oxidation of phenolic compounds by polyphenol oxidase (PPO), in the presence of oxygen into quinones (Macheix et al., 1990). Quinones undergo further oxidation and polymerisation leading to the appearance of the characteristic brown compounds (Macheix et al., 1990). For this process of oxidation to occur, the cell must have ruptured in order to have exposed the contents to O_2.

The flesh browning (FB) disorder of 'Cripps Pink' apples was classified into three disorders based on the visual assessment of the location of browning within the fruit.

The radial flesh browning (RFB) disorder of 'Cripps Pink' apples is characterised by browning of the vascular tissue of the fruit (Chapter 1, Figure 1.1). In contrast, the diffuse flesh browning (DFB) disorder of 'Cripps Pink' apples is characterised by browning of the cortex tissue of the fruit with the vascular tissue remaining relatively unaffected (Chapter 1, Figure 1.1). The third type of FB, CO_2 injury, is characterised by the development of lens shaped pits and cavities (Chapter 1, Figure 1.1). The variation in the location and type of damage between the three expressions of FB of 'Cripps Pink' apples suggested that the process responsible for the rupturing of the cells varied between the different types.

In apple fruit, preharvest factors including climatic conditions, fruit maturity, rootstock, crop load and mineral nutrition can have an impact on the stability of cells and in turn alter the fruit's susceptibility to developing disorders during storage. Postharvest conditions including the storage temperature and the composition of the storage atmosphere can also weaken the structural stability of cells, increasing the susceptibility of the fruit to developing disorders during storage.

3.2.1 Plant cells

Cells are the building blocks of all living organisms. By understanding the functions of cells and their components, a more thorough understanding of postharvest changes can be gained. The presence of the cell wall and large central vacuole are two of the key characteristics recognisable in plant cells that distinguish them from animal cells. Plant cells vary considerably in size, organisation, function and postharvest response. The postharvest response of plant cells is generally the result of the collective responses of the subcellular organelles. Cell structures of particular importance to postharvest physiology are the cell wall, cell membrane, mitochondria and vacuole. These cellular components are associated with the postharvest development of chilling injury (CI), senescent changes, high CO_2 injury, low O_2 injury and quality changes in apples during long term storage (Fahn, 1990; Kays, 1991; Raven et al., 2005).

3.2.1.1 Cell wall

The cell wall is the primary characteristic separating plant cells from animal cells and provides the mechanical and structural support of the plant. The rigidity and structure of most harvested products is predominantly a result of the presence of the cell wall.

The cell wall is largely responsible for determining the size and shape of the cell as well as the texture of the tissue. Types of cells can often be identified by the characteristics of the cell wall, reflecting the close relationship that exists between cell structure and function. The cell wall is predominantly composed of cellulose, but also contains hemicellulose, pectin, proteins, water and other organic and inorganic substances. The cellulose component of the primary cell wall is relatively stable and does not undergo significant postharvest alteration. The secondary cell wall, formed on the interior of the primary cell wall, also contains lignin and provides the cell with greater rigidity. The cell wall was once thought to be an inactive part of the cell, but is now recognised as having several functions such as the absorption, transport and secretion of molecules (Fahn, 1990; Kays, 1991; Raven et al., 2005).

The area between the walls of neighbouring cells is known as the middle lamella. The middle lamella contains a high concentration of pectins that act like cement, holding adjacent cells together. During the postharvest period and especially during fruit ripening and senescence, pectins can be solubilised eroding the cement that holds neighbouring cells together. Separation of the middle lamella is a common feature of senescence in apples and is the process that is primarily responsible for the change in texture of apple fruit during ripening resulting in the 'mealy' texture of the fruit. (Fahn, 1990; Jackson, 2003; Kays, 1991; Raven et al., 2005).

3.2.1.2 Cellular membranes

Within the cell wall, the contents of the cell are contained within the plasma membrane. Similarly to other membranes found within the cell, the plasma membrane is composed of a viscous lipid bi-layer. Within the membrane and on the membrane surface are a number of other molecules (such as proteins and sterols) that have a range of specific functions. Some mediate the transport of molecules across the membrane, while others catalyse reactions. This indicates that the membrane is not simply a barrier, but a dynamic and functional system. The composition of the plasma membrane provides a flexible and fluid-like structure under normal conditions. At low temperatures however, the fluidity of the plasma membrane can be greatly reduced. (Fahn, 1990; Jackson, 2003; Kays, 1991; Raven et al., 2005).

Changes to the fluidity of the plasma membrane are the primary causes of cell degradation associated with senescence and CI. During senescence, there is a

progressive loss of membrane integrity, a reduction of membrane fluidity and an increase in membrane rigidity. The change in membrane fluidity during senescence is associated with leakiness and the loss of compartmentalisation resulting in the death of the cell. Phase changes in the plasma membrane are also thought to be the primary event resulting in the development of symptoms of CI. When the temperature is reduced below the threshold temperature, the plasma membrane changes phase from liquid-crystalline to a gel phase. This phase change is also associated with a reduction in fluidity of the membrane increasing the likelihood of the loss of compartmentalisation and cell death. The secondary event of CI is thought to be the lateral phase separation of the lipid bi-layers that make up the cell membrane. Phase separations are the cause of irreversible symptoms of CI (Fahn, 1990; Kays, 1991; Marangoni et al., 1996; Raven et al., 2005).

Another important aspect of membrane stability is the concentration of calcium. Calcium has a number of functions relating to the strength and stability of cells and these are discussed further in section 3.2.2.1.2, however it is important to note that the concentration of calcium has a direct relationship to the stability of membranes. A high concentration of calcium in apple fruit has been found to increase the stability of the plasma and vaculoar membranes following storage. Calcium is thought to reduce the influences of senescence and CI on membrane viscosity and fluidity. Fruit with a high concentration of calcium were found to have maintained membrane integrity following 4 months of storage, whereas fruit with a low calcium concentration showed membrane breakdown (Jackson, 2003; Kays, 1991).

As the processes of senescence and CI are both involved with modification of the plasma membrane, it is likely that these two conditions will interact with each other during the postharvest period. Fruit that have begun to senesce will have initiated changes in the fluidity of the membranes that are likely to decrease the tolerance of the membrane to phase changes and separations, increasing the likelihood of the fruit developing CI.

3.2.1.3 Mitochondria

The mitochondria are another key organelle. They are bound by a double membrane and are of particular importance to cell functionality. The mitochondria are the primary site of respiration, involving the release of energy from organic molecules and its conversion to adenosine triphosphate (ATP). The energy conversion

processes of the tricarboxylic acid (TCA) and the electron transport system occur within the mitochondria. Consequently, the mitochondria are of critical importance in the recycling of stored energy following harvest. Located within the mitochondrial matrix and inner membrane are many of the enzymes associated with the TCA cycle, such as succinate dehydrogenase. Due to the requirement of the mitochondria by the cell for energy production, the mitochondria has been found to be one of the last organelles to breakdown during senescence (Fahn, 1990; Kays, 1991; Raven et al., 2005).

During controlled atmosphere (CA) storage, the increased concentration of CO_2 and decreased concentration of O_2 have an effect on the rate of respiration. Respiration may be either stimulated or inhibited, depending on the particular product and the specific tissue. For the long term storage of apples, CA storage is employed to reduce the rate of respiration and extend the storage life of the fruit. One consequence of the reduced rate of respiration is the build up of toxic quantities of succinate dehydrogenase accumulating in the cells (Fahn, 1990; Kays, 1991; Murray, 1997; Raven et al., 2005). Due to the role of the mitochondria in cellular respiration, it is likely that the development of CO_2 and O_2 injury of apples resulting from CA storage are related to the functionality of this organelle.

3.2.1.4 Vacuole

Along with the cell wall, the vacuole is one central feature of most mature plant cells that distinguishes them from animal cells. The vacuole has 3 primary functions within the cell. It is a site for containing the waste materials from the cytoplasm, it maintains the turgor pressure of the cell and it also contains a large number of hydrolytic enzymes for the recycling of cytoplasmic compounds. The vacuole is bound by a membrane, known as the tonoplast which is semi permeable to various molecules. In addition to inorganic ions such as calcium, potassium, sodium and chloride ions, the vacuole also contains sugars, organic acids and amino acids. The movement of these molecules and ions in and out of the vacuole is closely controlled. The hydrolytic compounds in the vacuole include proteases, lipases, nucleases and phosphatases. When the tonoplast is ruptured as a consequence of injury or senescence, these hydrolytic enzymes are released into the cytoplasm. Once in the cytoplasm, these enzymes can then attack a number of the other constituents of the cell and as a result can speed up the process of de-compartmentalisation and cell death (Fahn, 1990; Kays, 1991; Raven et al., 2005). One of the common

consequences resulting from rupturing of the tonoplast is the oxidation of phenolic compounds in the cytoplasm by polyphenol oxidase. This reaction leads to the characteristic browning of the flesh of apples that has been damaged through injury.

While the cells are self contained and partially self sufficient units, they are associated in various ways with each other forming structural and functional tissues. The investigation of physiological disorders of fruits often examines the effects of numerous variables on the plant or whole fruit. However, investigations at the level of tissue and cell can be equally revealing. For example, orchard conditions can alter cell structure and physiology and as a result alter the susceptibility of the tissue to postharvest injury.

3.2.2 Orchard conditions influencing apple structure

The cultivar as well as the conditions under which the fruit are grown can have substantial effects on the development, physiology and stability of the cells in an apple fruit. Orchard conditions that can influence apple fruit physiology include mineral nutrition, tree rootstock, tree crop load, and orchard climatic conditions.

3.2.2.1 Mineral nutrition

There is a wide variation in the mineral nutrition of apple fruit between cultivars, seasons, regions, trees, orchard practices, fruit size and fruit position on the tree (Ferguson and Watkins, 1992; Jackson, 2003; Little and Holmes, 2000; Mengel and Kirkby, 1982; Neilson and Neilson, 2003; Tough et al., 1996; Volz et al., 1993). Some minerals are essential for the development of the fruit as well as for maintaining cell structure and function (Jackson, 2003; Little and Holmes, 2000; Mengel and Kirkby, 1982; Neilson and Neilson, 2003). As a consequence the mineral nutrition of apple fruit can have a large influence on their postharvest behaviour and on the development of disorders. Minerals of specific importance in apple fruit include boron, calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc. Nitrogen is another key element for plant growth and development (Jackson, 2003; Mengel and Kirkby, 1982; Volz et al., 1993), however the focus of this research was on minerals that have previously been reported to be associated with the development of postharvest storage disorders of apples.

3.2.2.1.1 Boron

One of the key elements in cell physiology is boron, which is required in relatively low concentrations in both the apple tree and fruit (Neilson and Neilson, 2003). Boron has been shown to maintain cell wall stability and is also involved in the transport of sucrose within the plant (Neilson and Neilson, 2003). Boron deficiency in apples can result in misshapen fruit and the development of areas of 'corky' tissue (Jackson, 2003). Boron deficiency in 'Cripps Pink' apples has been associated with the development of the bulge browning disorder (Chapter 4) indicating that this element may also be involved in the flesh browning disorder.

3.2.2.1.2 Calcium

Low calcium concentrations in the fruit have also been associated with the development of several storage disorders of apples, including bitter pit, splitting, watercore and breakdown (Ferguson and Watkins, 1989; Ferguson and Watkins, 1992; Jackson, 2003; Neilson and Neilson, 2003). Calcium is not only associated with the development of disorders but can also influence apple quality and senescence (Jackson, 2003). One of the primary roles of calcium in cells relates to cell wall and cell membrane characteristics (Jackson, 2003; JongPil et al., 1999; Kays, 1991; Kovacs et al., 1999; Neilson and Neilson, 2003). Calcium was shown to bind pectin molecules in the middle lamella which increased the cell to cell strength in apple fruit even following extended periods of storage (Jackson, 2003). Fruit with a lower level of calcium showed degradation of the middle lamella to the point of cell separation (Jackson, 2003; Kovacs et al., 1999; Ribeiro et al., 2005). It is thought that the presence of calcium also increases the fluidity of membranes reducing the effects of senescence and CI and a high concentration of calcium can also reduce membrane breakdown during storage (Jackson, 2003; Marangoni et al., 1996).

Importantly, the calcium concentration of the apple flesh has been closely related to the development of some apple disorders (Ferguson and Watkins, 1989; Ferguson and Watkins, 1992; Jackson, 2003; Little and Holmes, 2000; Volz et al., 1993). Apples low in calcium have a higher susceptibility to developing bitter pit than apples with an optimal concentration (Ferguson and Watkins, 1992; Little and Holmes, 2000). One of the early signs of bitter pit in apples is the collapse of cell walls, leading to the development of cavities where several cells have collapsed and

occurring in zones of the fruit that are low in calcium (Jackson, 2003). This indicates the significance of calcium on the strength and stability of cell walls.

3.2.2.1.3 Magnesium

Magnesium's primary role within the cell related to protein synthesis and ATP metabolism (Jackson, 2003; Mengel and Kirkby, 1982; Neilson and Neilson, 2003). The application of magnesium has been found to increase the incidence of bitter pit in apples, however the relationship between magnesium and bitter pit is unclear (Ferguson and Watkins, 1989; Neilson and Neilson, 2003) however, It is likely to be related to interactions between magnesium and calcium. For example, the magnesium uptake in apple trees can be inhibited by calcium, manganese and potassium (Jackson, 2003; Neilson and Neilson, 2003).

3.2.2.1.4 Phosphorus

Another key element is phosphorus. Phosphorus plays a crucial role in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules, in energy transfer involving ATP and in many enzymatic processes in the cell (Jackson, 2003; Neilson and Neilson, 2003; Raven et al., 2005). A high phosphorus content has been found to decrease the incidence of low temperature breakdown, senescent breakdown, superficial scald and core flush (Jackson, 2003; Little and Holmes, 2000; Neilson and Neilson, 2003). High phosphorus concentrations have also been associated with an extended period of cell division in apples and a lower respiration rate of the fruit (Little and Holmes, 2000).

3.2.2.1.5 Potassium

Apples with calcium related disorders, such as bitter pit, often have a high concentration of potassium, however the calcium concentration appears to be the primary factor in their development (Neilson and Neilson, 2003). Potassium interacts with both calcium and magnesium uptake and excess potassium can inhibit the uptake of calcium and magnesium resulting in fruit with a high potassium concentration (Jackson, 2003). Potassium is involved in cell expansion and is found in high concentrations in the cytoplasm where it is involved in enzyme activation, pH stabilisation and protein synthesis (Jackson, 2003; Little and Holmes, 2000). Like many minerals, it is having the correct ratio of potassium with other nutrients that promotes optimal cell health.

3.2.2.1.6 Other minerals and mineral interactions

Several other minerals also have impacts on cell structure and function. An important minor nutrient is copper. Copper is involved in photosynthesis, is a component of several oxidative enzymes and is also essential for the lignification of xylem vessels (Jackson, 2003). In addition, copper is also necessary for chlorophyll synthesis and involved in enzyme reactions relating to oxidation (Neilson and Neilson, 2003).

Iron is another element that has been shown to influence apple fruit yield and quality (Neilson and Neilson, 2003), however few studies have examined any relationships between iron deficiency or toxicity and the development of storage disorders of apples.

Manganese toxicity tends to be more of a problem for apple trees than manganese deficiency (Jackson, 2003). Manganese is required for enzyme reactions involving photosynthesis and carbon assimilation (Neilson and Neilson, 2003).

Zinc is another mineral that is also essential for fruit development. The most important functions of zinc include enzyme activation, carbohydrate metabolism and cell membrane integrity (Jackson, 2003). Zinc functions in several of the enzyme systems and biochemical functions of plants including pH regulation, protection from oxidation and synthesis of RNA (Neilson and Neilson, 2003).

There are also many situations where the concentration of one mineral will affect the absorption, distribution or function of another mineral. One of the key influences in mineral interactions is the soil pH. For example, an increase in soil pH can reduce aluminium and manganese toxicity, but can reduce the availability of iron and zinc to the point of deficiency (Jackson, 2003; Little and Holmes, 2000). Ions can also interact during absorption into the roots of the plant. Calcium can inhibit manganese absorption, and potassium can inhibit the absorption of calcium and magnesium (Jackson, 2003). The influence of low calcium concentration on the development of storage disorders in apples is often increased when accompanied by low boron or high potassium concentrations (Jackson, 2003). To determine the influence of mineral interactions, several ideal ratios of minerals are also recommended to reduce the development of postharvest disorders of apples.

3.2.2.2 Nutritional requirements of apples

Table 3.1 shows the concentrations of key minerals that are required for optimal fruit growth and postharvest quality. Fruit with low concentrations of these minerals have been found to be more susceptible to developing disorders during storage.

Table 3.1. Typical mineral concentrations in apple fruit grown in south eastern Australia at harvest (adapted from Little and Holmes, 2000)

	Mineral level in apple fruit at harvest (mg.100g $^{-1}$ fresh weight)		
Mineral	Low	Adequate	High
Nitrogen	21.75 - 34.8	36.25 - 50.75	52.20 - 65.25
Phosphorus	4.93 - 7.25	7.40 - 10.01	10.15 - 11.75
Potassium	65.25 - 94.25	95.70 - 130.50	131.95 - 159.5
Calcium	2.18 - 3.48	3.63 - 5.08	5.22 - 6.53
Magnesium	1.45 - 2.90	2.90 - 4.35	4.35 - 5.80
Sulphur	1.45	2.9	43.5
Iron	0.5	0.8	1.2
Zinc	0.5	0.8	1.2
Copper	0.1	0.2	0.4
Manganese	0.8	1.2	1.7
Boron	1	1.5	3
Sodium	<0.001	<0.01	>0.01

Ideal mineral ratios: Ca:N (1:10) Ca:P' (1:2.2) Ca:K (1:30) Ca:Mg (1:0.08)

3.2.2.3 Rootstock

Another factor that influences the physiology and morphology of apple fruit is the tree rootstock. The effects of the rootstock on the tree and fruit physiology are discussed in Chapter 2 (Section 2.2.2.3.1) and are interactive and multifaceted (Al-Hinai and Roper, 2004; Barden and Marini, 1992; Drake et al., 1993; Webster and Wertheim, 2003).

One import affect of the rootstock on the fruit physiology is the influence on calcium uptake. A wide variation between calcium uptake and rootstocks has been found, which is thought to be related to variation in the volume, density and distribution of the root system (Little and Holmes, 2000; Webster and Wertheim, 2003). There is a tendency for fruit grown on medium to low vigour rootstocks to have a higher concentration of calcium than those grown on vigorous rootstocks (Little and Holmes, 2000). This relationship is likely to be due to the lower degree of vegetative competition in low vigour rootstocks (Little and Holmes, 2000).

The rootstock can also influence the timing of fruit maturity, for example it is generally agreed that the M.9 rootstock (the M.9 rootstock is widely planted commercially and dwarfs the tree to around 30% of the seedling height) ripen up to a week earlier than more vigorous rootstocks, however such findings are difficult to establish due to the seasonal influences on ripening and maturity (Dennis, 2003; Webster and Wertheim, 2003).

The mechanisms by which the rootstock influences the scion are not well understood. It is thought that the mechanism is related to the control of plant growth hormones such as auxins, gibberellins and cytokinins (Webster and Wertheim, 2003). The rootstock can also influence the calcium uptake of the tree and consequently the concentration of calcium in the fruit (Little and Holmes, 2000; Webster and Wertheim, 2003). The rootstock can also influence the size of the fruit (Al-Hinai and Roper, 2004; Webster and Wertheim, 2003), however the tree crop load has been reported to have a greater impact on fruit size.

3.2.2.4 Crop load

The crop load is a measure of the number of fruit per tree and this measure can be used to indicate fruit size at harvest. As crop load decreases, fruit weight and size tend to increase along with cell number and size, however this relationship is cultivar dependent (Goffinet et al., 1995). Several studies have linked crop load to storage potential and the incidence of disorders in several apple cultivars (Elgar et al., 1999; Ferguson and Watkins, 1989; Ferguson and Watkins, 1992; Little and Holmes, 2000; Volz et al., 1993; Wunsche et al., 2000). For example, a low crop load in Jonathan apples was found to increase the susceptibility to breakdown during storage (Little and Holmes, 2000). One of the primary influences of crop load is fruit size and mineral nutrition (Ferguson and Watkins, 1992; Little and Holmes, 2000; Volz et al., 1993), which in turn affects the fruits susceptibility to storage disorders.

3.2.2.5 Climate

As discussed in Chapter 5, climatic conditions during fruit growth and development can have a substantial affect on the physiology of the fruit. Climatic conditions during fruit growth can also alter the uptake and distribution of minerals (Jackson, 2003). The interaction between climate, mineral nutrition and rootstock is complex as either

of these factors or the interaction between them can affect the susceptibility of the fruit to developing storage disorders.

3.2.3 Impact of cell structure on storage disorders

Storage disorders of apples are often the result of many interacting external and internal factors that impact on the stability of the cell. As discussed in Chapter 4, the susceptibility to senescent disorders, CI and high CO_2 or low O_2 injury are dependent on the structure and stability of cell walls, cell membranes, and cell components. As minerals can directly influence cell structure, function and stability, the susceptibilities to these disorders can be moderated through the concentrations of minerals of the fruit, resulting in increased or decreased susceptibility to injury.

3.2.3.1 Senescence

The mechanism of senescence is discussed in Chapter 4; the process primarily involves the progressive breakdown of the tonoplast, the loss of fluidity of the plasma membrane and the breaking down of the middle lamella. An example of the impact of senescence on apple cell structure and stability has been reported for 'Braeburn' apples. During senescence, the cell shape in 'Braeburn' and 'Jonica' apples was seen to change from an even and round shape to an irregular shape (Schotsmans et al., 2004). The change in shape was associated with an increase in intercellular spaces, however did not relate to an increase in the diffusivity of gases (Schotsmans et al., 2004). During senescence, the intercellular spaces may become compromised due to leakage from damaged cells resulting in slower diffusion of O_2 and CO_2 and an increased susceptibility to O_2 and CO_2 injury developing during storage (Kays, 1991; Lau, 1998; Schotsmans et al., 2004).

3.2.3.2 High CO_2 and low O_2 injury

High CO_2 and low O_2 injury of apples are related to the concentrations of these gases within the fruit. One of the factors influencing the concentrations of CO_2 and O_2 is their rate of diffusion through the flesh of the fruit (Kays, 1991; Lau, 1998; Schotsmans et al., 2004). In apples, gas diffusion primarily occurs in intercellular spaces that result either from the breakdown of entire cells or from the splitting of the middle lamella between neighbouring cells (Goffinet et al., 1995; Schotsmans et al., 2004). The amount of intercellular space is cultivar dependent and also varies in distribution within the fruit (Goffinet et al., 1995; Schotsmans et al., 2004). High CO_2

and low O_2 injury of apples primarily involve metabolic degradation, reduced cytoplasmic pH and the build up of toxic compounds such as succinate dehydrogenase in the case of CO_2 injury and acetyl aldehyde in the case of low O_2 injury (Chapter 4).

3.2.3.3 Chilling injury

The mechanism of CI is discussed in Chapter 4. Chilling injury of apples initially involves the progressive loss of fluidity of the plasma membrane resulting from bulk lipid phase changes and become irreversible following the lateral phase separation of the plasma membrane lipid bi-layer (Aloia and Boggs, 1985; Kays, 1991; Lyons, 1973; Marangoni et al., 1996).

3.3 Aims

- To determine the distribution of tissue affected by RFB and DFB within the fruit.
- To determine the structural characteristics of the RFB and DFB disorders of 'Cripps Pink' apples.
- To establish the relationship between fruit mineral composition and the incidence of RFB and DFB disorders of 'Cripps Pink' apples.
- To establish the relationship between tree crop load, mineral nutrition and the development of RFB of 'Cripps Pink' apples.

3.4 Materials and methods

3.4.1 Fruit sources

In the 2004 season, 'Cripps Pink' apples were harvested from commercial orchards in four different growing regions: Batlow (New South Wales), the Huon Valley (Tasmania), the Goulburn Valley (Victoria) and Manjimup (Western Australia). Details on these orchards are provided in Chapter 4. In the 2005 and 2006 seasons, fruit were sourced from Batlow (New South Wales) and the Huon Valley (Tasmania). All fruit were harvested at the optimal maturity for long term storage.

3.4.2 Crop load

Crop load was measured on ten trees from each of five orchard blocks in Batlow (New South Wales) in the 2005 season. The average number of fruit on 10 branches per tree were counted to give the average number of fruit per branch, and then multiplied to give the number of fruit per tree. The circumference of the trunk of the tree was measured and trunk cross sectional area (TCSA) calculated. The crop load was estimated using the following equation:

$$Cropload = \frac{No.fruit}{TCSA(cm^2)} \quad (1)$$

3.4.3 Distribution of flesh browning

Fruit were assessed for flesh browning at three locations: the stem end, the middle and the calyx end of transverse sections of the fruit. Flesh browning was visually assessed as the area of the cut surface affected. Flesh browning assessment was completed on 'Cripps Pink' apples of optimal harvest maturity (SPI of 3.5) grown in Batlow and the Huon Valley. 'Cripps Pink' apples were stored in air at 0^{o}C for 4 months and were assessed for flesh browning following a further 7 days at 20^{o}C to allow symptoms to develop. From Batlow, 177 fruit were assessed for the area of radial flesh browning at each of the three fruit positions (531 observations). From the Huon Valley, 101 fruit were assessed for the area of diffuse flesh browning at each of the 3 fruit positions (303 observations). A lower number of fruit from the Huon Valley were assessed as fruit from this region had a higher incidence of rots during the 4 months of storage; fruit that had been affected by rots were not assessed for flesh browning.

3.4.4 Scanning electron microscopy

In the 2005 season, 'Cripps Pink' apples were stored for 9 months in air at 0^{o}C then kept at 20^{o}C for 7 days prior to preparation for microscopic examination. Fruit stored at 3^{o}C were not examined due to the low incidence of flesh browning observed at this storage temperature. Sections of vascular and cortex tissue from undamaged fruit and fruit affected by radial and diffuse types of flesh browning and CO_2 injury were analysed.

Sections of tissue were fixed with a 25 $g.l^{-1}$ gluteraldehyde solution (0.1M phosphate buffer, pH 7.2) for 1 hr, postfixed with 10 $g.l^{-1}$ OsO_4 solution (1 hr) and dehydrated using a graded ethanol series (500, 700, 800, 900, 950, 990 and 1000 $g.kg^{-1}$). Samples were subjected to critical point drying (BAL-TEC CPD 030) and then gold coated with an Edwards sputter coating machine (E306A) for 40 seconds. The specimens were examined with a Philips SEM 505 (Philips Eindhoven) at an accelerating voltage of 10 kV.

3.4.5 Mineral analysis

Sections of peeled flesh were used for mineral analysis. Samples consisted of two wedges from each of five fruit per orchard block in each district. In the 2006 season, wedges were sectioned into three sections, the stem, middle and calyx areas. Wedges were freeze dried and sent to an analytical laboratory for Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES) analysis (Advanced Analytical, North Ryde, New South Wales, Australia). As a nitric acid extraction method was used, the concentration of nitrogen in the tissue samples was not determined.

3.4.6 Density

Following the method of Tustin (2004), two sections of the cortex of the fruit midway between the epidermis and the cortex were removed from each fruit, one from each side of the fruit. Each sample was impaled on a fine needle and weighed (W_0). The sample volume was measured by weight of its displacement of water. The weight of water displaced (W_1) was recorded when the sample was weighed fully submerged on the pin with the sample holder mounted on the weighing pan. In the 2006 season, sections of the cortex were sampled from the top third of the fruit (stem end), the middle of the fruit (middle) and the lower third of the fruit (calyx end). Fruit density ($kg.m^{-3}$) was calculated using the following equation:

$$Density = \frac{W_0}{W_1} \quad (2)$$

3.4.7 Statistical analysis

For incidence of flesh browning, fruit density, crop load and mineral concentrations, an analysis of variance was performed and least significant differences (5%) calculated using the general analysis of variance procedure in GenStat statistical software (9th edition, version 9.1.0.147, Lawes Agricultural Trust, supplied by VSN International Ltd). Flesh browning incidence were transformed to angles ($Y = \sin^{-1} \sqrt{\%/100}$) for analysis and back-transformed to % for presentation (Sokal and Rohlf, 1995). Correlations between mineral concentrations and incidence (% of fruit with symptoms, regardless of severity) of flesh browning were calculated in GenStat.

3.5 Results

3.5.1 Distribution of flesh browning

The distribution of affected tissue was found to vary between RFB and DFB (Figure 3.1). Radial flesh browning showed a high area of browning at the stem end of the fruit, decreasing in the middle section of the fruit and decreasing further at the calyx end of the fruit (Figure 3.1). In contrast to this, the area of browning in fruit affected by DFB showed a high area of browning at the stem end of the fruit, a low area of browning in the middle of the fruit and the highest area of browning was observed at the calyx end of the fruit (Figure 3.1). This variation in the distribution of tissue affected by RFB and DFB gave an indication that the physiological causes of these two disorders may be different.

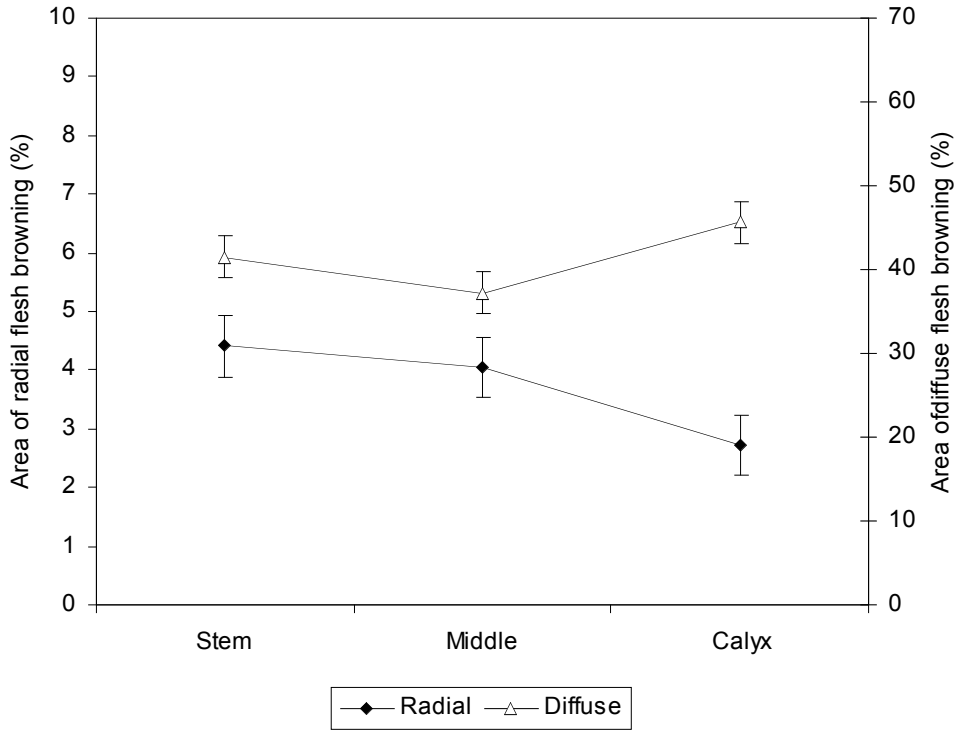

Figure 3.1 Area of tissue affected by radial flesh browning and diffuse flesh browning at the stem, middle and calyx positions of the 'Cripps Pink' apples. Bars represent standard error.

Fruit with radial flesh browning were grown in Batlow (New South Wales) in the 2004 season, 177 fruit were assessed from this region. Fruit with diffuse flesh browning were grown in the Huon Valley (Tasmania) in the 2004 season, 101 fruit were assessed from this region. All fruit were harvested at optimal maturity for long term storage (SPI 3.5) and stored in air for 4 months at 0^{o}C.

3.5.2 Structure of flesh browning

Scanning electron microscopy (SEM) was used to determine the structural characteristics of RFB, DFB and CO_2 injury.

3.5.2.1 Radial flesh browning

Figure 3.2 shows the structure of tissue samples taken from 'Cripps Pink' apples grown in Batlow (New South Wales) in 2005, with no symptoms of RFB following 9 months of air storage at 0^{o}C. In these images, the cells of the vascular (left) and cortex (right) tissue appear undamaged and the structure is well organised. Figure 3.3 shows the structure of tissue samples taken from 'Cripps Pink' apples from the same treatment that showed visual symptoms of RFB. The image on the left shows channels of fractured cell walls in the tissue adjacent to the vascular tissue, in contrast the image on the right of a section of cortex tissue shows no clearly identifiable cell damage.

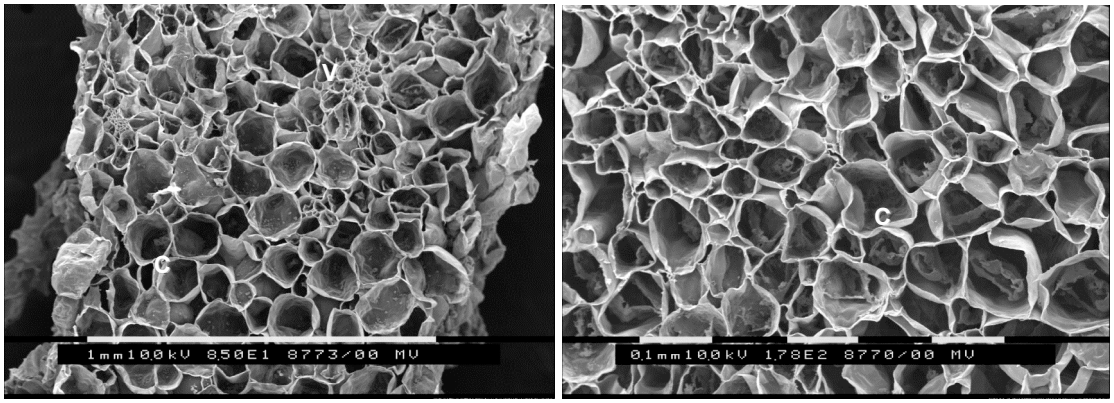

Figure 3.2 Scanning electron microscope (SEM) micrographs showing ultrastructure of apple vascular tissue (left) and cortex tissue (right) of 'Cripps Pink' apple grown in Batlow (New South Wales) following 9 months or air storage at 0^{o}C. Fruit had no visual symptoms of radial flesh browning (RFB). V: vascular cells, C: cortex cells.

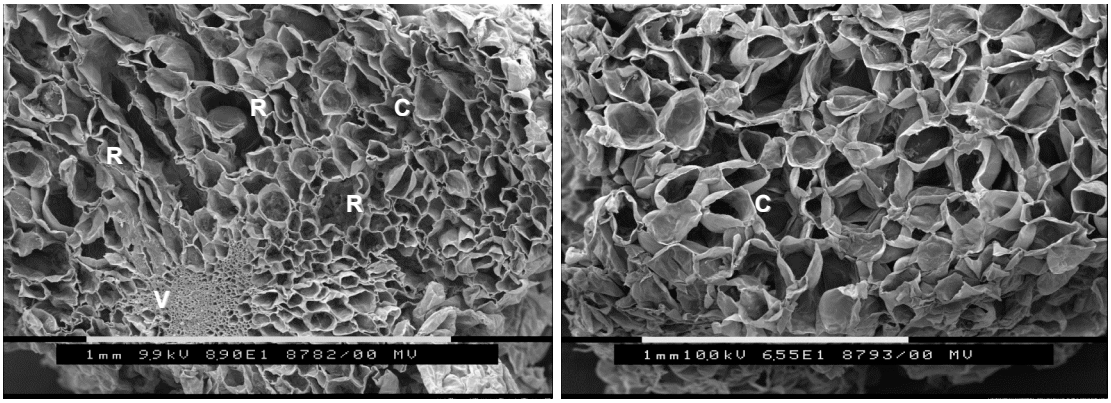

Figure 3.3 Scanning electron microscope (SEM) micrographs showing ultrastructure of apple vascular tissue (left) and cortex tissue (right) of 'Cripps Pink' apple grown in Batlow (New South Wales) following 9 months or air storage at 0^{o}C. Fruit had visual symptoms of radial flesh browning (RFB); sections of tissue were taken from the affected areas. V: vascular cells, C: cortex cells, R: cell wall fracture associated with radial RFB.

3.5.2.2 Diffuse flesh browning

Figure 3.4 shows the structure of tissue samples taken from 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in 2005, with no symptoms of DFB following 9 months of air storage at 0^{o}C. In these images, the cells of the vascular (left) and cortex (right) tissue appear undamaged and the structure has remained well organised. Figure 3.5 shows the structure of tissue samples taken from 'Cripps Pink' apples from the same treatment that showed visual symptoms of DFB. The image on the left shows no clearly identifiable cell damage of the vascular tissue or of cells in the adjacent area. In contrast, the figure on the right, taken from the same fruit, shows extensive cell collapse of the cortex tissue, disorganisation of the cells and the appearance of large intercellular spaces.

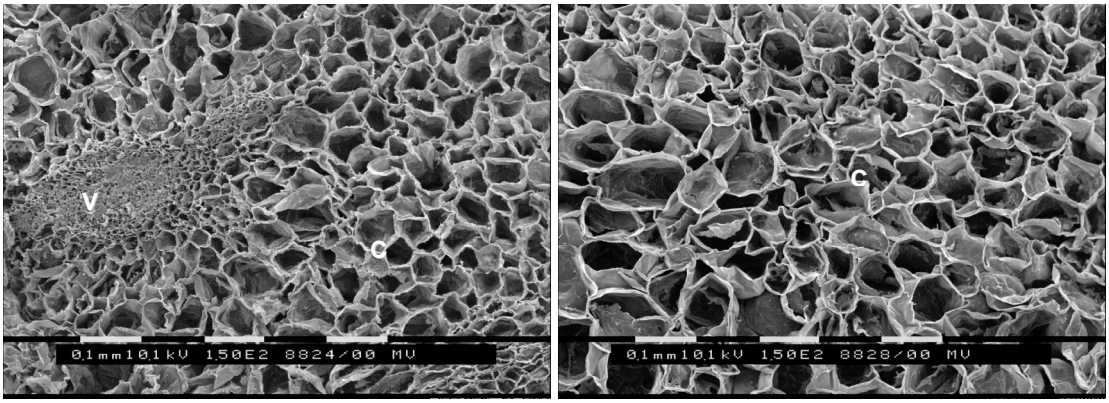

Figure 3.4 Scanning electron microscope (SEM) micrographs showing ultrastructure of apple vascular tissue (left) and cortex tissue (right) of 'Cripps Pink' apple grown in the Huon Valley (Tasmania) following 9 months of air storage at 0°C. Fruit had no visual symptoms of diffuse flesh browning (DFB). V: vascular cells, C: cortex cells.

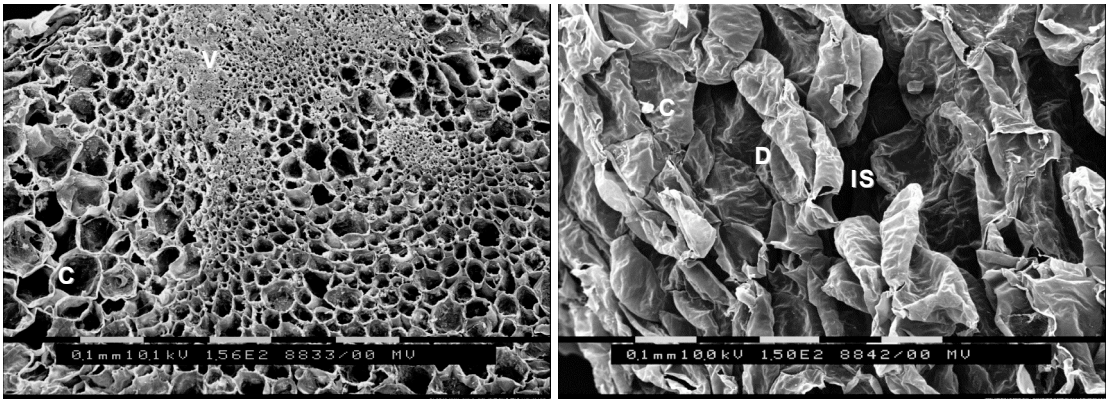

Figure 3.5 Scanning electron microscope (SEM) micrographs showing ultrastructure of apple vascular tissue (left) and cortex tissue (right) of 'Cripps Pink' apple grown in the Huon Valley (Tasmania) following 9 months of air storage at 0°C. Fruit had visual symptoms of diffuse flesh browning (DFB); sections of tissue were taken from the affected areas. V: vascular cells, C: cortex cells, D: cell collapse associated with DFB, IS: intracellular space.

3.5.2.3 CO_2 injury

Figure 3.6 shows the structure of a cavity that developed as a result of CO_2 injury in a 'Cripps Pink' apple grown in Batlow (New South Wales) in 2005 following 9 months of air storage at 0°C. This fruit had symptoms of CO_2 injury only and had no symptoms of DFB or RFB. The formation of the cavity is observed as the separation of the vascular tissue creating a large cavity (Figure 3.6, left). As seen in Figure 3.6 (right), the cavity was observed to originate at a core line vascular bundle and extended into the cortex tissue of the fruit. The cavity is hypothesised to have formed along the vascular tissue that radiates from the core line vascular bundle towards the skin of the apple.

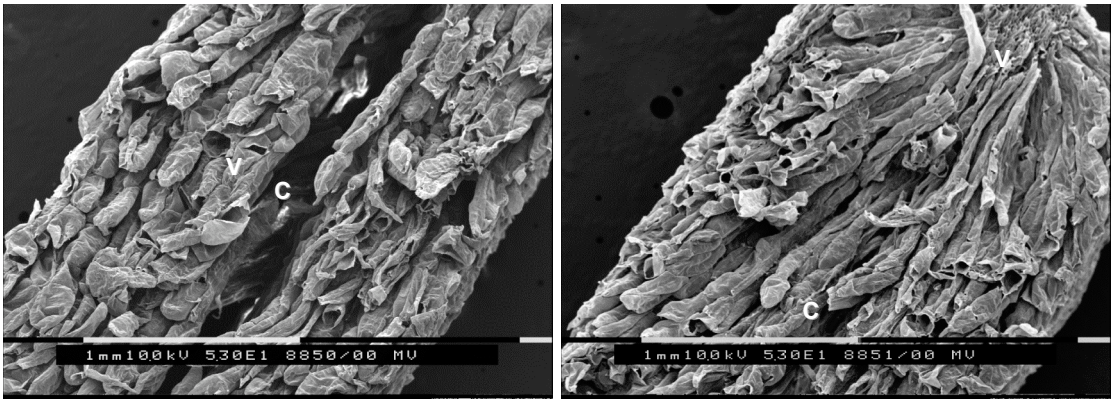

Figure 3.6 Scanning electron microscope (SEM) micrographs showing ultrastructure of CO_2 injury cavity formation of 'Cripps Pink' apple grown in Batlow (New South Wales) following 9 months of air storage at 0^oC. V: vascular tissue, CV: cavity formed from CO_2 injury.

3.5.3 Mineral nutrition and flesh browning

For each mineral analysed, a significant variation was found in the concentration between districts and seasons (Table 3.2). Similarly, a significant variation in mineral ratios was also found between seasons and districts (Table 3.2).

Correlations between the incidence of RFB and the concentrations of minerals in 'Cripps Pink' apples grown in Batlow in the 2004, 2005 and 2006 seasons showed no significant correlation between the incidence of RFB and any mineral or mineral ratio. Similarly, correlations between the incidence of DFB and the concentrations of minerals in 'Cripps Pink' apples grown in the Huon Valley in the same seasons also showed no significant correlation between the incidence of DFB and any mineral. However, a significant negative correlation ($P<0.05$) was found between the incidence of DFB and the ratios of calcium to potassium ($R^2=0.999$) and calcium to magnesium ($R^2=0.998$).

Table 3.2 Mineral composition of 'Cripps Pink' apples at harvest, grown in Batlow (New South Wales), the Huon Valley (Tasmania), the Goulburn Valley (Victoria) and Manjimup (Western Australia)

	Fruit mineral content at harvest (mg.100g^{-1} fresh weight)										Mineral ratios		
District	B	Ca	Cu	Fe	K	Mg	Mn	P'	S	Zn	Ca:P'	Ca:K	Ca:Mg
Batlow													
2004	0.37	8.34	0.07	0.23	152.83	5.97	0.04	9.91	4.08	0.11	1.19	18.33	0.72
2005	0.27	6.19	0.06	0.17	133.00	4.60	0.03	9.81	3.66	0.10	1.58	21.49	0.74
2006	0.20	3.81	0.06	0.43	118.97	3.65	0.02	8.44	2.56	0.03	2.21	31.22	0.96
Huon Valley													
2004	1.33	11.00	0.05	0.34	173.33	7.30	0.06	18.00	4.67	0.18	1.64	15.76	0.66
2005	0.86	6.10	0.05	0.23	120.00	4.50	0.04	12.50	3.70	0.12	2.05	19.67	0.74
2006	0.44	3.00	0.04	0.33	109.37	3.29	0.03	9.04	2.61	0.04	3.02	36.50	1.10
Goulburn Valley													
2004	1.13	6.80	0.06	0.20	196.67	6.80	0.05	18.67	5.30	0.11	2.75	28.92	1.00
Manjimup													
2004	0.92	6.13	0.04	0.20	156.67	5.23	0.05	18.33	4.57	0.11	2.99	25.54	0.85
LSD	0.09	0.44	0.01	0.21	23.18	0.57	0.01	1.96	0.53	0.03	0.97	11.00	0.30
Significance	D*** S*** DxS***	D*** S*** DxS***	D*** S***	S***	D*** S*** DxS**	D*** S*** DxS***	D*** S*** DxS***	D*** S*** DxS***	D*** S***	D** S*** DxS**	D*** S***	D*** S***	D*** S**

,* Significant at P<0.01 or <0.001 respectively where D=district and S=season

Table 3.3 shows the mineral composition and incidence of fruit with RFB for five orchard blocks in Batlow (New South Wales). A significant variation in the incidence of RFB was found between the orchard blocks (P<0.05) and the concentrations of boron (P<0.01), calcium (P<0.01), potassium (P<0.01) and phosphorus (P<0.05) as well as between mineral ratios between calcium and phosphorus (P<0.01) and calcium and potassium (P<0.05). However, no significant correlation was found between the incidence of RFB and the concentration of any mineral or mineral ratio. However a trend of increasing incidence of RFB with decreasing crop load and calcium concentration was observed. Fruit from block 5, the block with the lowest crop load, were found to have the lowest concentrations of calcium and potassium (Table 3.3). Conversely fruit from block 1, the block with the highest crop load had the highest concentration of calcium, block 1 was also found to have the lowest incidence of fruit with RFB (Table 3.3).

Table 3.3 Mineral composition, tree crop load, fruit density of optimally harvested 'Cripps Pink' apples at harvest, from 5 orchard blocks in Batlow (New South Wales) in 2005 and incidence of RFB following 7 months air storage at 0°C

				Fruit mineral content at harvest (mg.100g^{-1} fresh weight)					Mineral ratios		
Block	Crop load	Density (kg.m^{-3})	RFB (%)	B	Ca	K	Mg	P'	Ca:P'	Ca:K	Ca:Mg
1	4.06	0.81	40.00	0.26	8.05	140.00	4.85	9.60	1.19	17.39	0.60
2	3.84	0.82	44.20	0.31	6.15	160.00	4.60	11.00	1.79	26.02	0.75
3	3.92	0.79	55.80	0.26	6.55	130.00	4.70	9.35	1.43	19.85	0.72
4	3.42	0.79	90.00	0.24	5.20	120.00	4.35	9.50	1.83	23.08	0.84
5	2.98	0.80	57.50	0.31	5.00	115.00	4.50	9.60	1.92	23.00	0.90
LSD	1.00		31.15	0.04	1.63	18.18	0.57	1.04	0.03	0.39	0.02
Significance	*	ns	*	**	*	**	ns	*	**	*	ns

Crop load: fruit per square centimetre of trunk cross sectional area
Radial flesh browning (RFB) was determined as the % fruit with symptoms of RFB regardless of severity. Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation. Data is from optimally harvested, air stored at 0°C for 7 months storage treatment.
*,** Significant at P<0.05 or <0.01 respectively

Table 3.4 shows the mineral composition and density of tissue from the stem end, middle and calyx end of 'Cripps Pink' apples grown in Batlow (New South Wales) and the Huon Valley (Tasmania). The density of 'Cripps Pink' apples grown in Batlow was highest at the calyx end and lowest in the middle section of the fruit, in contrast the density of 'Cripps Pink' apples grown in the Huon Valley was highest in the middle section of the fruit and lowest at the stem end (Table 3.4). Calcium and magnesium as well as the ratios of calcium and phosphorus, calcium and potassium and calcium and magnesium were all significantly different between the stem, middle and calyx positions (P<0.001 for all minerals and mineral ratios). In both districts, the concentration of calcium was highest at the stem end of the fruit and lowest at the calyx end of the fruit, in contrast the concentration of magnesium was lowest at the stem end of the fruit and highest at the calyx end (Table 3.4).

Table 3.4 Mineral composition and density of tissue from the stem end, middle and calyx ends of 'Cripps Pink' apples grown in Batlow (New South Wales) and the Huon Valley (Tasmania) in 2006.

	Density	Fruit mineral content at harvest (mg.100g^{-1} fresh weight)					Mineral ratios		
	(kg.m^{-3})	B	Ca	K	Mg	P'	Ca:P'	Ca:K	Ca:Mg
Batlow									
stem	0.804	0.21	4.33	117.20	3.26	7.98	1.84	27.07	0.75
middle	0.784	0.19	3.99	119.80	3.69	8.20	2.06	30.03	0.92
calyx	0.821	0.20	3.11	119.90	4.01	9.13	2.94	38.55	1.29
Huon Valley									
stem	0.810	0.45	3.29	108.20	3.05	8.64	2.63	32.89	0.93
middle	0.875	0.42	3.15	108.90	3.38	8.57	2.72	34.57	1.07
calyx	0.835	0.45	2.55	111.00	3.44	9.92	3.89	43.53	1.35
LSD	0.06	0.06	0.59	16.23	0.34	1.59	0.71	0.01	0.18
Significance	D*	D***	D*** P***	D*	D*** P***	ns	D** P***	D* P***	D*** P***

*,**,*** Significant at P<0.05, <0.01 or <0.001 respectively where D=district and P=position

3.6 Discussion

The visual observation of symptoms of flesh browning of 'Cripps Pink' apples (Chapter 1, Figure 1.1) identified that the RFB and DFB had contrasting areas of affected tissue. In preliminary research it was also observed that the distribution of affected tissue within the fruit varied with the different expressions of flesh browning. However, these relationships had not been quantified and the RFB and DFB disorders could not be definitively classified as separate disorders.

The expression of CO_2 injury in 'Cripps Pink' apples throughout this research was only found to occur during CA storage where the concentration of CO_2 in the storage atmosphere exceeded 1%. The visual expression of CO_2 injury of 'Cripps Pink' apples as the development of pits and cavities was comparable to findings of CO_2 injury in other apple cultivars (Lau, 1998; Meheriuk et al., 1994; Smock, 1977; Watkins, 2003). In California, where 'Cripps Pink' apples were not found to be susceptible to developing either RFB or DFB during storage, CO_2 injury was observed in 'Cripps Pink' apples stored in CA storage (De Castro et al., 2007). This indicated that the 'Cripps Pink' apple had a susceptibility to developing CO_2 injury in CA storage. The recommended control for the prevention of CO_2 injury of 'Cripps Pink' apples was found to be similar as has been found for other apple cultivars, maintaining the level of CO_2 in the storage atmosphere below the level resulting in injury (Burmeister and Dilley, 1995; Clark and Burmeister, 1999; Elgar et al., 1998; Grant et al., 1996; Park and Lee, 1991; Volz et al., 1998). The development of CO_2 injury in 'Cripps Pink' apples lead to the development of the hypothesis that the flesh

browning disorder of ‘Cripps Pink’ apples was similar in physiology to the ‘Braeburn’ browning disorder (BBD). The BBD has been linked to increased fruit density as well as reduced gas diffusivity (Lau, 1998; Wunsche et al., 2000).

The examination of the distribution of affected tissue within the fruit (Figure 3.1) indicated that the distribution of affected tissue was different for the RFB and DFB disorders. The area of tissue affected by RFB was highest at the stem end of the fruit, decreasing toward the calyx end of the fruit. It was hypothesised that this distribution of affected tissue may be related to the density of the fruit and to a reduced gas diffusivity resulting in the build up of CO_2 at the stem end of the fruit causing damage to the cells. However, the measurement of density at the stem, middle and calyx positions of the fruit (Table 3.4) did not find that the stem end had the highest density. In contrast, the calyx end (the section with the lowest mean area of RFB) had the highest fruit density. However, fruit density does not have a direct relationship to gas diffusivity (Kays, 1991; Schotsmans et al., 2004). Rather, it is the connectivity of intercellular spaces that allows for the diffusion and transport of gasses through the apple fruit (Kays, 1991; Schotsmans et al., 2004; Wunsche et al., 2000). It is possible that there is a slower diffusion of gases at the stem end of the fruit, resulting in the accumulation of toxic concentrations of CO_2 leading to the increased are of affected tissue at this location within the fruit.

In contrast to the distribution of affected tissue that was observed with the development of RFB, DFB was shown to have a high area of affected tissue at the stem and calyx sections of the fruit with the lowest area of affected tissue observed at the middle section of the fruit (Figure 3.1), in particular it was observed that it was the core area of the fruit that remained less affected by DFB. This distribution of affected tissue was hypothesised to be the result of a CI, as the core area of the fruit has the benefit of insulation from the cool storage temperatures by the surrounding dense flesh. The core area of the fruit will take the longest amount of time to remove the field heat from as a result of the internal transfer of energy (Kays, 1991). It is possible that the delayed establishment of cool storage conditions in the core area of the fruit may lessen the symptoms of CI at this location within the fruit. The contrasting distribution of affected tissue was the first indication that the RFB and DFB disorders had contrasting physiological mechanisms.

The examination of sections of tissue affected by RFB and DFB by SEM was found to confirm the initial visual description of symptoms of RFB affecting the vascular tissue of the fruit and DFB affecting the cortex tissue of the fruit.

The damage observed by SEM in association with the development of RFB of 'Cripps Pink' apples has not been clearly established and no previous studies using SEM reported symptoms similar to those have observed in this research. Microscopic analysis revealed that RFB was related to the fracturing of the cell walls of those cells that were adjacent to the vascular tissue of the fruit with no damage observed in the cortex tissue (Figure 3.3). The fracturing of cell walls as opposed to the separation of neighbouring cells indicates that the middle lamella region has remained in tact (Kays, 1991; Kovacs et al., 1999; Ribeiro et al., 2005; Tu et al., 1997). As a result, the cells maintained an organised structure. It is possible that the fracturing of cell walls, hypothesised to be a result of senescence, resulted in the leaking of the cellular contents into the intracellular spaces, reducing the diffusion of gases through the fruit (Schotsmans et al., 2004) and leading to the build up of toxic quantities of CO_2.

The tissue affected by DFB was observed by SEM as the extensive collapse of cortex cells and the development of large intracellular spaces (Figure 3.5). As had been observed visually, the damage was not associated with the vascular tissue or with the cortex cells adjacent to the vascular tissue (Figure 3.5). It is possible that the vascular cells, having thickened cell walls (Fahn, 1990; Raven et al., 2005), are more structurally stable and less likely to collapse as a result of CI. Cell collapse has been observed in relation to the development of CI in other horticultural crops suggesting that DFB is the result of CI (Luza et al., 1992; Marangoni et al., 1996). In contrast to the development of RFB, DFB was shown to result in the separation of neighbouring cells resulting in a lack of cellular organisation and the appearance of large intracellular spaces. The separation between cells that was observed with DFB also indicated that the middle lamella region had been broken down (Kays, 1991; Tu et al., 1997).

The concentration of calcium in apple fruit has been closely linked to degradation of the middle lamella during long term storage (Jackson, 2003; JongPil et al., 1999; Kays, 1991; Kovacs et al., 1999). Apple fruit with a high calcium concentration were found to have a lower level of cell separation than fruit with a low calcium concentration, indicating the importance of this mineral in cell wall stability (Jackson,

2003). A low calcium concentration in apple fruit has been linked to a high susceptibility of the fruit to develop disorders during storage (Ferguson and Watkins, 1989; Ferguson and Watkins, 1992; Tough et al., 1996; Volz et al., 1993). Mineral analysis indicated that ratios of calcium to potassium and calcium to magnesium varied significantly ($P<0.001$) between growing seasons in the Huon Valley (Table 3.2). The season with the lowest ratios of calcium to potassium and calcium to magnesium (2004) was also the season with the highest incidence of DFB (Chapter 5, Table 5.3). As a high concentration of potassium or magnesium can inhibit the uptake of calcium in apple fruit (Neilson and Neilson, 2003), the ratios of calcium to potassium or magnesium are often found in association with disorders that are directly related to the concentration of calcium (Jackson, 2003). This suggests that a low concentration of calcium could be associated with an increased susceptibility of 'Cripps Pink' apples developing DFB.

The relationship between calcium and the development of RFB in 'Cripps Pink' apples is not clearly understood. No significant correlation between mineral concentrations or mineral ratios and the incidence of RFB was found. However, when analysing the incidence of RFB and the concentrations of minerals from 5 orchard blocks with varying crop load, a general trend of increasing incidence of RFB with decreasing crop load was found (Table 3.3). Fruit from trees with a low crop load were also found to have a lower concentration of calcium than those from trees with a high crop load (Table 3.3). This relationship between crop load and fruit calcium concentration has been reported for other apple cultivars and has been associated with the development of disorders of apples during storage (Ferguson and Watkins, 1992; Jackson, 2003; Volz et al., 1993). It is possible that the concentration of calcium in the fruit is related to the development of RFB, with fruit with a low concentration of calcium having an increased susceptibility to developing RFB during storage. However, more data is required to confirm this relationship.

The development of CO_2 injury of 'Cripps Pink' apples was found to be similar to the symptoms and development of CO_2 injury reported for other apple cultivars. However, despite the fact that CO_2 injury of 'Cripps Pink' apples was relatively straightforward to identify, the RFB and DFB disorders were less clear-cut. This research has indicated that the RFB and DFB disorders of 'Cripps Pink' apples have contrasting physiological and structural characteristics, a clear indication that these disorders are unique despite having similar risk factors. In Chapter 4, the impacts of

storage conditions on the development of the RFB and DFB disorders of ‘Cripps Pink’ apples are examined.

3.7 Summary

- Radial flesh browning
  - ⇒ Area of tissue affected by RFB was found to be highest at the stem end of the fruit, decreasing towards the lowest area of affected area at the calyx end of the fruit.
  - ⇒ Cell damage was associated with cells adjacent to the vascular tissue and was characterised as cell wall fractures.
  - ⇒ The cellular structure of damaged tissue remained well organised indicating a strong middle lamella between neighbouring cells.
  - ⇒ No consistent relationship between fruit mineral concentrations and the incidence of RFB was found.

- Diffuse flesh browning
  - ⇒ Area of tissue affected by DFB was highest at the stem and calyx ends of the fruit with the lowest area affected in the middle section of the fruit.
  - ⇒ Cell damage was associated with the cortex tissue and was characterised as cell collapse.
  - ⇒ The structure of damaged disuse showed cellular disorganisation indicating a weak middle lamella between neighbouring cells.
  - ⇒ The incidence of DFB was found to increase with decreasing ratios of calcium to potassium and calcium to magnesium.

3.8 References

Al-Hinai, Y.K.and T.R. Roper. 2004. Rootstock effects on growth, cell number, and cell size of 'Gala' apples. Journal of the American Society of Horticultural Science. 129: 37-41.

Aloia, R.C.and J.M. Boggs. 1985. Membrane fluidity in biology, volume 4. Cellular aspects, Academic Press, Orlando.

Barden, J.A.and M.E. Marini. 1992. Maturity and quality of 'Delicious' apples as influenced by rootstock. Journal of the American Society of Horticultural Science. 117: 574-550.

Burmeister, D.M.and D.R. Dilley. 1995. A 'scald-like' controlled atmosphere storage disorder of Empire apples - a chilling injury induced by CO_2. Postharvest Biology and Technology. 6: 1-7.

Clark, C.J.and D.M. Burmeister. 1999. Magnetic resonance imaging of browning development in 'Braeburn' apple during Controlled-Atmosphere storage under high CO_2. HortScience. 34: 915-919.

De Castro, E., B. Biasi, E. Mitcham, S. Tustin, D. Tanner, and J. Jobling. 2007. Carbon dioxide-induced flesh browning in Pink Lady apples. Journal of the American Society for Horticultural Science. 132: 713-719.

Dennis, F. 2003. Flowering, pollination and fruit set and development, p. 153-166. In: D. Ferree and I. Warrington (eds.). Apples: Botany, Production and Uses. CABI Publishing, UK, USA.

Drake, S.R., F.E. Larsen, J.K. Fellman, and S.S. Higgins. 1993. Rootstocks affect Goldspur maturity, quality, storage. Good Fruit Grower. 44: 53-55.

Elgar, H.J., D.M. Burmeister, and C.B. Watkins. 1998. Storage and handling effects on a CO_2-related internal browning disorder of 'Braeburn' apples. HortScience. 33: 719-722.

Elgar, H.J., C.B. Watkins, and N. Lallu. 1999. Harvest data and crop load effects on carbon dioxide-related storage injury of 'Braeburn' apple. HortScience. 34: 305-309.

Fahn, A. 1990. Plant Anatomy, 4th Edition. Pergamon Press, Sydney.

Ferguson, I.B.and C.B. Watkins. 1989. Bitter pit in apple fruit. Horticultural Reviews. 11: 289-355.

Ferguson, I.B.and C.B. Watkins. 1992. Crop load affects mineral concentrations and incidence of bitter pit in 'Cox's Orange Pippin' apple fruit. Journal of the American Society of Horticultural Science. 117: 373-376.

Goffinet, M.C., T.L. Robinson, and A.N. Lakso. 1995. A comparison of 'Empire' apple fruit size and anatomy in unthinned and hand-thinned trees. Journal of Horticultural Science. 70: 373-387.

Grant, J., B. Mitcham, B. Biasi, and S. Chinchiolo. 1996. Late harvest, high CO_2 storage increase internal browning of Fuji apples. California Agriculture. 50: 26-29.

Jackson, J.E. 2003. Biology of apples and pears. Cambridge University Press, Melbourne.

JongPil, C., H. YongSoo, L. JaeChang, and D.J. Huber. 1999. Cell wall component changes during maturation and storage in 'Tsugaru' and 'Fuji' apple fruits. Journal of the Korean Society for Horticultural Science. 40: 705-710.

Kays, S. 1991. Postharvest physiology of perishable plant products. Thomas Nelson Australia, Melbourne.

Kovacs, E., P. Sass, and K. Al-Ariki. 1999. Cell wall analysis of different apple cultivars. Acta Horticulturae. 485: 219-224.

Lau, O.L. 1998. Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. Postharvest Biology and Technology. 14: 131-141.

Little, C.R.and R. Holmes. 2000. Storage technology for apples and pears. Department of Natural Resources and Economics, Victoria.

Luza, J.G., R. van Gorsel, V.S. Polito, and A.A. Kader. 1992. Chilling injury in peaches: a cytochemical and ultrastructural cell wall study. Journal of the American Society for Horticultural Science. 117: 114-118.

Lyons, J.M. 1973. Chilling injury in plants. Annu. Rev. Plant Physiol. 24: 455-466.

Macheix, J.J., A. Fleuriet, and J. Billot. 1990. Fruit Phenolics. CRC Press.

Marangoni, A.G., T. Palma, and D.W. Stanley. 1996. Membrane effects in postharvest physiology. Postharvest Biology and Technology. 7: 193-217.

Meheriuk, M., R.K. Prange, P.D. Lidster, and S.W. Porritt. 1994. Agriculture Canada Publication 1737/E: Postharvest disorders of apples and pears. Minister of Supply and Services Canada.

Mengel, K.and E.A. Kirkby. 1982. Principles of plant nutrition, 3rd edition. International Potash Institute, Bern.

Murray, D.R. 1997. Carbon dioxide and plant responses. Research Studies Press Ltd, Somerset.

Neilson, G.H.and D. Neilson. 2003. Nutritional Requirements of Apple, p. 585-615. In: D. Ferree and I. Warrington (eds.). Apples. Botany, Production and Uses. CABI Publishing.

Park, D.G.and S.K. Lee. 1991. Susceptibility of 'Fuji' apples to low-oxygen injury and high-carbon dioxide injury during CA storage. Journal of the Korean Society for Horticultural Science. 33: 38-43.

Raven, P.H., R.F. Evert, and S.E. Eichhorn. 2005. Biology of plants, 7th Edition. W. H. Freeman and Company, New York.

Ribeiro, C.J.O., E.A.S. Rosa, P. Bandeira-Tavares, O. Meireles, and A. Silva. 2005. Using scanning electron microscopy for quality evaluation of apples after cold storage. Acta Horticulturae. 682: 715-721.

Schotsmans, W., B. Verlinden, J. Lammertyn, and B.M. Nicolai. 2004. The relationship between gas transport properties and the histology of apple. Journal of the Science of Food and Agriculture. 84: 1131-1140.

Smock, R.M. 1977. Nomenclature for internal storage disorders of apples. HortScience. 12: 306-308.

Sokal, R.R.and F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. W. H. Freeman, New York.

Tough, H.J., D.G. Park, K.J. Crutchley, F.B. Bartholomew, and G. Craig. 1996. Effect of crop loan on mineral status, maturity and quality of 'Braeburn' (*Malus domestica* Borkh.) apple fruit. Postharvest 1996. p. 53-57.

Tu, K., K. Waldron, L. Ingham, T. De Barsy, and J. De Baerdenmaeker. 1997. Effect of picking time and storage conditions on 'Cox's Orange Pippin' apple texture in relation to cell wall changes. Journal of Horticultural Science. 72: 971-980.

Tustin, D.S. 2004. The seasonal development and biophysical structure of Pink Lady™ apple fruit grown in Australia, New Zealand, USA and Italy, and the association to susceptibility to flesh browning, p. 36-40. In: J. Jobling and H. James (eds.). Final report HAL AP02009: Understanding the flesh browning disorder of Pink Lady™ apples.

Volz, R.K., W.V. Biasi, J.A. Grant, and E.J. Mitcham. 1998. Prediction of controlled atmosphere-induced flesh browning in 'Fuji' apple. Postharvest Biology and Technology. 15: 97-107.

Volz, R.K., I.B. Ferguson, J.H. Bowen, and C.B. Watkins. 1993. Crop load effects on fruit mineral nutrition, maturity, fruiting and tree growth of 'Cox's Orange Pippin' apple. Journal of Horticultural Science. 68: 127-137.

Watkins, C.B. 2003. Principles and practices of postharvest handling and stress, p. 585-615. In: D. Ferree and I. Warrington (eds.). Apples. Botany, production and uses. CABI Publishing.

Webster, A.D.and S.J. Wertheim. 2003. Apple Rootstocks, p. 91-124. In: D. Ferree and I. Warrington (eds.). Apples. Botany, Production and Uses. CABI Publishing.

Wunsche, J.N., J.W. Palmer, and D.H. Greer. 2000. Effects of crop load on fruiting and gas-exchange characteristics of 'Braeburn'/M.26 apple trees at full canopy. Journal of the American Society of Horticultural Science. 125: 93-99.

4

The influence of storage conditions on the development of radial and diffuse types of flesh browning of 'Cripps Pink' apples

4.1 Abstract

For optimal long term storage, most apple cultivars should be harvested prior to the initiation of the ethylene climacteric and stored at 0^{o}C in controlled atmosphere (CA) storage. In comparison, late harvest, low temperature storage and modification of the storage atmosphere can disrupt cell function and lead to the development of disorders in susceptible apple cultivars. Late harvested fruit have a higher susceptibility to senescent related loss of membrane integrity. Low temperature storage can result in permanent membrane damage expressed as chilling injury (CI) in susceptible apple cultivars. High CO_2 and low O_2 in CA storage can also disrupt cell metabolism and lead to the development of disorders in susceptible apple cultivars. Harvest maturity, storage atmosphere composition and storage temperature were examined for their influence on the development of radial flesh browning (RFB) and diffuse flesh browning (DFB) disorders of 'Cripps Pink' apples grown in four regions in Australia over three consecutive seasons. A wide variation in the incidence of RFB and DFB was found between districts and seasons implicating seasonal climate in the development of both disorders. Increasing the concentration of CO_2 in the storage atmosphere to 1% had an inconsistent effect on the incidence of RFB however previous work found that increasing the concentration of CO_2 resulted in an increased incidence of RFB. The increase in atmospheric CO_2 reduced the incidence of DFB indicating that it is not related to high CO_2 injury. Late harvested fruit tended to have a greater susceptibility to both RFB and DFB suggesting that senescence increases the sensitivity to injury. Increasing the storage temperature was found to be successful for reducing the incidence of RFB and DFB; however the effectiveness depended on the seasonal incidence. Increasing the storage temperature from 0^{o}C to 1^{o}C was effective for preventing the development of RFB in a low risk season. Increasing the storage temperature from 0^{o}C to 3^{o}C was required for the prevention of DFB in a low risk season indicating that fruit with DFB are less tolerant of low temperature storage.

4.2 Introduction

Since the beginning of the 19th century, methods for the storage of apples have been developed to maintain quality, reduce postharvest losses and to satisfy the market demand for a year round supply of apples. Early research identified that the storage temperature and the storage atmosphere were factors that could be controlled in order to increase the storage life of apples. More recent research has focused on the postharvest application of ripening inhibitors such as 1-methylcyclopropene (1-MCP) for the extension of storage life and the prevention of postharvest losses.

Losses associated with storage can be the result of a range of factors. As a living product, the processes of ripening and senescence continue to progress following harvest and eventually result in degradative processes ultimately leading to cell death. The decline in quality during storage, such as changes in appearance, texture, firmness, flavour and aroma that occur as a result of ripening and senescence reduce the overall acceptability of the fruit by the consumer. Maintaining acceptable quality during long term storage is of utmost importance for the economic viability of apple storage.

Postharvest losses can cover a broad spectrum of problems including decay, quality loss and the development of physiological disorders. Physiological storage disorders are different from those that are the result of pathogens that result in decay. Physiological disorders are often the result of changes to the metabolic processes of the fruit. Modification and malfunction of metabolic processes can result in a wide range of physical symptoms in apple fruit, often resulting in cell degradation or death.

Storage disorders can be influenced by both pre and postharvest conditions. Orchard conditions such as crop load and nutrition can also influence fruit susceptibility to storage disorders, these factors are discussed in Chapter 3. Orchard climatic conditions have also been found to influence the development of storage disorders in some apple cultivars. The influence of climate on the development of storage disorders of apples is discussed in Chapter 5. This chapter will address the influence of storage conditions and fruit maturity on the development of physiological storage disorders of ‘Cripps Pink’ apples.

4.2.1 Storage conditions

Maintaining quality without inducing the development of physiological disorders requires the delicate balancing of storage conditions. The balance of storage conditions and quality is cultivar specific with some cultivars having sensitivities to specific storage conditions. Some cultivars have a relatively limited storage potential and can only be stored for 2 or 3 months in air storage before falling below acceptable quality limits. For example, 'Gala' and 'Braeburn' apples can generally only be stored for up to 3 months before the development of disorders or the loss of quality occurs (Little and Holmes, 2000). Other cultivars, such as 'Democrat' and 'Lady Williams' apples are more tolerant of long term storage and can be successfully stored for up to 9 months (Little and Holmes, 2000).

A number of techniques have been developed for extending the storage life of apples; two of the most common methods used commercially include reducing the storage temperature and modifying the storage atmosphere. Both of these methods act in different ways to increase the storage potential of apples. Whilst modification of the storage conditions can increase the length of storage time before quality losses become apparent, such modifications can also detrimentally affect the physiology of the fruit resulting in the development of disorders.

4.2.1.1 Storage temperature

Refrigerated storage at 0^{o}C can extend the storage life of apples by up to 6 months in some apple cultivars (Little and Holmes, 2000; Watkins et al., 2002). One of the effects of reducing the storage temperature is a reduction in the respiration rate of the fruit and suppression of the respiratory climacteric, both of which prolong the life of the fruit (Little and Holmes, 2000; Watkins, 2003). Refrigerated storage can also slow down the onset of ethylene production in apples (Watkins, 2003). By delaying the production of ethylene, the processes of ripening are delayed, extending the time before quality losses occur. The tolerance to storage temperature is cultivar specific with a wide variation in tolerances between different cultivars. Some cultivars, including 'Boskoop', 'Elstar' and 'McIntosh' are highly susceptible to injury at 0^{o}C and require storage at a higher temperature, usually between 2 and 4^{o}C (Little and Holmes, 2000; Watkins, 2003). Other cultivars, such as 'Granny Smith', 'Delicious' and 'Sundowner' are less susceptible to CI and can be successfully stored at 0^{o}C (Little and Holmes, 2000; Watkins et al., 2002). Cripps et al. (1993) recommended that the 'Cripps Pink' cultivar be stored at 0-1^{o}C for 4 months in air. However, Hundall

and Fourie (2003) recommend storage of 'Cripps Pink' apples at -0.5^{o}C. It is likely that cultivar tolerance to CI is determined by cellular physiology. The impact of chilling injury on cell structure and viability is discussed in Chapter 3.

4.2.1.2 Storage atmosphere

The storage potential of apples can be increased up to 12 months through the use of CA storage. Worldwide, the apple is the predominant horticultural commodity stored under CA conditions. The aim of CA storage is to increase the concentration of CO_2 and reduce the concentration of O_2 in the storage atmosphere to levels that will reduce the rate of respiration of the fruit and reduce ethylene production and action. The understanding that modification of the storage atmosphere can extend storage by maintaining apple quality was established in early studies of apple storage in the 1930s (Kidd and West, 1932; Kidd and West, 1936; Magness and Diehl, 1924). More recently, the use of CA storage for the commercial storage of apples has become common practice. Controlled Atmosphere storage generally refers to storing fruit in atmospheres containing low O_2 (1-3%) and high CO_2 (1-3%) levels compared to atmospheric conditions. Tolerance to CA conditions also varies between apple cultivars. Less tolerant cultivars include 'Braeburn' and 'Empire', which have been found to develop disorders when stored in certain CA conditions (Burmeister and Dilley, 1995; DeEll et al., 2005; Elgar et al., 1998; Lau, 1998; Watkins, 2003; Wunsche et al., 2000). For tolerant cultivars, such as 'Gala', 'McIntosh' and 'Golden Delicious', the optimal atmospheric composition is cultivar specific and often also specific to the region where the fruit are grown (Little and Holmes, 2000; Watkins, 2003). The recommended atmosphere for the storage of 'Cripps Pink' apples is a minimum concentration of 1.5% O_2 and a maximum concentration of 1% CO_2 at 0.5^{o}C for 8 months (Hurndall and Fourie, 2003; Little and Holmes, 2000).

The time taken to establish the atmospheric conditions is critical for the success of this method for maintaining apple quality throughout the storage period. Prior to the mid 1970s, 8 to 10 days were often required to load a CA room and a further 15 to 20 days were required to establish the atmosphere (Little and Holmes, 2000; Watkins et al., 2002). This was due to the fact that the establishment of the atmosphere used the fruits own respiration to reduce the concentration of O_2 and increase the concentration of CO_2. This delay before establishment often resulted in poor quality fruit at outturn. However, rapid CA has since been developed and is now standard practice. Rapid CA relies on nitrogen flushing to reduce the level of oxygen to below 5%, which can be achieved in a shorter time frame (Watkins et al., 2002). The delay

in the establishment of CA conditions meant that often fruit would have begun to ripen before the CA conditions were imposed. Once ripening has been initiated, the process cannot be stopped. The rapid establishment of CA conditions ensures that this doesn't happen. However it is also important that the fruit are harvested at the correct maturity stage for optimal long term storage.

4.2.1.3 Fruit maturity

In order to maintain optimal quality throughout the storage period, it is essential that the fruit be harvested at the correct maturity prior to being placed into long term storage. As discussed in Chapter 2, apples are a climacteric fruit and the autocatalytic production of ethylene often occurs around the time of optimal harvest for long term storage. However, the timing of the ethylene climacteric is cultivar specific. Optimal picking date in 'McIntosh' apples is the beginning of the respiratory climacteric (Plotto et al., 1995). In other varieties, such as 'Golden Delicious', the ethylene and respiratory climacterics do not correlate well to optimal harvest maturity as they do not show an increase in respiration or ethylene production during the harvest window (Watkins, 2003). This makes the measurement of ethylene less relevant in such cultivars for determining optimal maturity. The determination of optimal maturity is often based on a number of harvest indices including starch pattern index, skin colour, flesh firmness, internal ethylene concentration, respiration rate, soluble solids and date; however these are specific to cultivar, season and growing region. The determination of optimal harvest maturity is discussed in Chapter 2. Storage at the optimal maturity can also reduce the development of senescent disorders through delay and suppression of the respiration and ethylene climacterics (Elgar et al., 1999; Little and Holmes, 2000; Watkins et al., 2002). By harvesting fruit at the correct maturity for long term storage, the potential for postharvest losses is reduced.

4.2.2 Postharvest losses

4.2.2.1 Injury in cool storage

Temperature plays a key role in many aspects of plant metabolism. The use of refrigeration is vital for the commercial storage of many horticultural products, including apples. In Australia, refrigerated storage has been used commercially for the storage of apples since the 1890s (Little and Holmes, 2000). Decreasing temperature lowers fruit metabolism and consequently can result in the extension of storage life. The reduction of the storage temperature directly reduces the rate of

respiration and suppresses the respiratory climacteric, extending the storage life of the fruit (Watkins, 2003). Most apple cultivars are not sensitive to injury during low temperature storage and should be stored as close to 0^{o}C as possible in order to increase the storage potential of the fruit. However, some cultivars have been found to be sensitive to low temperatures and storage under such conditions can result in the development of disorders. Chilling injury is a physiological disorder that is induced by low temperatures. Chilling injury is different from freezing injury, which results from the formation of ice crystals at temperatures below freezing (Wills et al., 1998). The freezing point in apples is between -1.4 and -2.3^{o}C, depending on the cultivar (Meheriuk et al., 1994), CI occurs at temperatures above the freezing point. Symptoms of CI are varied and can be observed in a number of horticultural commodities including avocado, tomato, pineapple and melon (Wills et al., 1998). Chilling Injury in apples can be seen in several cultivars, including the 'McIntosh' and 'Cortland' cultivars (Alwan and Watkins, 1999; DeEll and Prange, 1998; Watkins et al., 2002).

4.2.2.1.1 Mechanism of chilling injury

The mechanism of CI is thought to be related to alterations to cell membranes as a result of exposure to low temperatures. Plant cell membranes are fluid bi-layers of phospholipids containing embedded proteins and sterols (Fahn, 1990; Kays, 1991; Marangoni et al., 1996; Raven et al., 2005). It has been suggested that the sterols act as stabilisers of membrane fluidity maximising membrane function over a range of temperatures (Leshem, 1992). The presence of both sterols and proteins can affect the fluidity of membranes, which can influence both membrane function and deterioration (Marangoni et al., 1996). The loss of fluidity of cell membranes is thought to be a leading cause of CI. Chilling injury was thought to be the result of bulk lipid-phase membrane transitions occurring at a critical temperature (Lyons, 1973). However, more recent research has shown that such transitions do not take place, rather the membranes undergo liquid-crystalline to gel-phase transitions (Kays, 1991; Marangoni et al., 1996). It appears likely that membranes of chilling sensitive produce undergo alterations in the biophysical properties related to their composition that results in an altered functionality (Marangoni et al., 1996).

Such phase transitions have been observed to occur at higher temperatures in chilling sensitive membranes of tomatoes as compared to their chilling tolerant counterparts (Marangoni and Stanley, 1989). This suggests that the membranes of chilling tolerant fruit maintain the liquid-crystalline state at a lower temperature than

those from chilling sensitive cultivars (Marangoni et al., 1996) This indicates the importance of cultivar specific traits in horticultural produce.

Aside from phase transitions, lateral phase separations are also thought to be the result of CI (Aloia and Boggs, 1985). Phase separations are different from phase transitions and involve separation of the lipid bi-layers that make up the cell membrane (Kays, 1991). Phase separations in avocado fruit subjected to chilling treatments were found to occur however they could be reversed following re-warming (Aloia and Boggs, 1985). Lateral phase separations may be reversible up to a point in time where lipid degradation induces irreversible membrane damage (Marangoni et al., 1996). It is suggested that phase transition changes are the primary event in CI, which would be reversible upon rewarming until secondary events, such as phase separations, had caused modifications to the membrane properties that caused permanent damage (Kays, 1991; Marangoni et al., 1996). Permanent and extensive symptoms of CI may be due to the irreversible phase of the reaction and post-chilling recovery may be possible if the threshold of irreversibility has not been reached (Kays, 1991; Marangoni et al., 1996).

The reversibility of symptoms of CI supports some findings of CI in apples. Intermittent warming has been found to reduce the development of Scald in 'Law Rome' apples and reduced the incidence of breakdown in 'Cortland' apples (Alwan and Watkins, 1999). Both of these disorders have symptoms that are thought to be caused by CI however, such effects have not been found to be consistent across other cultivars (Alwan and Watkins, 1999). Intermittent warming may allow for membrane repair to take place and prevent the lateral phase separations from occurring and irreversible damage taking place. Intermittent warming may also assist in cellular acclimation to chilling temperatures, which lowers the temperature that damage occurs or which protects the cells from damage.

It has been shown that sensitive fruits can be acclimated to chilling temperatures that would otherwise result in membrane damage. When the fruit are exposed to a low (but not CI inducing temperature) prior to refrigerated storage, their tolerance is increased (Marangoni et al., 1996). This supports the practice of stepwise cooling in apples where the temperature is gradually reduced down to the long-term storage temperature over several weeks in order to prevent or minimise the development of CI (Little and Holmes, 2000).

4.2.2.1.2 Chilling injury of apples

Specific storage disorders of apples that are affected by the storage temperature include core browning (CB), low temperature breakdown (LTB) and internal browning (IB). Chilling injury of apples often results in the browning of the flesh of the fruit and can result in severe damage and large losses. The classification of CI in apples can be dependent on the symptoms as well as the cultivar. The identification of CI in apples can be a difficult process as several disorders have similar symptoms and can have different expressions in different apple cultivars. There are also discrepancies in the literature over the description of symptoms as well as the name for each disorder, with some disorders being known by several different names. The common link between CI of apples is that increasing the storage temperature reduces or prevents the development of symptoms.

For example, CB, a type of CI also known as core flush and brown core, affects 'McIntosh' and 'Summerland' cultivars (Smock, 1977) as well as 'Granny Smith', 'Gravenstein', 'Newton' (Meheriuk et al., 1994) and 'Cortland' cultivars (DeEll and Prange, 1998). Symptoms of CB include browning of the core area of the fruit (Little and Holmes, 2000; Watkins et al., 2002) but can extend out beyond the carpels with increasing severity (Smock, 1977; Watkins et al., 2002). The affected tissue is brown, but moist and the symptoms increase in severity with increasing time in storage (Smock, 1977). Core browning usually develops in susceptible fruit stored between -1 and 2^{o}C (Smock, 1977) however symptoms can occur at temperatures as high as 4.5^{o}C (Meheriuk et al., 1994). Storage of 'Cortland' apples at 3^{o}C has been found to reduce the symptoms of CB (DeEll and Prange, 1998). Core browning can be managed in some cultivars with stepwise cooling, intermittent warming or delayed cool storage (Smock, 1977). Core browning can by exacerbated by CA storage in some apple cultivars (Smock, 1977). The incidence of CB in 'Granny Smith' apples has been reduced from as high as 90% to 30% with intermittent warming throughout the storage period (Watkins et al., 2000).

Low temperature breakdown is another form of CI of apples and has been described as a metabolic disorder rather than a senescent disorder due to the influence of low temperature on the metabolic processes of the fruit (Meheriuk et al., 1994). Symptoms of LTB include browning of the vascular bundles and browning of the flesh with a defined area of unaffected tissue underneath the skin of the fruit (Meheriuk et al., 1994; Smock, 1977; Watkins et al., 2002). Low temperature breakdown occurs in

susceptible fruit when stored below 3°C (Smock, 1977). 'Bramley's Seedling' apples have also been found to be susceptible to LTB when stored at 2.8°C with a lower incidence in fruit that were stored at 5°C (Knee and Bubb, 1975). Symptoms of LTB can vary in onset and severity between cultivars. For example 'Jonathan' apples have been found to develop symptoms of LTB after 12 weeks of storage at -1°C while 'Cox's Orange Pippin' apples have developed symptoms in less time at 0°C (Smock, 1977). The 'Cortland' apple cultivar has been found to be susceptible to LTB as well as CB (DeEll and Prange, 1998). The incidence of LTB had reached 31.8% following 8 months of storage at 0°C, however no incidence was observed in 'Cortland' apples stored at 3°C (DeEll and Prange, 1998). Storage atmosphere or CA conditions had no effect on the development of LTB in 'Cortland' apples (DeEll and Prange, 1998).

Another CI of apples is IB. Internal browning of apples involves discolouration of the flesh (Little and Holmes, 2000; Watkins et al., 2002) and often develops first in the vascular regions of the fruit (Smock, 1977) but can affect the cortex flesh of the fruit as well (Watkins et al., 2002). However, Merheriuk et al (1994) describes IB as not affecting the vascular tissue of the fruit, but affecting the cortex and core tissue. Cultivars susceptible to IB include 'Cox's Orange Pippin', 'Delicious', 'Jonagold' and 'Jonathan' (Meheriuk et al., 1994). Internal browning occurs in susceptible fruit when stored below 3°C (Smock, 1977). Later harvested fruit also tend to have a higher susceptibility to developing IB during storage (Meheriuk et al., 1994). 'Sunrise' apples have been found to develop IB when harvested late and stored for longer than 1 month (Lau and Lane, 1998). Similarly to CB, storing susceptible fruit at higher temperatures can reduce the severity and incidence of IB (Smock, 1977).

A common method for controlling the development of CI of apples is to store the fruit at an increased temperature. However, increasing the temperature can reduce the storage potential of the fruit due to the loss of fruit quality. Another method for the commercial storage of apples that increases the storage potential by maintaining fruit quality is CA storage.

4.2.2.2 Injury in controlled atmosphere storage

The goal of CA storage is to reduce the rate of metabolism through modification of the storage atmosphere. The rate of metabolism is a key factor in the storage life of apples. While modification of the storage atmosphere has been shown to be beneficial in the storage of a wide selection of crops including apples, broccoli,

lettuce, cherries and avocado (Kays, 1991), the action of increased CO_2 and reduced O_2 on the extension of storage life is not clearly understood. It is thought that the modification of the atmosphere may extend the storage life of the fruit by reducing the rate of respiration (Kays, 1991; Watkins, 2003) as well as reducing ethylene biosynthesis and action (Beaudry, 1999; Watkins, 2003). The respiration rate of apples varies considerably with cultivar, from 3.8 $ml.kg^{-1}hr^{-1}$ in 'Granny Smith' up to 6.2 $ml.kg^{-1}hr^{-1}$ in 'Cox's Orange Pippin' (Little and Holmes, 2000). The reduction in respiration appears to be primarily through the progressive decline in succinate dehydrogenase with increasing CO_2 (Kays, 1991). However, the reduction in respiration is also achieved through the reduction in oxygen availability in the storage atmosphere (Kays, 1991). As oxygen is required for respiration, limiting its availability limits the rate of the reaction.

As had been found for cool storage, the optimal storage atmosphere varies based on the apple cultivar. Sensitivity can be to either the increased level of CO_2, reduced level of O_2, or to an interaction between both gases (Watkins, 2003). Apples can be classified as being either tolerant or sensitive to modification of the atmosphere and this will determine the optimal application of CA storage. For example, 'Gala' and 'Golden Delicious' cultivars have been shown to be able to tolerate high levels (up to 5%) of CO_2 in the storage atmosphere (Little and Holmes, 2000; Watkins, 2003). In comparison, sensitive cultivars include 'Fuji' and 'Braeburn', these cultivars should be stored below 0.5% CO_2 to avoid injury (Elgar et al., 1998; Grant et al., 1996; Lau, 1998; Little and Holmes, 2000; Park and Lee, 1991; Volz et al., 1998; Watkins, 2003). These cultivars tend to have a high fruit density and as a result have reduced gas diffusivity (Lau, 1998; Watkins, 2003). Such fruit tend to accumulate increased levels of internal CO_2, which will often exceed the tolerance threshold resulting in the development of injury.

4.2.2.2.1 Mechanism of CO_2 injury

Increasing the concentration of CO_2 can have beneficial effects on fruit quality and the extension of storage life. For some cultivars however, modifications of the storage atmosphere can result in the development of disorders. Elevated levels of CO_2 in the storage atmosphere can result in damage in several horticultural commodities. Some examples include surface blemish of tomato, internal browning of capsicum, brown stain of lettuce and discolouration of mushrooms (Kays, 1991).

The mode of action of high CO_2 on the development of injury is not clearly understood. One hypothesis is that high CO_2 inhibits the rate of respiration, resulting in the production of stress metabolites (Kays, 1991). The inhibitory effect is not due to permanent tissue damage however as the respiration rate often returns to normal following the removal of CO_2 (Kays, 1991). High CO_2 can also inhibit the conversion of succinate to malate and malate to pyruvate in apple fruit tissue and as a result disrupts respiration (Kays, 1991). High concentrations of CO_2 (up to 15%) have been found to result in toxic levels of succinate accumulating in apple tissue resulting in cell damage (Kays, 1991; Watkins, 2003). This work indicates that the same mechanism may occur under CA conditions. Carbon dioxide inhibition of the tricarboxylic acid (TCA) cycle may also lead to the accumulation of toxic quantities of acetaldehyde and ethanol resulting in poisoning due to the inhibition of alcohol dehydrogenase (Kays, 1991; Lidster et al., 1985; Park and Lee, 1991; Volz et al., 1998; Watkins, 2003).

Elevated levels of CO_2 have also been shown to induce secondary effects on cell function. One such effect is a reduction in the cytoplasmic pH with increasing levels of CO_2 (Kays, 1991). The effect of CO_2 on cellular pH is complex and the change in pH is often reversible upon return to a normal environment (Kays, 1991).

4.2.2.2.2 CO_2 injury of apples

In carbon dioxide sensitive apple cultivars, CA storage can lead to the development of a range of disorders. For example, brown heart and CO_2 injury are disorders of apples that are induced with CA storage (Meheriuk et al., 1994; Smock, 1977). Controlled atmosphere conditions can also interact with storage temperature conditions and can exacerbate the symptoms of other storage disorders of apples.

Carbon dioxide injury can be expressed as the development of pits and cavities in the flesh of the fruit. The tissue affected by CO_2 tends to dry out and collapse, forming the cavities (Meheriuk et al., 1994; Smock, 1977; Watkins et al., 2002). The cavities are often surrounded by damaged, brown tissue (Smock, 1977). Depending on severity, cavities can be as large as 18 mm in diameter (Smock, 1977). The development of cavities is often more prevalent near the calyx end of the fruit (Smock, 1977) this may be the result of a cell density gradient that exists within the fruit. This type of CO_2 injury can be seen in most apple cultivars when stored in conditions exceeding their tolerance to CO_2. However, cultivars that more commonly

develop cavities include ‘Jonathan’, ‘Golden Delicious’ and ‘Granny Smith’ (Smock, 1977).

A common physiological disorder resulting from CO_2 injury from CA storage is brown heart (BH). Symptoms of this disorder include browning of the vascular tissue extending through the mid-cortex of the fruit (Meheriuk et al., 1994; Smock, 1977). Initially, the injured tissue remains firm but is generally not moist (Smock, 1977). Brown heart is often more severe in fruit stored at 0^{o}C than fruit that were stored at higher temperatures (Smock, 1977). Symptoms of BH can also be present on the skin of the fruit, observed as irregular, sunken, dry patches of skin, usually occurring on the green portion of the skin in bi-coloured apples (Smock, 1977). Cultivars that have shown susceptibility to BH include ‘Jonathan’, ‘McIntosh’, ‘Cortland’, ‘Delicious’ and ‘Granny Smith’ (Smock, 1977). In ‘Elstar’ apples, the highest incidence of BH was observed when stored in 5% CO_2 with 1.2% O_2 (Streif and Saquet, 2003). However, a high variation in the incidence of BH in ‘Elstar’ apples was observed between different orchards (Streif and Saquet, 2003), indicating that pre-harvest conditions play a role in the development of this disorder.

Pre-harvest factors were also found to be involved in the development of flesh browning during CA storage of ‘Fuji’ apples (Volz et al., 1998). Large differences in incidence were found between different orchard locations as well as harvest dates (Volz et al., 1998). Seasonal variation in the incidence of CA disorders has also been shown in ‘Golden Delicious’ apples (Volz et al., 1998).

Another example is the ‘Braeburn’ cultivar, which is particularly susceptible to injury during CA storage. The ‘Braeburn’ browning disorder (BBD) occurs during air and CA storage and is thought to be due to the high density and skin resistance of this cultivar resulting in increased levels of internal CO_2 (Clark and Burmeister, 1999; Lau, 1998). As with IB of ‘Fuji’ and ‘Elstar’ apples, a wide variation in the incidence of BBD is reported between districts and seasons (Clark and Burmeister, 1999; Elgar et al., 1999). Cool districts in New Zealand tend to have a higher incidence of BBD than warmer growing districts (Elgar et al., 1999; Lau, 1998), further implicating climate and regional effects on the development of CA related disorders.

A ‘scald like’ disorder of ‘Empire’ apples has been associated with CA storage and is also affected by the storage temperature (Burmeister and Dilley, 1995). The disorder is characterised by the appearance of brown patches on the skin of the fruit that have

a similar appearance to superficial scald (Burmeister and Dilley, 1995). Unlike superficial scald however, this disorder is not present in air stored fruit or diminished with low O_2 storage (Burmeister and Dilley, 1995). The disorder was more prevalent at 1^oC than at 3^oC and it is hypothesised that the disorder is the result of a complex interaction between the storage atmosphere and temperature (Burmeister and Dilley, 1995).

4.2.2.2.3 Mechanism of O_2 injury

The low level of O_2 that is used in CA storage can also induce physiological disorders. Low O_2 stress (0-0.5% O_2 for 5-10 days) can be beneficial for the control of superficial scald and the maintenance of flesh firmness in 'Granny Smith' apples (Lau et al., 1998; Little and Holmes, 2000). However, the use of this treatment carries a risk for the development of low O_2 injury in some apple cultivars. The extent of injury is closely related to the length of exposure to anaerobic conditions. Generally, the longer the exposure, the greater the accumulation of stress metabolites and the more severe the subsequent injury (Kays, 1991). However, as the reactions for the conversion of pyruvate to ethanol are reversible upon re-establishment of aerobic conditions, most plants are capable of recycling the stress metabolites that were formed and consequently can prevent further injury from occurring (Kays, 1991).

During low O_2 storage, the TCA cycle is inhibited, however the glycolytic pathway is not blocked (Kays, 1991; Raven et al., 2005). This leads to a build up of acetaldehyde and ethanol that are phytotoxic to the cell (Kays, 1991). As these metabolites accumulate, they disrupt cellular organisation and eventually lead to cell death (Kays, 1991). For example, a build up of ethanol may act on the stability and function of cell membranes (Kays, 1991).

Low O_2 stress can be categorised into three general classes. Severe stress occurs when anaerobic conditions are reached within the product and produces a rapid and irreversible decline in product quality (Kays, 1991). Moderate stress occurs at O_2 concentrations above those required for anaerobiosis, however these conditions can considerably impair the quality of the product (Kays, 1991). In particular, moderate O_2 stress can produce undesirable changes in flavour and aroma (Kays, 1991; Little and Holmes, 2000; Watkins, 2003). However, mild O_2 stress does not result in injury and can act to increase longevity and maintain overall fruit quality (Kays, 1991). Mild O_2 stress can be beneficial to maintaining apple quality during storage. Benefits of low O_2 include a decreased rate of softening, weight loss and skin colour change as

well as delayed onset of ethylene production (Kays, 1991; Little and Holmes, 2000; Watkins, 2003).

4.2.2.2.4 O_2 injury of apples

Low O_2 injury of apples is associated with fermentation and the development of alcohol when stored below 1% O_2. Low O_2 injury is initially observed as pinkish brown flesh that remains moist (Smock, 1977; Watkins et al., 2002). In some cases, brown areas may also appear on the skin of the fruit in addition to the internal symptoms (Smock, 1977). Apples affected by low O_2 injury often become soft and the flesh of the fruit can split (Smock, 1977; Watkins et al., 2002). This disorder can also occur in conjunction with CO_2 injury (Smock, 1977). Low O_2 injury can also be associated with a loss of flavour and the development of fermentation odours (Little and Holmes, 2000; Smock, 1977; Watkins et al., 2002).

4.2.2.3 Senescence

Another physiological consideration in the development of storage disorders of apples is the process of senescence. Fruit maturity at harvest is an important consideration in the long term storage of apples for the maintenance of quality and the prevention of senescent disorders. The storage of over mature fruit can result in the development of senescent disorders and can also exacerbate the development of CI and CO_2 injury. Senescent disorders are thought to be influenced by fruit calcium concentration (Jackson, 2003; Watkins, 2003) but are also the result of over storage and can present a wide range of symptoms in different apple cultivars. Senescence can be affected by a number of factors. For example, stress resulting from low temperature storage and CA storage as well as humidity, mechanical damage and mineral imbalances can all accelerate the process of senescence in apples (Kays, 1991).

4.2.2.3.1 Mechanism of senescence

Ripening is considered to be a process of senescence. Consequently, harvesting fruit at the correct stage of maturity is essential for optimal long term storage. Senescence is generally accepted to be a genetically programmed event in the development of plant tissue that results in the breaking down of cellular integrity (Kays, 1991; Wills et al., 1998). At the commencement of the respiratory climacteric changes occur in protein synthesis and are associated with nucleic acid alterations (Kays, 1991). As ethylene has the ability to initiate such biochemical and

physiological changes, it is evident that ethylene action is regulated at the level of gene expression (Alexander and Grierson, 2002; Kays, 1991; Picton et al., 1995; Wills et al., 1998; Xin-Jian and Jiarui, 2000). Once thought to be an organisational collapse of the cell, senescence is now believed to be a tightly controlled developmental sequence of events (Kays, 1991). Senescence is an active process, requiring energy; consequently any process that acts to suppress the respiration rate will consequently reduce the rate of senescence (Kays, 1991).

During senescence, there is a progressive loss of membrane integrity and consequently a loss of regulatory control by the cell (Kays, 1991). The cell membranes change in fluidity, becoming more rigid and result in the loss of compartmentalisation (Kays, 1991; Leshem, 1992; Marangoni et al., 1996). The mitochondrial, plasmalemma, nuclear and vacuolar membranes are retained until the very late stages of senescence due to their essential functionality (Kays, 1991; Raven et al., 2005).

Changes that occur in fruit as a result of senescence often diminish the quality and acceptability of the product. Loss of chlorophyll during senescence results in colour changes in the product (Kays, 1991; Watkins, 2003), in apples this is seen through the yellowing of the skin. During senescence, the concentration of certain enzymes increases, most of these enzymes are hydrolytic in nature and are involved in the dismantling of large molecules and this causes changes in the texture of the fruit (Kays, 1991).

4.2.2.3.2 Senescent disorders of apples

The generic form of senescent breakdown is known as ‘mealy breakdown’ as a result of the textural changes of the fruit flesh (Smock, 1977). As well as the textural changes, browning of the flesh can also be present. The browning usually extends outwards from the vascular bundles of the fruit (Smock, 1977). Some cultivars, including ‘Jonathan’ and ‘McIntosh’ have specific classifications of senescent breakdown as the symptoms have been found to be cultivar specific. Intermittent warming was found to reduce the incidence of senescent breakdown in ‘Cortland’ apples (Alwan and Watkins, 1999). However intermittent warming increased the incidence of senescent breakdown in ‘Delicious’ apples and had an inconsistent effect on senescent breakdown of ‘Law Rome’ apples (Alwan and Watkins, 1999). Such variation in cultivar responses to disorders is not uncommon.

4.2.3 Postharvest treatments for the prevention physiological disorders

As modification of the storage conditions has been found to lead to the development of disorders in several apple cultivars, postharvest treatments have been developed to attempt to reduce or prevent their development. As with the modification of storage conditions, the application of postharvest treatments varies with different apple cultivars.

4.2.3.1 Diphenylamine

Diphenylamine (DPA) is an antioxidant that is used to inhibit the development of superficial scald. Much debate continues as to the physiological causes of superficial scald in apples, although DPA has been found to be successful for inhibiting its development in many situations. The effect of DPA on the development of scald varies between different apple cultivars (Little and Holmes, 2000; Watkins, 2003; Zanella, 2003). For example, scald was successfully controlled in 'Jonathan' apples with less than half the rate of application of DPA that was required for a similar control of scald in 'Granny Smith' apples (Little and Holmes, 2000). Alternative treatments for the prevention of superficial scald in apples are being sought due to increasing concern from growers, environmentalists and consumers about the use of chemicals (Zanella, 2003). One such alternative is 1-Methylcyclopropene (1-MCP).

4.2.3.2 1-Methylcyclopropene

1-MCP (Smartfresh™, manufactured by Agrofresh Inc.) is a relatively new compound that is structurally similar to ethylene (Watkins, 2006). It is thought that 1-MCP interacts with ethylene receptors and consequently prevents ethylene dependent responses, including ripening (Sisler and Serek, 2003; Watkins, 2006). The application of 1-MCP has been found to have several beneficial effects on quality maintenance during apple storage. 1-MCP noticeably inhibits ripening of apples through prevention or delay of the climacteric phase of ripening (Watkins, 2006). Specifically, the application of 1-MCP can maintain fruit firmness and acidity, reduce superficial scald, peel greasiness and can also prevent the development of CI (DeEll et al., 2005; Watkins, 2006). The maintenance of firmness through the use of 1-MCP has made it possible to store apples for extended periods of time without the use of CA storage, however it is most beneficial when used in conjunction with CA storage (Watkins, 2006). The effectiveness of 1-MCP depends on cultivar and subsequent storage conditions (Watkins, 2006). However, 1-MCP has also been found to increase the susceptibility of some apple cultivars to CO_2 injury (DeEll et al., 2005).

4.2.4 The interactions of storage disorders of apples

Storage conditions can interact with one another to increase susceptibility to a disorder. For example, susceptibility to low O_2 injury is increased with low temperature storage and CO_2 injury is more prevalent with reduced O_2 storage (Watkins et al., 2002). While the final cause of cell degradation can be attributed to CI, metabolic degradation or senescence, it is often the case that flesh browning disorders of apples have a range of factors that contribute to their development. Pre harvest factors including maturity, nutrition and climate are involved in the development of disorders during storage and are discussed in chapter 2, 4 and 5 respectively. However, these factors influence the development of storage disorders through their influence on cell structure and stability as well as the process of senescence.

4.2.5 Flesh browning of 'Cripps Pink' apples

Symptoms of flesh browning of 'Cripps Pink' apples share some similarities with symptoms resulting from CI, CO_2 injury and senescent breakdown. One of the aims of this research is to establish the physiological cause of the radial and diffuse types of flesh browning in 'Cripps Pink' apples and to accurately describe each disorder as being a CI, a high CO_2 or low O_2 induced metabolic injury or a senescent disorder. 'Cripps Pink' apples have been found to develop CO_2 related cavities during storage (Chapter 3) indicating that the cultivar was susceptible to CO_2 injury. However, the pattern of flesh browning within the fruit was not consistent between districts or seasons. Preliminary studies completed by Jobling et al. (2004) and Zanella (2004) implicated the storage atmosphere, storage temperature and fruit maturity as being related to the development of the flesh browning disorder of 'Cripps Pink' apples.

4.3 Aims

- To determine the effect of modification of the storage atmosphere on the development of RFB and DFB disorders of 'Cripps Pink' apples.
- To determine the effect of the storage temperature on the development of RFB and DFB disorders of 'Cripps Pink' apples.
- To determine the effect of fruit maturity at harvest on the development of RFB and DFB disorders of 'Cripps Pink' apples.
- To establish the relationship between storage time, storage temperature and quality of 'Cripps Pink' apples.

4.4 Materials and methods

4.4.1 Fruit sources

In 2004, 'Cripps Pink' apples were harvested from four commercial apple growing districts in Australia: Batlow (New South Wales), the Goulburn Valley (Victoria), the Huon Valley (Tasmania) and Manjimup (Western Australia). In 2005 and 2006, fruit were sourced from Batlow and the Huon Valley.

4.4.1.1 Batlow

'Cripps Pink' apples were harvested from 7 year old trees on a commercial orchard (35°31'S 148°09'E) in Batlow. The trees were grown and managed using current commercial practices. The trees were on M9 rootstocks, it is unfortunate that due to commercial restraints the trees in Batlow were grown on different rootstocks to those grown in other regions. Tree rootstocks can influence tree and fruit physiology (Chapter 3) and this was taken into consideration in the results. Fruit were harvested from 5 orchard blocks. Each block consisted of 10 trees.

4.4.1.2 Manjimup

'Cripps Pink' apples were harvested from trees on a commercial orchard (34°15'S 116°10'E) in Manjimup. The trees were grown and managed using current commercial practices. The trees were on MM106 rootstocks.

4.4.1.3 Goulburn Valley

'Cripps Pink' apples were harvested from a commercial orchard (36°30'S 145°20'E) in the Goulburn Valley. The trees were grown and managed using current commercial practices. The trees were on MM106 rootstocks.

4.4.1.4 Huon Valley

'Cripps Pink' apples were harvested from 8 year old trees on a commercial orchard in the Huon Valley (43°16'S 146°92'E). The trees were grown and managed using current commercial practices. The trees were on MM106 rootstocks with M9 interstems for vigour control. In 2006, fruit were harvested from a different orchard in the same region.

4.4.2 Harvest maturity

In each season, 'Cripps Pink' apples were harvested at 2 maturities (SPI 3.5 and 8.5 on CTIFL 10 point SPI scale; except fruit from Manjimup in the 2004 season and fruit from the Huon Valley in the 2006 season, which were harvested at a SPI of 3.5 only). Fruit harvested at an SPI of 3.5 are pre-climacteric and suited to long term storage; fruit harvested at an SPI of 8.5 are post-climacteric and are not suited to long term storage.

4.4.3 Pre-storage wax

Half the fruit from each district were double waxed with a commercial apple wax (APL LUSTR® 331) using a commercial packing line before being placed in storage, except for Cripps Pink apples from Huon Valley in 2006 when no wax treatment was used. The wax treatment is not a commercially recommended treatment and was used to induce symptoms of flesh browning (Lau, 1998).

4.4.4 Storage conditions

4.4.4.1 Controlled atmosphere

In 2004 'Cripps Pink' apples were stored at 0^{o}C in 60l sealed plastic drums in a randomised block design under an atmosphere of regular air or air + 1% CO_2 using a flow through system with a flow rate of 200ml per minute. Four replicates per district, maturity, wax and atmosphere combination with 50 fruit per replicate (10 fruit per orchard block) were assessed for flesh browning. The flesh browning assessment was carried out after 4 and 7 months of storage, following 7 days at 20^{o}C. In 2004 a total of 7,200 fruit were assessed for flesh browning.

4.4.4.2 Storage temperature

In 2005 'Cripps Pink' apples were stored in regular air at 0 or 3^{o}C in a randomised block design at each temperature. Four replicates per district, maturity, wax and temperature combination with 50 fruit per replicate (10 fruit per orchard block in Batlow) were assessed for flesh browning. The flesh browning assessment was carried out after 7 months of storage, following seven days at 20^{o}C. In 2005 a total of 3,200 fruit were assessed for flesh browning.

In 2006 'Cripps Pink' apples were stored in regular air at 0, 1 or 3^{o}C or stepwise cooled (2 weeks at 3^{o}C, 2 weeks at 2^{o}C then stored at 1^{o}C) in a randomised block

design at each temperature. Four replicates per district, maturity, wax and temperature combination with 25 fruit per replicate (5 fruit per orchard block in Batlow) were assessed for flesh browning. The flesh browning assessment was carried out after 1, 3, 5 and 7 months of storage at 0 and 3^{o}C and after 7 months of storage for apples stored at 1^{o}C or stepwise cooled, following 7 days at 20^{o}C. In 2006 a total of 5,000 fruit were assessed for flesh browning.

4.4.5 Maturity and Quality evaluation

In 2006 'Cripps Pink' apples were assessed for quality and maturity attributes including flesh firmness, skin greasiness and internal ethylene concentration. Details of the methods for maturity and quality evaluation are described in Chapter 2. Twenty fruit per district, maturity and storage temperature combination were assessed for maturity and quality characteristics after 1, 3, 5 and 7 months of storage at 0^{o}C and 3^{o}C and after 7 months of storage for apples stored at 1^{o}C or stepwise cooled. Assessments were completed on the day that the fruit were removed from storage (allowing 3 hrs for the fruit to warm to room temperature (20^{o}C)) and following 7 days at 20^{o}C.

4.4.6 Flesh browning determination

Fruit were assessed for flesh browning at three locations: the stem end, the middle and the calyx end of transverse sections of the fruit. Flesh browning was assessed as the percentage of fruit with symptoms, regardless of severity.

4.4.7 Statistical analysis

For internal ethylene concentration, flesh firmness and incidence of flesh browning an analysis of variance was performed and least significant differences (5%) calculated using the general analysis of variance procedure in GenStat statistical software (9th edition, version 9.1.0.147, Lawes Agricultural Trust, supplied by VSN International Ltd). Internal ethylene concentration was transformed using a log transformation to normalise the data. Flesh browning incidence were transformed to angles ($Y = \sin^{-1} \sqrt{\%}/100$) for analysis and back-transformed to % for presentation. Starch pattern index and greasiness score data were analysed using an ordinal logistic regression using GenStat statistical software. The results for each experiment within a season have been presented on figures with the same Y-axis scale to allow for simple comparison of the data.

4.5 Results

4.5.1 Response of flesh browning to controlled atmosphere storage

4.5.1.1 Radial flesh browning

As discussed in Chapter 5, RFB occurs in districts accumulating greater than 1400 growing degree days (GDD) >10^{o}C between full bloom and optimal commercial harvest date. In this experiment, three of the districts assessed were within this climacteric range and developed symptoms of RFB. These districts were Batlow (New South Wales), Manjimup (Western Australia) and the Goulburn Valley (Victoria). As the climatic conditions during fruit growth and development influence the development of RFB, a significant difference (P=0.019) in the incidence of RFB between the districts was observed. In order to establish the effect of the storage atmosphere, each district was examined independently.

4.5.1.1.1 Batlow

The incidence of RFB increased significantly (P=0.002) between the 4 and 7 month removal times for 'Cripps Pink' apples grown in Batlow (New South Wales). Following 4 months of storage (Figure 4.1), there was no significant difference between treatment means for the storage atmosphere (P=0.117), harvest maturity (P=0.28) or wax treatment (P=0.752). Following 7 months of storage (Figure 4.2), no significant difference was observed between the storage atmosphere (P=0.778), harvest maturity (P=0.747) or wax treatment (P=0.208).

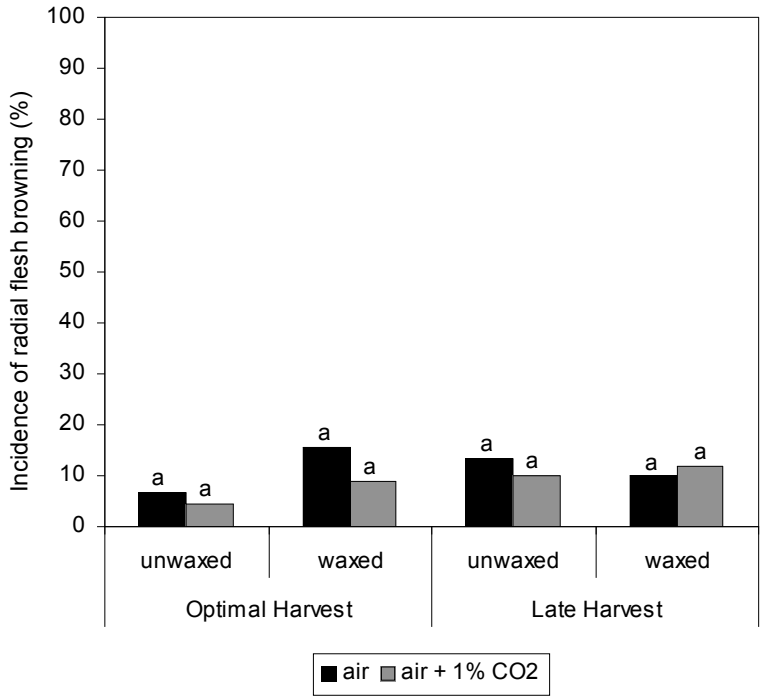

Figure 4.1 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in Batlow (New South Wales) after 4 months storage at 0°C. Percentage data were transformed to angles ($Y = \sin^{-1} \sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

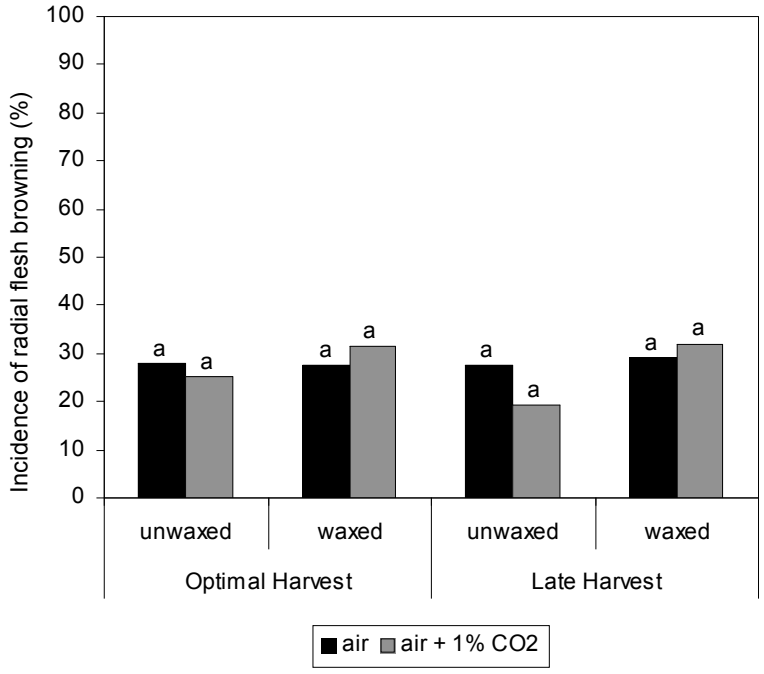

Figure 4.2 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in Batlow (New South Wales) after 7 months storage at 0°C. Percentage data were transformed to angles ($Y = \sin^{-1} \sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

4.5.1.1.2 Manjimup

The incidence of RFB increased significantly (P<0.001) between the 4 and 7 month removal times for fruit grown in Manjimup (Western Australia). Following 4 months of storage (Figure 4.3), no significant difference was found between treatment means for the storage atmosphere (P=0.266) or wax treatment (P=0.973). Following 7 months of storage (Figure 4.4), a significant interaction between the storage atmosphere and pre storage waxing was found (P=0.032). The highest incidence of RFB was observed in apples that were waxed and stored in air + 1% CO_2 (52.28%). There was no significant difference between the other treatment means (Figure 4.4).

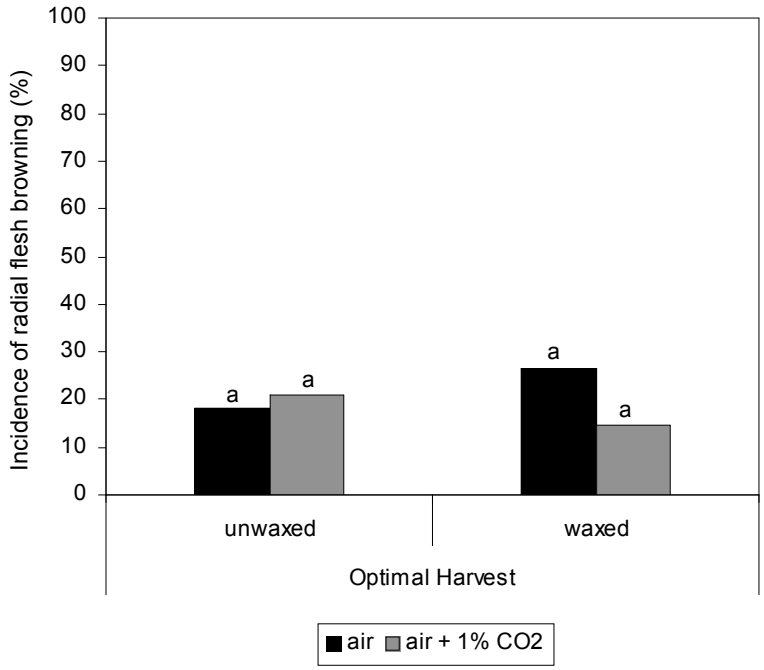

Figure 4.3 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in Manjimup (Western Australia) after 4 months storage at 0°C.
Percentage data were transformed to angles ($Y = \sin^{-1} \sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5

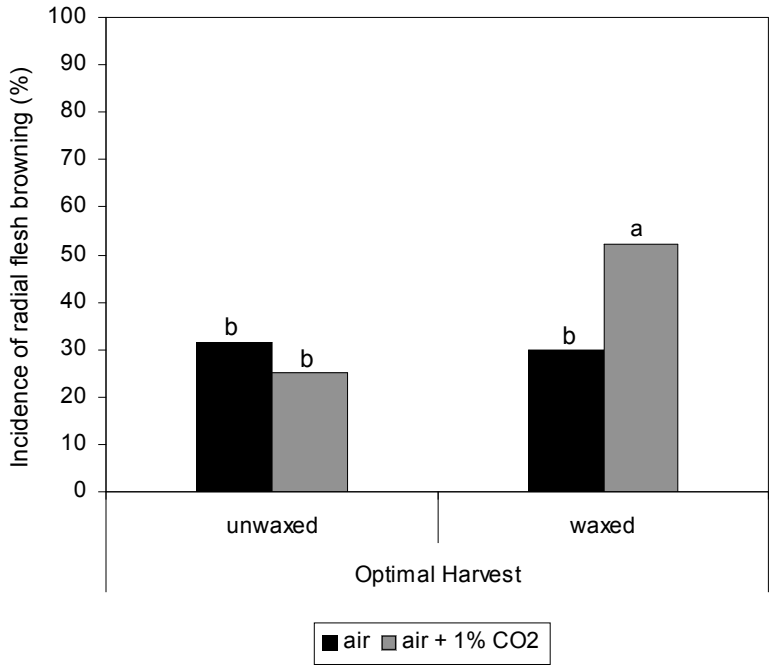

Figure 4.4 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in Manjimup (Western Australia) after 7 months storage at 0°C. Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5

4.5.1.1.3 Goulburn Valley

The incidence of RFB increased significantly (P=0.005) between the 4 and 7 month removal times for Cripps Pink apples grown in the Goulburn Valley (Victoria). Following 4 months of storage (Figure 4.5), there was no significant difference between treatment means for the storage atmosphere (P=0.208), harvest maturity (P=0.375) or wax treatment (P=0.559). Following 7 months of storage (Figure 4.6), no significant difference was observed between the storage atmosphere (P=0.26) or wax treatment (P=0.268).

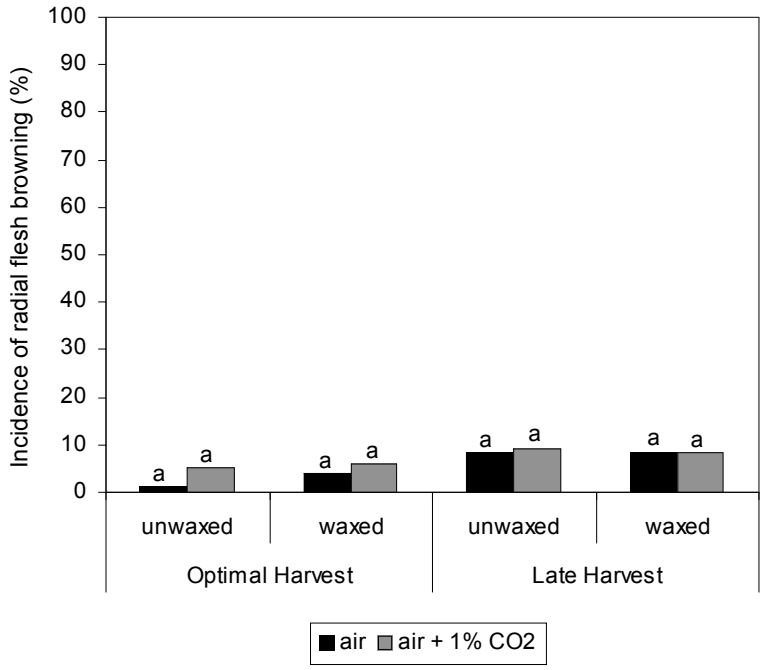

Figure 4.5 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in the Goulburn Valley (Victoria) after 4 months storage at 0°C. Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

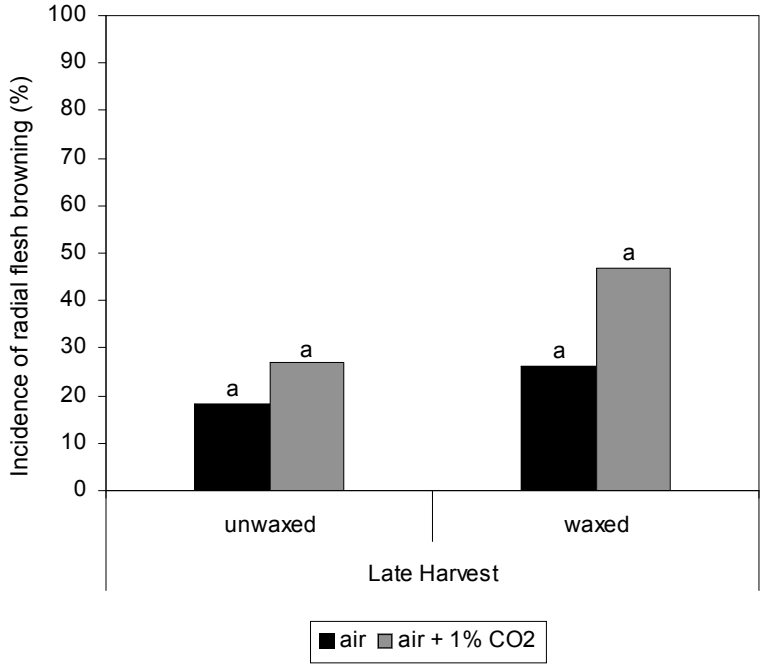

Figure 4.6 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in the Goulburn Valley (Victoria) after 7 months storage at 0°C. Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5

4.5.1.2 Diffuse flesh browning

As discussed in Chapter 5, DFB occurs in districts accumulating less than 1100 GDD >10^{o}C between full bloom and optimal commercial harvest date. In this experiment, one of the districts assessed, the Huon Valley (Tasmania) was within this climacteric range and developed symptoms of DFB

4.5.1.2.1 Huon Valley

In contrast to RFB, the incidence of DFB did not change significantly (P=0.713) between the 4 and 7 month removals. Following 4 months of storage (Figure 4.7) waxed fruit had a significantly lower (P=0.029) incidence of DFB (76.4%) compared to unwaxed fruit (83.5%). However there was no clear relationship between treatments and the incidence of DFB (Figure 4.7). Following 7 months of storage (Figure 4.8), there was no significant effect on the incidence of DFB of the storage atmosphere (P=0.12), harvest maturity (P=0.734) or wax treatment (P=0.995).

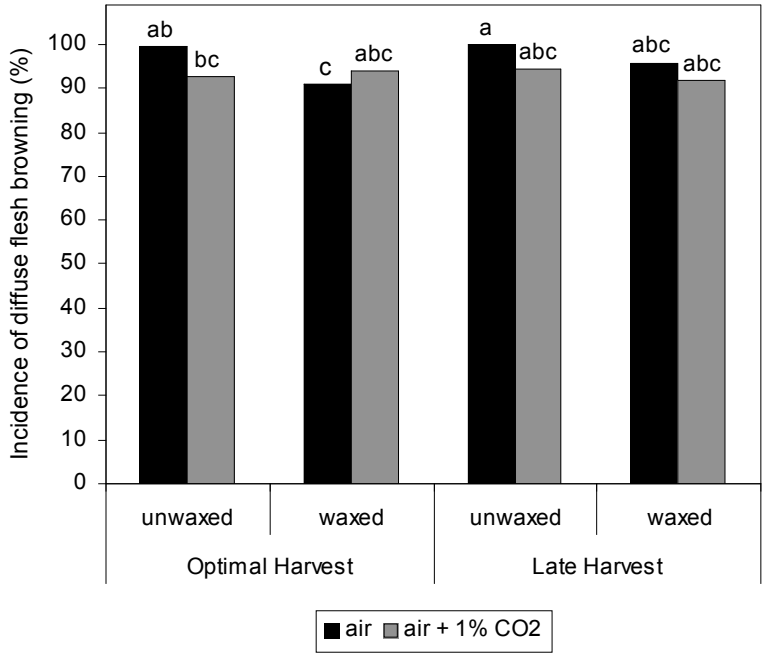

Figure 4.7 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on diffuse flesh browning of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) after 4 months storage at 0^{o}C.
Percentage data were transformed to angles (Y = $\sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

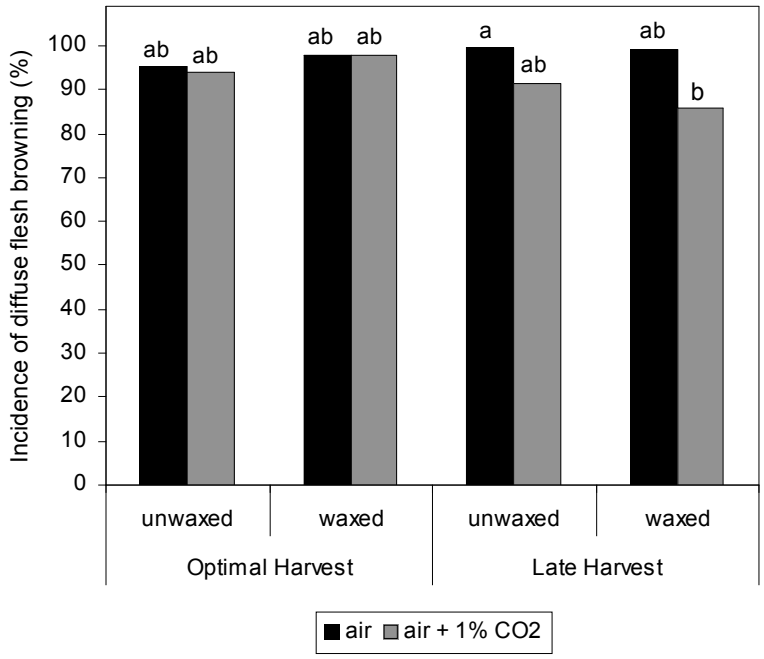

Figure 4.8 Effect of storage atmosphere, pre storage wax treatment and fruit maturity on diffuse flesh browning of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) after 7 months storage at 0°C. Percentage data were transformed to angles ($Y = \sin^{-1} \sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

4.5.2 Response of flesh browning to storage temperature

Storage experiments examining the influence of the storage temperature were completed with 'Cripps Pink' apples grown in two regions, Batlow (NSW) and the Huon Valley (Tasmania), districts susceptible to RFB and DFB respectively. Storage experiments to determine the influence of the storage temperature were completed in two seasons (2005 and 2006). As discussed in Chapter 5, the 2005 season was a high risk season for developing RFB and a moderate risk season for developing DFB, whereas the 2006 season was a low risk season for the development of RFB and DFB.

In the 2005 season, 2 storage temperatures were examined, 0 and 3°C. In the following season, 2006, 2 additional storage temperature treatments were included in the experiment in addition to the 0°C and 3°C storage temperatures. These were a 1°C and a stepwise cooling treatment.

4.5.2.1 Radial flesh browning

In both the 2005 and 2006 seasons, the storage temperature had a significant effect on the incidence of RFB ($P<0.001$ for both the 2005 and 2006 seasons). In the 2005

season (Figure 4.9) the highest incidence of RFB (following 7 months of air storage) was observed in late harvested and waxed fruit stored at 0°C (85.65%). A significant interaction between the storage temperature and the harvest maturity was found (P=0.012). When main effects were analysed, fruit stored at 3°C had a significantly lower (P<0.001) incidence of RFB (32.7%) than fruit stored at 0°C (57.6%) (Figure 4.9). Fruit harvested at a late maturity had a significantly higher (P=0.021) incidence of RFB (48.9%) than fruit harvested at optimal maturity (41.4%).

The 2006 season was a low risk season and the incidence of RFB was lower than was observed in the 2005 season (Figure 4.10). After 7 months of storage, no RFB was detected in 'Cripps Pink' apples that were harvested at optimal maturity when stored at 1°C, 3°C or stepwise cooled, regardless of wax treatment (Figure 4.10). A significant interaction between storage temperature and harvest maturity (P=0.013) was found. When main effects were analysed, the storage temperature (P<0.001) and harvest maturity (P<0.001) were found to have a significant affect on the development of RFB. The highest incidence of RFB was observed in late harvested fruit that were waxed and stored at 0°C (18.98%). Late harvested fruit developed RFB when stored at 1°C or stepwise cooled (Figure 4.10). The 3°C storage temperature was able to prevent the development of RFB in late harvested fruit (Figure 4.10).

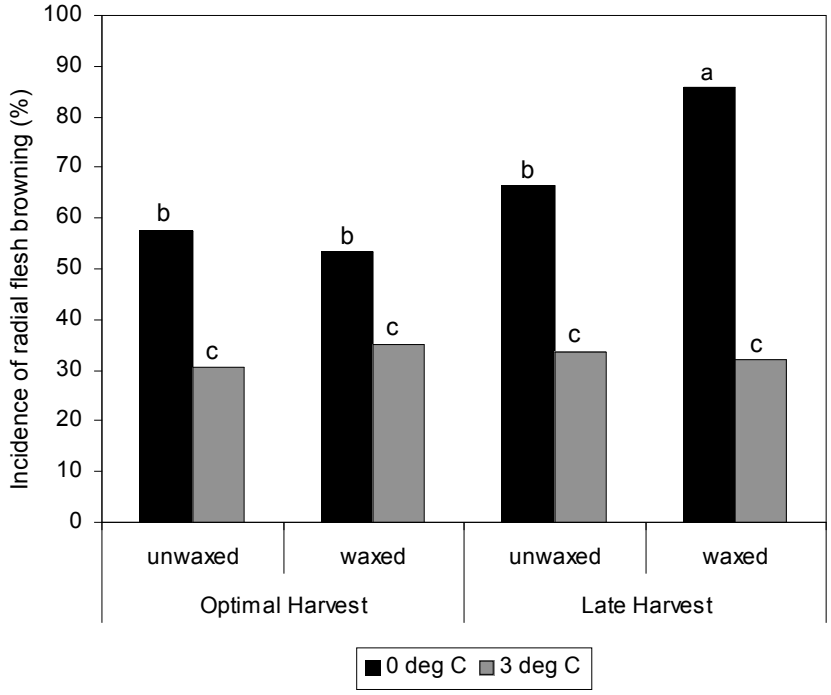

Figure 4.9 Effect of storage temperature, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in Batlow (New South Wales) after 7 months at 0°C or 3°C.
Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

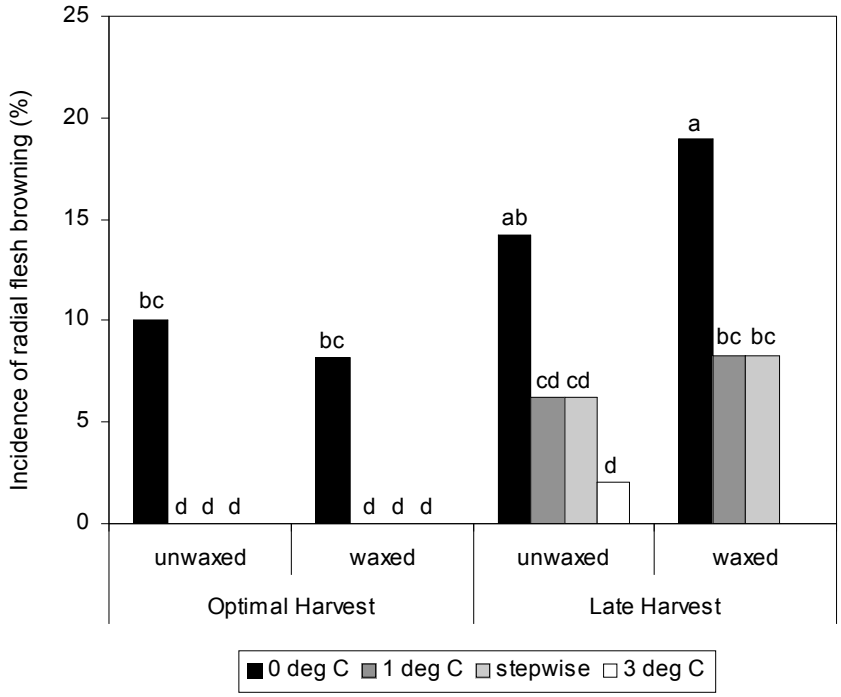

Figure 4.10 Effect of storage temperature, pre storage wax treatment and fruit maturity on radial flesh browning of 'Cripps Pink' apples grown in Batlow (New South Wales) after 7 months at 0°C, 1°C, stepwise cooled (2 weeks at 3°C, 2 weeks at 2°C, 6 months at 1°C) or 3°C.
Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

4.5.2.2 Diffuse flesh browning

Similarly as for RFB, storing 'Cripps Pink' apples that are susceptible to developing DFB at 3°C significantly reduced the incidence of DFB than was observed at 0°C in both the 2005 and 2006 seasons ($P<0.001$ for both the 2005 and 2006 seasons). In the 2005 season (Figure 4.11) the highest incidence of DFB, following 7 months of air storage, was observed in late harvested fruit that were waxed and stored at 0°C (75.73%). There was a significant 3-way interaction between the storage temperature, fruit maturity at harvest and wax treatment ($P=0.008$). When main effects were analysed, fruit stored at 0°C had a significantly higher ($P<0.001$) incidence of DFB than fruit stored at 3°C (Figure 4.11).

As in Batlow, the 2006 season in the Huon Valley was a lower risk season for the development of DFB. The incidence of DFB was lower in the 2006 season compared to the 2005 season for all storage treatments (Figure 4.12). After 7 months of storage, the storage temperature had a significant effect on the development of DFB ($P=0.007$). The highest incidence of DFB (16.52%) was observed in fruit that were stored at 0°C (Figure 4.12). Storage at 1°C (3.08%) or stepwise cooling (3.08%) significantly reduced ($P=0.007$) the incidence of DFB compared to storage at 0°C. Apples stored at 3°C in the 2006 season did not develop any symptoms of DFB following 7 months of air storage (Figure 4.12). The influence of harvest maturity was not assessed in the Huon Valley in this season due to a lack of availability of suitable fruit in 2006.

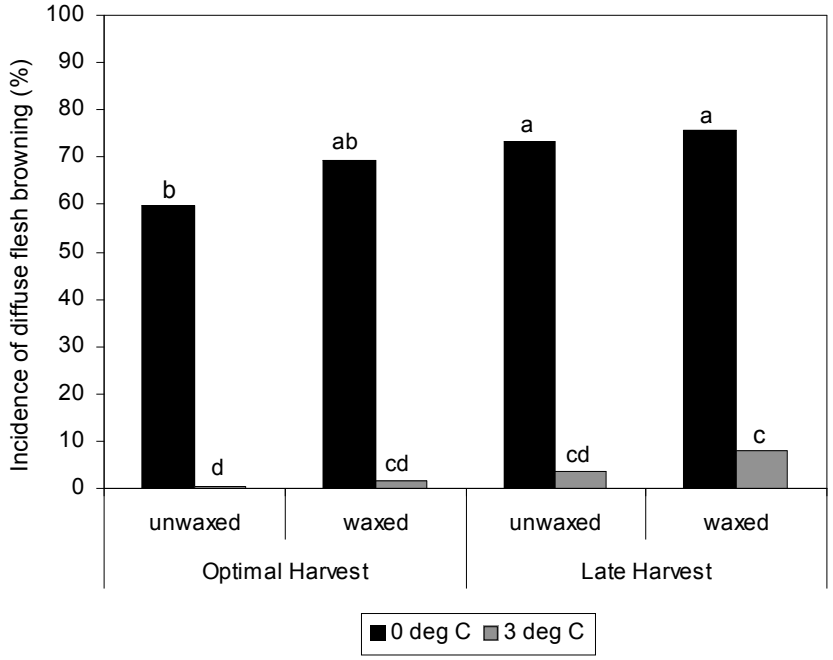

Figure 4.11 Effect of storage temperature, pre storage wax treatment and fruit maturity on diffuse flesh browning of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) after 7 months storage at 0°C or 3°C.
Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5, Late Harvest had a starch pattern index of 8.5

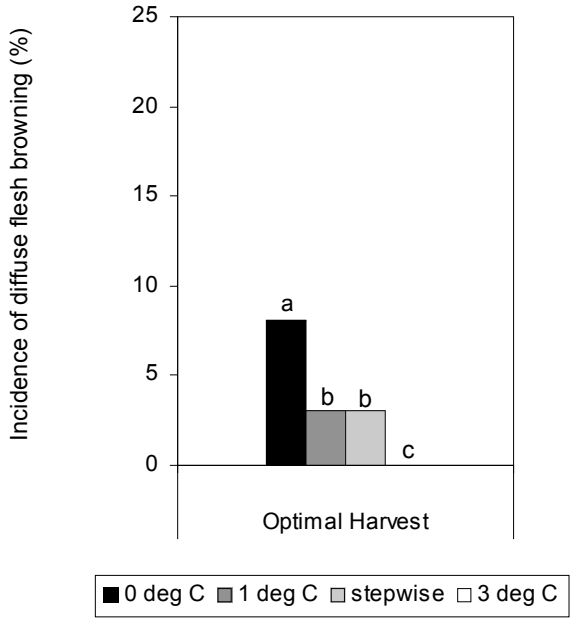

Figure 4.12 Effect of storage temperature on diffuse flesh browning of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) after 7 months storage at 0°C, 1°C, stepwise cooled (2 weeks at 3°C, 2 weeks at 2°C, 6 months at 1°C) or 3°C.
Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation.
Any two means not followed by the same letter are significantly different at 5% level of significance
Optimal Harvest had a starch pattern index of 3.5

4.5.3 Relationship between storage temperature and fruit quality

The quality and maturity measurements made on the fruit included flesh firmness (FF), skin greasiness (SG) and internal ethylene concentration (IEC). These characteristics are used commercially to determine the acceptability of the fruit for marketing. As 'Cripps Pink' apples were found to be sensitive to cool storage and CA storage conditions in the 2005 experiment, the recommendation for prevention of flesh browning would be to store in air and to store at an increased temperature. However, this recommendation could reduce the length of time in storage before quality falls below acceptable limits. In 2006, the quality assessments were included in the storage trial to validate this recommendation.

4.5.3.1 Flesh firmness

Flesh firmness decreased with increasing time in storage for 'Cripps Pink' apples grown in Batlow (Table 4.1) and the Huon Valley (Table 4.2). The influence of the storage temperature had an inconsistent effect on FF with storage time and between the two districts. In Batlow, fruit stored at 0^{o}C were significantly firmer than those stored at 3^{o}C following 1 and 3 months of storage (Table 4.1). Following 7 months of storage, fruit stored at 3^{o}C had the highest FF (74.6 N) and fruit stored at 1^{o}C had the lowest FF (62.6 N). The change in FF observed in the Huon Valley (Table 4.2) followed a similar trend.

Table 4.1 Effects of storage temperature and duration on the flesh firmness (N) of 'Cripps Pink' apples grown in Batlow (New South Wales) following up to 7 months in air.

	Firmness (N)							
	1 month		3 months		5 months		7 months	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0^{o}C	87.1	83.1	78.4	69.5	68.9	64.0	69.2	63.4
3^{o}C	76.0	78.3	72.3	71.6	70.6	72.5	74.6	71.4
1^{o}C							62.6	58.5
stepwise							72.0	68.4
L.S.D.	5.7		5.6		5.2		5.5	
Significance	D***		DxT*		T**		T***,D**	

*,**,***Significant at P<0.05, 0.01 or 0.001 respectively where T=storage temperature and D=day

Table 4.2 Effects of storage temperature and duration on the flesh firmness (N) of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) following up to 7 months in air.

	Firmness (N)							
	1 month		3 months		5 months		7 months	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0°C	85.0	81.7	72.3	67.3	61.9	60.2	59.0	57.6
3°C	83.5	78.1	65.9	63.9	62.1	60.9	62.3	60.4
1°C							61.3	58.6
stepwise							63.3	59.3
L.S.D.	5.9		3.9		5.8		4.1	
Significance	D*		T***,D*		ns		D*	

*, ***Significant at P<0.05 or 0.001 respectively where T=storage temperature and D=day

4.5.3.2 Skin greasiness

Skin greasiness increased with increasing time in storage for 'Cripps Pink' apples grown in Batlow (Table 4.3) and the Huon Valley (Table 4.4). In Batlow, SG was higher in fruit stored at 3°C than fruit stored at 0°C at all removal times.

Table 4.3 Effects of storage temperature and duration on the skin greasiness of 'Cripps Pink' apples grown in Batlow (New South Wales) following up to 7 months in air.

	Skin Greasiness (score 1-3)							
	1 month		3 months		5 months		7 months	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0°C	0	0	0.1	0	0.1	0.6	0.1	0.4
3°C	0.1	0.5	0.3	1.3	1.3	0.9	1.7	1.5
1°C							0.8	1.3
stepwise							1.2	1.4
Significance	***		***		***		***	

Score data (1-not greasy, 2-slightly greasy, 3-very greasy) was analysed using ordinal logistic regression

***Significant at P<0.001 as significantly different from reference treatment (0°C Day 1)

Table 4.4 Effects of storage temperature and duration on the skin greasiness of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) following up to 7 months in air.

	Skin Greasiness (score 1-3)							
	1 month		3 months		5 months		7 months	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0°C	0	0.8	0.5	1.4	0.3	0.9	1.2	1.4
3°C	0	0.4	0.6	0.8	0.9	1.3	1.2	1.3
1°C							0.7	1.3
stepwise							0.7	1.6
L.S.D.								
Significance	***		***		***		***	

Score data (1-not greasy, 2-slightly greasy, 3-very greasy) was analysed using ordinal logistic regression

***Significant at P<0.001 as significantly different from reference treatment (0°C Day 1)

4.5.3.3 Internal ethylene concentration

Internal ethylene concentration increased with increasing time in storage for 'Cripps Pink' apples grown in Batlow (Table 4.5) and the Huon Valley (Table 4.6). 'Cripps Pink' apples from Batlow that were stored at 3°C had a significantly higher IEC following 1, 3and 5 months of storage. 'Cripps Pink' apples from the Huon Valley that were stored at 3oC had a significantly higher IEC following 1, 5 and 7 months of storage (plus 7 days at room temperature).

Table 4.5 Effects of storage temperature and duration on the internal ethylene concentration of 'Cripps Pink' apples grown in Batlow (New South Wales) following up to 7 months in air.

	Internal Ethylene Concentration (μL L^{-1})							
	1 month		3 months		5 months		7 months	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0°C	64.4	59.8	109.5	99.8	74.9	154.0	148.0	160.9
3°C	96.5	120.2	127.1	157.6	114.8	343.8	121.9	326.0
1°C							225.9	436.1
stepwise							229.7	218.5
L.S.D.	24.6		29.6		77.3		84.8	
Significance	D***		T***		TxD**		TxD***	

,*Significant at P<0.01 or 0.001 respectively where T=storage temperature and D=day

Table 4.6 Effects of storage temperature and duration on the internal ethylene concentration of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) following up to 7 months in air.

	Internal Ethylene Concentration (μL L^{-1})							
	1 month		3 months		5 months		7 months	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0°C	184.8	217.3	145.7	286.0	251.3	233.4	409.9	300.5
3°C	124.6	292.2	209.2	207.4	245.7	538.8	203.4	722.7
1°C							238.4	371.2
stepwise							356.0	408.9
L.S.D.	92.2		80.4		81.5		172.3	
Significance	TxD*		TxD*		TxD*		TxD***	

*,***Significant at P<0.05 or 0.001 respectively where T=storage temperature and D=day

4.5.4 Development of flesh browning over time

In the 2004 season, 'Cripps Pink' apples grown in Batlow (New South Wales) and the Huon Valley (Tasmania) were assessed for RFB and DFB respectively following 4 and 7 months of storage. The 2004 season was a high DFB risk season in the Huon Valley and a moderate RFB risk season in Batlow, based on climatic conditions (seasonal risk is discussed in Chapter 5). The 2006 season was a low risk season in both districts. In the 2006 season, 'Cripps Pink' apples from the same growing districts were assessed following 1, 3, 5 and 7 months of storage. These removal times aimed to establish the development of RFB and DFB over time during storage.

4.5.4.1 Radial flesh browning

In ‘Cripps Pink’ apples grown in Batlow (New South Wales) in 2004 (moderate RFB risk season), the incidence of RFB in optimally harvested, unwaxed air stored apples had reached 7% after 4 months of storage (Figure 4.1). The incidence increased to 28% following 7 months of storage (Figure 4.2).

In 2006 (low RFB risk season), no RFB was detected after 1 or 3 months of storage (Table 4.7) in ‘Cripps Pink’ apples grown in Batlow (New South Wales). Following 5 months of storage, the incidence of RFB had reached 10% for optimally harvested fruit stored at 0^{o}C and 16% for late harvested waxed fruit stored at 0^{o}C. After 5 months of storage, no RFB was detected in fruit stored at 3^{o}C, regardless of wax or maturity (Table 4.7).

Table 4.7 Effects of storage temperature and duration on diffuse flesh browning of ‘Cripps Pink’ apples grown in Batlow (New South Wales) following up to 7 months in air.

	Incidence of radial flesh browning (%)			
	1 month	3 months	5 months	7 months
Optimal Harvest				
0^{o}C	0	0	10	10
3^{o}C	0	0	0	0
Late Harvest				
0^{o}C	0	0	2	8
3^{o}C	0	0	0	0

4.5.4.2 Diffuse flesh browning

In ‘Cripps Pink’ apples grown in the Huon Valley (Tasmania) in 2004 (high DFB risk season), the incidence of DFB in optimally harvested, unwaxed, air stored apples had reached 100% after 4 months of storage (Figure 4.7). The incidence of DFB did not change significantly following 7 months of storage in this season (Figure 4.8).

In 2006 (low DFB risk season), no DFB was detected following 1 or 3 months of storage in ‘Cripps Pink’ apples grown in the Huon Valley (Tasmania) (Table 4.8). Following 5 months of storage, the incidence of DFB was measured at 10%. The incidence of DFB did not change significantly following 7 months of storage (Table 4.8).

Table 4.8 Effects of storage temperature and duration on diffuse flesh browning of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) following up to 7 months in air.

	Incidence of diffuse flesh browning (%)			
	1 month	3 months	5 months	7 months
Optimal Harvest				
0°C	0	0	10	8
3°C	0	0	0	0

4.6 Discussion

The FB disorder of 'Cripps Pink' apples was first identified in the year 2000 in a shipment of 'Cripps Pink' apples originating in Tasmania and the disorder was first described in 2003 (Brown et al., 2003). Preliminary research completed by Jobling et al. (2004) and Zanella et al. (2004) indicated that both the storage atmosphere and the storage temperature influenced the development of FB of 'Cripps Pink' apples. When this preliminary work was completed, the FB disorder had not been classified as RFB and DFB disorders. Soon after the preliminary work, the Australian industry began to call the disorders RFB and DFB based on the visual appearance of the symptoms (Chapter 1, Figure 1.1). A similar situation has been reported for 'Jonathan' apples, this cultivar has been found to be susceptible to both FB and IB during storage (Little, 1973; Little et al., 1973). One of the aims of this work was to establish the differences in storage behaviour between RFB and DFB.

Flesh browning of 'Cripps Pink' apples has been shown to be the result of a complex interaction between a number of preharvest and postharvest conditions. Climatic conditions during fruit growth and development (Chapter 5) as well as fruit ripening (Chapter 2) have been shown to have an effect on the development of the both disorders. However, while preharvest conditions have been shown to predispose 'Cripps Pink' apples to developing FB, it is the storage conditions that have been found to promote and exacerbate symptoms of FB. No symptoms of either RFB or DFB have been observed in 'Cripps Pink' apples at harvest. However, another form of internal browning of 'Cripps Pink' apples known as bulge browning (East et al., 2005) can be seen in fruit at harvest and has been confused with the RFB and DFB disorders. It is thought that the bulge browning (BB) disorder of 'Cripps Pink' apples is related to the fertilisation of the fruit. 'Cripps Pink' apples that develop BB can be recognised by their bulging shape and as such as also known as 'fat ladies'. Bulge browning is also thought to be related to fruit boron concentrations (Chapter 3), as boron deficiency in apples can be identified by misshapen fruit (Jackson, 2003).

It is often the case that a storage disorder does not have a clearly defined cause, but is influenced by a number of pre and postharvest conditions. To complicate this relationship, pre and postharvest factors will often interact with one another to alter the incidence of a disorder.

A number of postharvest browning disorders of other apple cultivars helped to establish the research directions for investigating the FB disorder of 'Cripps Pink' apples. Storage disorders resulting from CO_2 injury, low temperature injury and senescence in other apple cultivars shared some similarities with RFB and DFB in 'Cripps Pink' apples.

4.6.1 Radial flesh browning

Radial flesh browning has been shown to be characterised by browning of the vascular areas of the fruit, extending through the cortex of the fruit with increasing severity (Chapter 3). Radial flesh browning was observed in three growing districts throughout the storage trials, these included Batlow (New South Wales), Manjimup (Western Australia) and the Goulburn Valley (Victoria). The greatest variation in the incidence of RFB was observed between these districts and between growing seasons rather than between any storage treatments. This seasonal and regional variation indicates that climatic conditions during fruit growth and development strongly influence the development of RFB. The role of climatic conditions on the development of RFB is discussed in Chapter 5. Within a district and season, variation in the incidence of RFB was found between fruit maturity at harvest, the storage atmosphere and the storage temperature.

'Cripps Pink' apples grown in Batlow (New South Wales) and harvested at a late maturity were found to have a higher incidence of RFB than those harvested at an optimal maturity for long term storage (Figure 4.9 and Figure 4.10). This result confirms the result from the preliminary study where advanced fruit maturity was found to increase the incidence of RFB (Jobling et al., 2004). Advanced fruit maturity has been found to be involved in the development of other storage disorders of apples (Brown et al., 2003; Grant et al., 1996; Toivonen et al., 2003; Volz et al., 1998). In late harvested fruit, the process of ripening will often have been initiated prior to the establishment of optimal storage conditions. As a consequence, such fruit are likely to develop symptoms associated with senescence. One of the main

changes associated with senescence is the loss of membrane integrity (Kays, 1991), this loss of membrane fluidity may be responsible for the collapse of cells and the development of symptoms of RFB.

Storage of fruit with advanced maturity can also result in the degradation of quality characteristics such as FF and SG. The maintenance of fruit quality is a high priority of apple storage. Losses in quality can result in a large financial loss for the grower as poor quality fruit will not achieve a premium price. In 2006, quality assessments aimed at establishing the relationship between fruit quality, fruit maturity and storage temperature. Skin greasiness was found to be higher on fruit stored at 3^{o}C than fruit stored at 0^{o}C in Batlow (Table 4.3) and the Huon Valley (Table 4.4). Skin greasiness is an important quality consideration in the export of 'Cripps Pink' apples to foreign markets. Skin greasiness is classified as a major defect in export quality 'Cripps Pink' apples and there is no tolerance for any detectable level of SG (Hurndall and Fourie, 2003). Skin greasiness was more prevalent in 'Cripps Pink' apples grown in the Huon Valley (Table 4.4) than in Batlow (Table 4.3) indicating a degree of climatic influence on this quality parameter. For fruit grown in Batlow, SG was only very minimal in fruit stored at 0^{o}C, however was present in fruit stored at 3^{o}C (Table 4.3) resulting in fruit that would not be acceptable for export. The relationship between storage temperatures, storage duration and flesh firmness was inconclusive in Batlow (Table 4.1) and the Huon Valley (Table 4.2). It is likely that the low number of replicates used in these experiments resulted in insignificant results; it is also possible that the FF measurements were affected by the presence of RFB in the fruit. Other research has indicated that storage at increased temperature will result in a loss of flesh firmness (Blankenship et al., 1997; DeEll et al., 2000; Little and Holmes, 2000; Watkins et al., 2002). These studies indicate that storage at increased temperature results in reduced fruit quality. Despite reducing the incidence of the flesh browning disorder, storage at increased temperature may reduce the length of effective storage time as a result of decreasing fruit quality. However, it is possible that the potential losses resulting from the decreased quality observed when fruit were stored at 3^{o}C, compared to those stored at 0 or 1^{o}C may result in a lower financial loss than the potential financial losses resulting from the development of the RFB disorder when fruit were stored at the decreased temperature. Future work may focus on the relationship between storage temperature and fruit quality with the aim of determining the cost-benefit analysis of quality loss versus the development of flesh browning.

Fruit maturity at harvest is of particular concern for 'Cripps Pink' apples as major Australian supermarkets had set a high specification of red blush (60%). This high specification often resulted in fruit being harvested over mature as growers were forced to delay harvest in an attempt to increase the level of red colouring on the skin of the fruit. By delaying harvest, the risk of developing RFB during storage is increased, negating some of the positive qualities of this cultivar. As a result of the findings of the link between fruit maturity at harvest and flesh browning, Australian retailers have reduced the percentage of red blush specifications to 45-50% which is an important difference for growers (James et al., 2005).

Fruit maturity at harvest can also increase the susceptibility to CO_2 injury (Watkins, 2003). In a preliminary study examining 'Cripps Pink' apples grown in Batlow in 2003, Jobling et al. (2004) found that the incidence of RFB increased with increasing CO_2 and decreasing O_2 in the storage atmosphere. In that study, the highest incidence of RFB was observed in late harvested fruit stored in an atmosphere of 2% O_2 and 1% CO_2 (Jobling et al, 2004). The lowest incidence of RFB was observed in fruit stored in 21% O_2 and 0.003% CO_2. Storage in 21% O_2 and 1% CO_2 or 2% O_2 and 0.03% showed an intermediate incidence of RFB (Jobling et al, 2004). The study indicated that the 'Cripps Pink' apple is susceptible to injury when stored in atmospheres containing low O_2 or high CO_2 and they are particularly susceptible to injury when stored in atmospheres containing low O_2 combined with high CO_2. A similar response to modified atmospheres has been observed in browning disorders in 'Braeburn' and 'Fuji' apple cultivars (Clark and Burmeister, 1999; Grant et al., 1996; Lau, 1998; Streif and Saquet, 2003; Volz et al., 1998).

While the preliminary study indicated a susceptibility to CA related disorders in the 'Cripps Pink' cultivar and suggested that the RFB disorder may be the result of CO_2 injury, this research had not established a clear or consistent relationship between the storage atmosphere and the development of RFB. In this work, the incidence of RFB in 2004 was not significantly affected by the addition of 1% CO_2 to the storage atmosphere in any district following 4 months (Figures 4.1, 4.3, 4.5) or 7 months (Figures 4.2, 4.4, 4.6) of storage. However, the development of cavities in the flesh of fruit stored in air + 1% CO_2 in all districts (data not shown) indicates that the 'Cripps Pink' apple is susceptible to CO_2 injury when stored in CA. Cavities were found to develop in fruit with symptoms of RFB as well as in fruit that showed no visual symptoms of RFB suggesting that RFB is not simply a CO_2 injury. In northern California, a district that is not susceptible to either RFB or DFB (Chapter 5), CO_2

injury has been observed in 'Cripps Pink' apples stored in CA storage (De Castro et al., 2007).

CO_2 injury in other apple cultivars is generally characterised as damage to the vascular tissue of the fruit (Little and Holmes, 2000; Meheriuk et al., 1994; Smock, 1977; Watkins, 2003). As discussed in Chapter 3, the vascular tissue of an apple consists of small and tightly packed cells with strengthened cell walls. It is possible that such cells result in a slow path for the diffusion of gases through the fruit. As a consequence, elevated levels of CO_2 are more likely to accumulate in the vascular tissue of the fruit rather than the cortex tissue which consist of much larger and more loosely packed cells (Raven et al., 2005). Elevated levels of CO_2 trapped in the vascular tissue may result in the type of damage that is symptomatic of CO_2 injury. Tissue damage occurring during storage in high CO_2 can be the result of membrane damage and metabolic disruption (Kays, 1991). As 'Cripps Pink' apples are susceptible to CO_2 injury during CA storage and the RFB disorder affected the vascular tissue of the fruit, it appears likely that RFB is associated with the level of CO_2 in the storage atmosphere.

However, inconsistency of the impact of the storage atmosphere on the development of RFB in 'Cripps Pink' apples may indicate that the storage atmosphere was a secondary influence on the development of RFB. The impact of the storage atmosphere may present as an additive factor, interacting with other pre and postharvest factors resulting in the development of RFB.

While alteration of the storage atmosphere was not found to have a consistent effect on the development of RFB, increasing the storage temperature was seen to significantly reduce the incidence of RFB. In a study completed by Zanella et al. (2004) increasing the storage temperature from 1.3^{o}C to 2.5^{o}C was found to significantly reduce the incidence of FB. This study was completed in Italy where DFB is the dominant form of FB observed; no study had examined the influence of the storage temperature on the development of RFB.

The impact of the storage temperature on RFB was examined in 2 seasons. The seasonal incidence of RFB varied between these two seasons as a result of the climatic conditions (discussed in Chapter 5). The effect of increasing the storage temperature from 0^{o}C varied depending on the seasonal severity of symptoms.

In 2005, the seasonal incidence of RFB in Batlow was high (Chapter 5, Table 5.2). In this season, increasing the storage temperature from 0°C to 3°C was found to significantly reduce the incidence of RFB in all storage treatments (Figure 4.9). However, despite being significantly reduced, the incidence of RFB was not prevented at this storage temperature in this season (Figure 4.9).

In 2006, the seasonal incidence of RFB in Batlow was low (Chapter 5, Table 5.2). The highest incidence of RFB in 2006 (20%) was observed in late harvested fruit that were waxed and stored at 0°C (Figure 4.10). Despite being the highest incidence observed in 2006, this was lower than the lowest incidence observed in the previous season (30%) (Figure 4.9). With the low seasonal incidence, storage at 1°C, stepwise cooling to 1°C or storage at 3°C in 2006 were treatments that were able to prevent the development of RFB of optimally harvested fruit (Figure 4.10).

Increasing the storage temperature had a greater impact on reducing the incidence of RFB than was observed with modifications to the storage atmosphere. However, the structural damage associated with RFB indicates that this disorder is not the result of CI (Chapter 3). Further to this, the time scale for the development of symptoms of RFB is longer than would generally be considered typical for a CI (Wills et al., 1998). It is likely that the primary event causing symptoms of RFB is senescence related loss of membrane integrity. As high CO_2 in the storage atmosphere and low temperature storage were also found to have an impact on the development of RFB, it is possible that high CO_2 induced metabolic degradation and CI are a secondary cause of RFB in 'Cripps Pink' apples. As the impact of increasing the storage temperature to 3°C was not consistent in preventing the development of RFB in both seasons, it is likely that seasonal climatic conditions play an important role in the development of RFB.

4.6.2 Diffuse flesh browning

In contrast to RFB, DFB is characterised by browning of the cortex tissue of the fruit with little or no damage associated with the vascular tissue (Chapter 3). In this research, one district (the Huon Valley) consistently developed symptoms of DFB during storage. As had been found with RFB, a large variation in the incidence of DFB was observed between growing seasons. The incidence of DFB of optimally harvested unwaxed 'Cripps Pink' apples stored in air at 0°C varied between 8% in 2006 (Figure 4.12) and 95% in 2004 (Figure 4.8). As with RFB, such a high variation

between seasons indicates that climatic conditions during fruit growth and development are involved in the development of DFB. The impact of climatic conditions on the development of DFB is discussed in Chapter 5. Within a season, variation in the incidence of DFB was found between fruit maturity at harvest and the storage temperature.

In a similar trend to what was observed with RFB, the incidence of DFB was found to increase with increasing fruit maturity at harvest (Figure 4.11). Increasing maturity at harvest is likely to result in ripening being initiated and the process of senescence being active. As senescence involves the progressive loss of membrane integrity (Kays, 1991), such fruit are likely to be less able to tolerate CI inducing conditions.

In contrast to RFB, the addition of 1% CO_2 in the storage atmosphere was found to decrease the incidence of DFB (Figures 4.7 and 4.8). It is possible that the increased level of CO_2 in the storage atmosphere inhibited the action of polyphenol oxidase (Rocha and Morais, 2001; Tian et al., 2004) and consequently reduced the severity of browning. However, the application of high CO_2 for prevention of DFB would not be recommended as a commercial treatment as the reduction in DFB was not large enough to be of any practical value.

For DFB, increasing the storage temperature was found to significantly reduce the incidence of symptoms in both the 2005 and 2006 seasons. This supports the findings of a preliminary study completed by Zanella et al. (2004) where increasing the storage temperature from 1.3^{o}C to 2.5^{o}C was found to significantly reduce the incidence of DFB of 'Cripps Pink' apples.

As was observed with RFB, storing fruit susceptible to DFB at an increased temperature was found to significantly reduce the incidence of the disorder (Figure 4.11 and Figure 4.12). In 2005, a season with a moderate incidence of DFB, storing optimally harvested fruit at 3^{o}C reduced the incidence of DFB from 60% to 1%, a value that is within commercially accepted levels. Storage at 3^{o}C was found to virtually prevent the development of DFB in 2005, even with late harvested fruit (Figure 4.11). In contrast to RFB, the effect of increasing the storage temperature remained consistent in preventing the development of DFB in the Huon Valley irrespective of the seasonal incidence. This indicates DFB is less likely to be the result of a complex interaction between multiple factors as was found for RFB, but more likely to be the direct result of CI. It is important to note that storage at 3^{o}C was

found to reduce fruit quality and storage potential as discussed earlier. An alternative treatment that has been found to reduce the incidence of low temperature disorders, without significant loss of quality is the reduction of the relative humidity during storage (Brackmann et al., 1999; Brackmann and Ceretta, 1999; Fidler et al., 1973; Kays, 1991; Lidster et al., 1977). Specifically, reducing the relative humidity from 93% to 87% has been found to be associated with a decrease in the development of LTB of both 'Cox's Orange Pippin' and 'Bramley's Seedling' apples (Fidler et al., 1973). The decrease in relative humidity is thought to disrupt the metabolic processes related to the ratio of volatiles (Wills and McGlasson, 1969a; Wills and Scott, 1968). This hypothesis links the accumulation of acetic acid in the fruit with the development of LTB (Wills and McGlasson, 1969b) and may be worthwhile examining in future work on 'Cripps Pink' apples as a potential storage treatment for the reduction of the development of DFB.

In 2006 (a low DFB risk season), storage at 3^{o}C prevented the development of DFB (Figure 4.12). In contrast to RFB, storage at 1^{o}C and stepwise cooling (storage temperatures that were found to be successful for the prevention of RFB in a low risk season) had a significantly higher incidence of DFB than the 3^{o}C storage temperature (Figure 4.12). This indicates that fruit from the Huon Valley are more susceptible to injury in cool storage temperatures than fruit from Batlow. It is possible that climatic conditions influence the structure and stability of cell membranes. In a study on the influence of climate on the compositional characteristics of membranes of sugar beet roots, Lindberg et al. (2005) found that plants grown under cool temperatures had a higher concentration of lipids that acted to reduce the concentration of sterols in comparison to plants grown under warm conditions. The presence of sterols in plant cell membranes act as stabilisers of membrane fluidity over a range of temperatures (Leshem, 1992). Consequently, membranes with a low concentration of sterols bought about through cool climatic conditions during development are likely to be more rigid, less tolerant to cool storage temperatures and more susceptible to CI. Such variation in membrane composition as a result of climatic conditions may explain the contrast in tolerance to storage temperature between 'Cripps Pink' apples grown in Batlow and the Huon Valley. These effects also highlight the importance of climatic conditions during fruit growth and development on the development of RFB and DFB in 'Cripps Pink apples'.

4.7 Summary

- Radial flesh browning
  - ⇒ Occurs after 5 months of storage in a low risk season.
  - ⇒ Increases in incidence with late harvest.
  - ⇒ Increases in incidence with addition of 1% CO_2 in the storage atmosphere.
  - ⇒ Decreases in incidence to within commercial tolerance with storage at 1°C, 3°C or stepwise cooling in a low risk season.
  - ⇒ Is a senescent disorder induced by seasonal climatic conditions and aggravated by CA conditions and low temperature storage.

- Diffuse flesh browning
  - ⇒ Occurs after 5 months of storage in a low risk season.
  - ⇒ Increases in incidence with late harvest.
  - ⇒ Decreases in incidence with addition of 1% CO_2 in the storage atmosphere.
  - ⇒ Decreases in incidence to within commercial tolerance with storage at 3°C in a low risk season.
  - ⇒ Is a chilling injury induced by seasonal climatic conditions.

4.8 References

Alexander, L.and D. Grierson. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. Journal of Experimental Botany. 53: 2039-2055.

Aloia, R.C.and J.M. Boggs. 1985. Membrane Fluidity in Biology, Volume 4. Cellular Aspects, Academic Press, Orlando.

Alwan, T.F.and C.B. Watkins. 1999. Intermittent warming effects on superficial scald development of 'Cortland', 'Delicious' and 'Law Rome' apple fruit. Postharvest Biology and Technology. 16: 203-212.

Beaudry, R.M. 1999. Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. Postharvest Biology and Technology. 15: 293-303.

Blankenship, S.M., M. Parker, and C.R. Unrath. 1997. Use of maturity indices for predicting poststorage firmness of 'Fuji' apples. HortScience. 32: 909-910.

Brackmann, A., G. Bortoluzzi, and L. Bortoluz. 1999. Control of flesh breakdown in Fuji apple with dynamic O2 and CO2 concentrations and low relative humidity during controlled atmosphere storage. Ciencia Rural. 29: 459-463.

Brackmann, A.and M. Ceretta. 1999. Effect of ethylene levels and relative humidity on the storability of 'Gala' apple in a controlled atmosphere. Pesquisa Agropecuaria Brasileira. 34: 2169-2174.

Brown, G., L. Schimanski, and D. Jannings. 2003. Investigating internal browning of Tasmanian 'Pink Lady' apples. Acta Horticulturae. 628: 161-166.

Burmeister, D.M.and D.R. Dilley. 1995. A 'scald-like' controlled atmosphere storage disorder of Empire apples - a chilling injury induced by CO2. Postharvest Biology and Technology. 6: 1-7.

Clark, C.J.and D.M. Burmeister. 1999. Magnetic resonance imaging of browning development in 'Braeburn' apple during Controlled-Atmosphere storage under high CO_2. HortScience. 34: 915-919.

Cripps, J.E.L., L.A. Rishards, and A.M. Mairata. 1993. 'Pink Lady' apple. HortScience. 28: 1057.

De Castro, E., W.V. Biasi, and E. Mitcham. 2007. Quality of Pink Lady apples in relation to maturity at harvest, prestorage treatments and controlled atmosphere during storage. HortScience. 42: 605-610.

DeEll, J.R., S. Khanizadeh, and D.P. Murr. 2000. Preharvest and postharvest factors influencing apple fruit firmness. New England Fruit Meetings. 106: 43-46.

DeEll, J.R., D.P. Murr, R. Mueller, L. Wiley, and M.D. Porteous. 2005. Influence of 1-methylcyclopropene (1-MCP), diphenylamine (DPA), and CO_2 concentration during storage on 'Empire' apple quality. Postharvest Biology and Technology. 38: 1-8.

DeEll, J.R.and R.K. Prange. 1998. Disorders in 'Cortland' apple fruit are induced by storage at 0^{o}C in controlled atmosphere. HortScience. 33: 121-122.

East, A.R., K.M. Maguire, J. Jobling, D.J. Tanner, and A.J. Mawson. 2005. The effect of harvest date on incidence of Pink LadyTM apple postharvest diseases and disorders. Acta Horticulturae.

Elgar, H.J., D.M. Burmeister, and C.B. Watkins. 1998. Storage and handling effects on a CO_2-related internal browning disorder of 'Braeburn' apples. HortScience. 33: 719-722.

Elgar, H.J., C.B. Watkins, and N. Lallu. 1999. Harvest data and crop load effects on carbon dioxide-related storage injury of 'Braeburn' apple. HortScience. 34: 305-309.

Fahn, A. 1990. Plant Anatomy, 4th Edition. Pergamon Press, Sydney.

Fidler, J., B. Wilkinson, K. Edney, and R. Sharples. 1973. The biology of apple and pear storage. Commonwealth Agricultural Bureaux, England.

Grant, J., B. Mitcham, B. Biasi, and S. Chinchiolo. 1996. Late harvest, high CO_2 storage increase internal browning of Fuji apples. California Agriculture. 50: 26-29.

Hurndall, R.and J. Fourie. 2003. The South African Pink Lady^{TM} handbook. South African Pink Lady Association.

Jackson, J.E. 2003. Biology of apples and pears. Cambridge University Press, Melbourne.

James, H., G. Brown, E. Mitcham, D. Tanner, S. Tustin, I. Wilkinson, A. Zanella, and J. Jobling. 2005. Flesh browning in Pink LadyTM apples: research results have helped to change market specifications for blush colour which is an added bonus for growers. Acta Horticulturae. 687: 175-180.

Jobling, J., G. Brown, E. Mitcham, D. Tanner, S. Tustin, I. Wilkinson, and A. Zanella. 2004. Flesh browning of Pink Lady^{TM} apples: why do symptoms occur? Results from an international collaborative study. Acta Horticulturae. 682: 851-858.

Kays, S. 1991. Postharvest physiology of perishable plant products. Thomas Nelson Australia, Melbourne.

Kidd, F.and C. West. 1932. Effects of ethylene and of apple vapours on the ripening of fruits, p. 55-58. Reports of the Food Investigation Board, London.

Kidd, F.and C. West. 1936. Gas storage of fruit IV. Cox's Orange Pippin apples. J. Pomol. Hortic. Sci. 14: 276–294.

Knee, M.and M. Bubb. 1975. Storage of Bramleys Seedling apples .2. Effects of source of fruit, picking date and storage conditions on incidence of storage disorders. Journal of Horticultural Science. 50: 121-128.

Lau, O.L. 1998. Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. Postharvest Biology and Technology. 14: 131-141.

Lau, O.L., C.L. Barden, S.M. Blankenship, P.M. Chen, E.A. Curry, J.R. DeEll, L. Lehman-Salada, E.J. Mitcham, R.K. Prange, and C.B. Watkins. 1998. A North American cooperative survey of 'Starkrimson Delicious' apple responses to 0.7% O_2 storage on superficial scald and other disorders. Postharvest Biology and Technology. 13.

Lau, O.L.and W.D. Lane. 1998. Harvest indices, storability, and poststorage refrigeration requirement of 'Sunrise' apple. HortScience. 33: 302-304.

Leshem, Y.Y. 1992. Plant membranes: A Biophysical Approach to Structure, Development and Senescence. Kluwer Academic Publishers, Dordrecht.

Lidster, P.D., G.D. Blanpied, and E.C. Lougheed. 1985. Factors affecting the progressive development of low-oxygen injury in apples. Proceedings of the 4th National CA Research Conference: 57-69.

Lidster, P.D., S.W. Porritt, and G.W. Eaton. 1977. The effect of storage relative humidity on calcium uptake by 'Spartan' apple. Journal of the American Society for Horticultural Science. 102: 394-396.

Lindberg, S., A. Banas, and S. Stymne. 2005. Effects of different cultivation temperatures on plasma membrane ATPase activity and lipid composition of sugar beet roots. Plant Physiology and Biochemistry. 43.

Little, C.R. 1973. Flesh browning in CA stored Jonathans. Victorian Horticulture Digest. 17: 17-21.

Little, C.R.and R. Holmes. 2000. Storage technology for apples and pears. Department of Natural Resources and Economics, Victoria.

Little, C.R., I.D. Peggie, and H.J. Taylor. 1973. Effect of maturity, carbon dioxide and storage duration on controlled atmosphere stored Jonathan apples. Australian Journal of Experimental Agriculture and Animal Husbandry. 13: 593-599.

Lyons, J.M. 1973. Chilling injury in plants. Annu. Rev. Plant Physiol. 24: 455-466.

Magness, J.R.and H.C. Diehl. 1924. Physiological studies on apples in storage. Journal of Agricultural Research. 27: 1-38.

Marangoni, A.G., T. Palma, and D.W. Stanley. 1996. Membrane effects in postharvest physiology. Postharvest Biology and Technology. 7: 193-217.

Marangoni, A.G.and D.W. Stanley. 1989. Phase transitions in microsomal membranes from chilling sensitive and chilling resistant plants and fruit. Phytochemistry. 28: 2293-2301.

Meheriuk, M., R.K. Prange, P.D. Lidster, and S.W. Porritt. 1994. Agriculture Canada Publication 1737/E: Postharvest disorders of apples and pears. Minister of Supply and Services Canada.

Park, D.G.and S.K. Lee. 1991. Susceptibility of 'Fuji' apples to low-oxygen injury and high-carbon dioxide injury during CA storage. Journal of the Korean Society for Horticultural Science. 33: 38-43.

Picton, S., J. Gray, and D. Grierson. 1995. Ethylene genes and fruit ripening. In: P. Davis (ed.). Plant hormones physiology, biochemistry and molecular biology. Kluwer Academic Publishers, the Netherlands.

Plotto, A., A.N. Azarenko, J.P. Mattheis, and M.R. McDaniel. 1995. 'Gala,' 'Braeburn,' and 'Fuji' Apples: maturity indices and quality after storage. Fruit Varieties Journal. 49: 133-142.

Raven, P.H., R.F. Evert, and S.E. Eichhorn. 2005. Biology of plants, 7th Edition. W. H. Freeman and Company, New York.

Rocha, A.M.C.N.and A.M.M.B. Morais. 2001. Influence of controlled atmosphere storage on polyphenoloxidase activity in relation to colour changes of minimally processed 'Jonagored' apple. International Journal of Food Science and Technology. 36: 425-432.

Sisler, E.C.and M. Serek. 2003. Compounds interacting with the ethylene receptor in plants. Plant Biology. 5: 473-480.

Smock, R.M. 1977. Nomenclature for Internal Storage Disorders of Apples. HortScience. 12: 306-308.

Streif, J.and A.A. Saquet. 2003. Internal flesh browning of 'Elstar' apples as influenced by pre- and postharvest factors. Postharvest unlimited. p. 523-527.

Tian, S., A. Jiang, Y. Xu, and Y. Wang. 2004. Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage. Food Chemistry. 87: 43-49.

Toivonen, P.M.A., P.A. Wiersma, Y. Gong, and O.L. Lau. 2003. Levels of antioxidant enzymes and lipid soluble antioxidants are associated with susceptibility of internal browning in 'Braeburn' apples. Acta Horticulturae. 600: 57-61.

Volz, R.K., W.V. Biasi, J.A. Grant, and E.J. Mitcham. 1998. Prediction of controlled atmosphere-induced flesh browning in 'Fuji' apple. Postharvest Biology and Technology. 15: 97-107.

Watkins, C.B. 2003. Principles and Practices of Postharvest Handling and Stress, p. 585-615. In: D. Ferree and I. Warrington (eds.). Apples. Botany, Production and Uses. CABI Publishing.

Watkins, C.B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnology Advances. 24: 389-409.

Watkins, C.B., W.J. Bramlage, J.G. Brookfield, S.J. Reid, S.A. Weis, and T.F. Alwan. 2000. Cultivar and growing region influence efficacy of warming treatments for amelioration of superfical scald development on apples after storage. Postharvest Biology and Technology. 19: 33-45.

Watkins, C.B., E. Kupferman, and D.A. Rosenberger. 2002. Apple. Postharvest quality management guidelines. In: K.C. Gross, Y. Wang, and M. Saltveit (eds.). The commercial storage of fruits, vegetables and florist and nursery crops. USDA.

Wills, R., B. McGlasson, D. Graham, and D. Joyce. 1998. Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals, 4th edition. University of New South Wales Press Ltd, Sydney.

Wills, R.B.H.and W.B. McGlasson. 1969a. Association between loss of volatiles and reduced incidence of breakdown in Jonathan apples achieved by warming during storage. Journal of Food Science and Agriculture. 20: 446-447.

Wills, R.B.H.and W.B. McGlasson. 1969b. Effect of storage temperature on apple volatiles associated with low temperature breakdown. Journal of Horticultural Science. 46: 115-120.

Wills, R.B.H.and K.J. Scott. 1968. Relation between low-temperature breakdown and the volatiles given off by individual apples. Australian Journal of Biological Science. 21: 1307-1309.

Wunsche, J.N., J.W. Palmer, and D.H. Greer. 2000. Effects of crop load on fruiting and gas-exchange characteristics of 'Braeburn'/M.26 apple trees at full canopy. Journal of the American Society of Horticultural Science. 125: 93-99.

Xin-Jian, C.and F. Jiarui. 2000. Ethylene regulation of fruit ripening: molecular aspects. Plant Growth Regulation. 30: 193-200.

Zanella, A. 2003. Control of apple superficial scald and ripening - a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra low oxygen storage. Postharvest Biology and Technology. 27: 69-78.

Zanella, A. 2004. Results from the Agricultural Research Centre, Laimburg, Italy, p. 41-124. In: J. Jobling and H. James (eds.). Final report HAL AP02009: understanding the flesh browning disorder of Pink Lady™ apples.

5

The influence of climatic conditions during fruit growth on the development of radial and diffuse types of flesh browning of 'Cripps Pink' apples

5.1 Abstract

Variation in the expression of symptoms of flesh browning, observed during storage was found between different growing regions within Australia and around the world. Climatic conditions during fruit growth and development were hypothesised to cause the regional variation in the expression of symptoms. Radial flesh browning (RFB) was found in warm growing regions (accumulating between 1400 and 1700 GDD>10^{o}C for the entire season) whereas diffuse flesh browning (DFB) was found to occur in fruit that had been grown in cool growing districts (accumulating less than 1100 GDD>10^{o}C for the entire season). Fruit that were grown in a region accumulating 1100-1400 GDD>10^{o}C have not been classified as having a risk of RFB or DFB in this study as a lack of data from this climatic range prevented a definitive classification. Fruit that were grown in a hot region (accumulating above 1700 GDD>10^{o}C) did not develop any symptoms of FB during storage, in this research. The incidence of RFB and DFB was also found to vary significantly between different seasons within the same growing region. Cooler seasons within the climatic range that developed RFB had a higher incidence of RFB following storage than seasons at the high end of the RFB climatic range. Similarly, cool seasons in the climatic range that developed DFB were found to have a higher incidence of DFB than seasons at the warm end of the DFB climatic range. By analysing data from additional seasons, climatic conditions may provide a method for the prediction of the type and incidence of FB developing during storage.

5.2 Introduction

5.2.1 Apple growth and development

Apple growth occurs over a 2 year period. In Australia, flowering and bud initiation begin in September with fruit growth continuing through the summer with the harvest time of 'Cripps Pink' apples occurring in April. The trees are dormant from the end of April until the following spring when the cycle of growth and development repeats.

5.2.1.1 Bud induction, flowering and fruit set

Bud induction occurs in early summer and represents the change from vegetative to reproductive growth (Dennis, 2003). In most regions, the flower buds will become dormant by early autumn and will require winter chilling to initiate further growth the following year (Dennis, 2003). Bud dormancy is a mechanism for surviving cool winter temperatures without causing damage to the fragile buds (Jackson, 2003). Seasonal dormancy represents a complex physiological change which is not clearly understood, however as commercial apple production is increasingly spreading to more warm-temperate and even subtropical regions, the mechanism for breaking bud dormancy is of increasing economic importance (Jackson, 2003).

Flowering occurs during spring of the following year when the chilling requirement has been reached (Jackson, 2003; Little and Holmes, 2000). The chilling requirement is cultivar specific but is generally in the range of 800-1200 chill units (Palmer et al., 2003). The 'Cripps Pink' cultivar has been described as having a medium (Hurndall and Fourie, 2003) to low (Campbell, 2002) chilling requirement, indicating that it can be successfully grown in warm regions. The calculation of chill units can be based on several different models, however one of the most commonly used is the Utah model (Anderson and Seeley, 1993) which takes into account the hours in specific temperature ranges. When 60-80% of the flowers have opened, the tree is said to have reached the 'full bloom' developmental stage (Little and Holmes, 2000). Flowing of the 'Cripps Pink' cultivar generally occurs around the same time as flowering of 'Granny Smith', 'Gala', 'Fuji' and 'Red Delicious' cultivars (Campbell, 2002; Cripps et al., 1993). The timing and degree of the full bloom stage are complex relationships that can be influenced by shading, vigour, water relations, pruning, rootstock, cultivar, plant hormones and climatic conditions including maximum and minimum daily temperatures (Jackson, 2003). Cool temperatures can delay the onset of flowering whereas warm temperatures can accelerate flowering (Jackson, 2003). However, warm temperatures can also stimulate shoot growth and consequently reduce the degree of flowering (Jackson, 2003), indicating the complexity of the impact of climatic conditions.

The next stage is fruit set. Fruit set is the process that is required for the post anthesis growth of the fruit (Jackson, 2003). Fruit set is indicated by the growth of the ovary and surrounding receptacle tissue soon after fertilisation (Dennis, 2003). Typically, only 5-10% of the flowers develop into harvestable fruit, the rest fail to set

and are shed (Jackson, 2003). Cultivars differ considerably in fruit set; ‘Golden Delicious’ and ‘Fuji’ tend to set a higher proportion of flowers than do ‘McIntosh’ and ‘Delicious’ (Dennis, 2003). As a result, ‘Golden Delicious’ and ‘Fuji’ cultivars tend to be biennial bearers (Dennis, 2003). This means that unless the trees are managed correctly, the crop per tree will alternate between high and low yield from year to year.

5.2.1.2 Fruit development

5.2.1.2.1 Early fruit development

Fruitlet growth begins within 5-6 days of fertilisation. This developmental stage involves a period of cell division accompanied by an increase in the diameter of the fruit (Cutter, 1971). During the first 30-50 days after full bloom (DAFB), cell division is the primary cause of growth (Blanpied and Little, 1991; Cutter, 1971; Denne, 1963; Dennis, 2003; Little and Holmes, 2000). Following this point, increase in size is almost entirely the result of cell expansion and the expansion of intercellular spaces (Cutter, 1971; Dennis, 2003; Little and Holmes, 2000). As the process of cell enlargement progresses, the shape of the cortical cells changes from compact angular shape to a more spherical shape (Little and Holmes, 2000). As a result of the change in cell shape, the intercellular spaces enlarge and fill with gases reducing the density of the fruit (Little and Holmes, 2000). While cell expansion will be responsible for much of the final volume of the fruit, cell division is critical in determining the final size of the fruit (Dennis, 2003). Carpellary tissue stops growing approximately 6 weeks following bloom whereas the cortex tissue continues to expand until maturity is reached (Dennis, 2003). The growth resulting from cell division, cell enlargement and the increase in intercellular spaces typically results in an s-shaped curve with fruit weight over time (Little and Holmes, 2000).

5.2.1.2.2 Late fruit development and maturation

At the latter stage of development, the growth of apples is seen to plateau in some apple cultivars as the fruit changes from a predominately growth phase to the ripening phase (Little and Holmes, 2000). Apple cultivars differ considerably in the time of ripening; some cultivars (eg ‘Gravenstein’) will ripen within 110 DAFB while other cultivars (eg ‘Lady Williams’) require 225 days or more in order to reach maturity (Little and Holmes, 2000). The ‘Cripps Pink’ cultivar generally reaches maturity 200 DAFB (Cripps et al., 1993).

5.2.2 Climatic conditions influencing apple development

Many researchers have reported a seasonal variation in the incidence of storage disorders of apples (Clark and Burmeister, 1999; Elgar et al., 1999; Lau, 1998; Martin, 1954; Park, 1991; Sharples, 1975; Streif and Saquet, 2003; Volz et al., 1998; Watkins et al., 2004). Differences observed in the storage behaviour of apples between seasons and regions are often greater than variation observed between postharvest treatments. One of the factors hypothesised to cause this variation is the climactic conditions during fruit growth and development. Climatic conditions have been shown to influence fruit growth and development across the entire growing season as well as acting on specific periods of growth such as the early and late season stages of growth and maturity. This work focused on the first 50 DAFB as well as the last 60 days before harvest (DBH) and the entire season from full bloom to harvest as they had been reported as influencing the development of chilling injury (CI), senescent disorders and high CO_2 injury in other apple cultivars (Lau, 1998; Martin, 1954; Sharples, 1975).

5.2.2.1 First 50 days after full bloom

In apples, the first 50 DAFB are characterised by a period of cell division (Deene, 1960; Jackson, 2003; Little and Holmes, 2000). Climatic conditions that influence the periods of cell division and expansion can have an impact on the storability of the fruit. Warm temperatures early in the season can stimulate fruit growth and increase the size of the mature fruit (Martin, 1954; Warrington et al., 1999), but warm temperatures will decrease the period of cell division (Dennis, 2003), resulting in fruit with larger cells. Research has linked large cell size with a predisposition to several storage disorders and a shortened shelf life (Al-Hinai and Roper, 2004; Jackson, 2003; Little and Holmes, 2000). For example, low cell count and large cell size have been linked to low concentrations of minerals such as calcium which are implicated in the development of storage disorders (Chapter 3). Conversely, cool temperatures can extend the period of cell division resulting in fruit with a higher cell count (Jackson, 2003; Little and Holmes, 2000). Such fruit will also tend to have a higher density, predisposing them to high CO_2 and low O_2 injury during CA storage (Chapter 3).

5.2.2.2 Last 60 days before harvest

The last 60 DBH can influence the development of red blush on the skin of bi-coloured apple cultivars such as 'Cripps Pink' (Dennis, 2003; Jackson, 2003; Little and Holmes, 2000; Reay, 1998) as well at the timing and synchronisation of ripening and maturity (Chapter 2). Two of the most important climatic variables are solar radiation and the average, maximum and minimum temperatures (Dennis, 2003). Exposure to sunlight can increase the area and intensity of red colouration on the skin of the fruit and can also increase the sugar content (Dennis, 2003; Little and Holmes, 2000). Conversely, other research has shown that cool temperatures during spring and summer can delay maturation (Dennis, 2003; Martin, 1954). The maturation phase of apple development is thought to begin about half way through the process of fruit growth (Kays, 1991; Little and Holmes, 2000; Wills et al., 1998). A delay in maturation as a result of cool temperatures (Martin, 1954) can delay the processes of ripening and senescence and consequently exacerbate the development of senescent disorders during storage. The influence of fruit maturity on the development of senescent disorders is discussed in Chapter 4.

5.2.3 Climatic conditions influencing storage disorders of apples

Due to the interaction between multiple factors on the development of many storage disorders of apples, the accurate determination of the influence of climatic conditions is difficult. In order to establish the effect of the climatic conditions, other conditions such as maturity and nutrition ideally need to remain constant.

One of the earlier investigations into the impact of climatic conditions on the storage of apples was by West (1930) who examined the impact of climate on the development of low temperature breakdown (LTB) in 'Bramley's Seedling' apples. The report found that low rainfall and high temperatures during the four weeks prior to harvest were associated with a lower incidence of LTB (West, 1930). Another study by Sharples (1975) investigating LTB in 'Bramley's Seedling' apples found that a low incidence of the disorder was associated with warm temperatures during the 8 weeks prior to harvest. Low temperature breakdown in 'Cox's' apples was also found to be reduced with increasing temperatures during fruit growth (Sharples, 1975). Unlike 'Bramley's Seedling' however, it was found that temperatures during the entire growing season, rather than the last 4 or 8 weeks gave the best indication of the incidence of LTB developing during storage (Sharples, 1975). No relationship

between early season temperatures and the development of LTB of 'Cox's' apples was found (Sharples, 1975). Low temperature breakdown of apples is a CI (Smock, 1977), these climatic investigations indicate that the conditions during the entire season and the late season can influence the development of CI, however early season temperatures appear to have less of an impact on the development of CI of apples during storage.

Core browning is also a CI (Smock, 1977) and has similarly been found to be influenced by climatic conditions during fruit growth. Core browning was found to be more severe in apples harvested following a cool summer than those grown in warm conditions (Sharples, 1975). Other disorders of apples that also fit under the CI classification are soft scald and soggy breakdown (Watkins et al., 2004). These disorders are further examples of CI that have been found to vary in incidence between growing region and season (Watkins et al., 2004). These disorders have tended to be higher in incidence in fruit grown in New York, than in fruit grown in Michigan and Massachusetts which are warmer regions of the United States of America (Watkins et al., 2004). These studies indicate that climatic conditions can predispose apples to developing CI during storage.

Aside from the development of CI, climatic conditions can also influence the development of CO_2 injury. The 'Braeburn' browning disorder (BBD) is known to be stimulated by high levels of CO_2 in the storage atmosphere (Clark and Burmeister, 1999; Elgar et al., 1998; Lau, 1998) and has been examined in more detail in order to establish the link between climatic conditions and the development of this disorder during storage. Cool growing conditions during the first 50 DAFB extend the period of cell division (Jackson, 2003) and consequently increase the density and reduce the gas diffusivity of the fruit (Lau, 1998; Schotsmans et al., 2004). Research has found that BBD is more prevalent in fruit grown in cool and high altitude regions in New Zealand (Elgar et al., 1999; Lau, 1998). Lau (1998) suggested that the increased incidence of BBD in such regions was a result of the cool growing conditions altering cellular metabolism, reducing skin and tissue diffusivity and consequently increasing the fruits susceptibility to elevated CO_2 and reduced O_2 during CA storage.

As the incidence and expression of the RFB and DFB disorders of 'Cripps Pink' apples were found to vary considerably between regions and seasons, climatic conditions were examined as part of this research. While many studies have reported variation in the susceptibility to storage disorders based on climatic conditions during

fruit growth and development, few studies have clearly determined the influence that the climatic conditions have on fruit physiology and the resulting increased susceptibility to injury during storage.

5.3 Aims

- To determine the impacts of seasonal temperatures on the development of RFB, DFB and CO_2 injury of 'Cripps Pink' apples.
- To assess the relationship between cool temperatures during the first 50 DAFB and fruit density.
- To establish if climatic conditions during fruit growth and development could be used to predict the development of RFB, DFB and CO_2 injury of 'Cripps Pink' apples during storage.

5.4 Materials and methods

5.4.1 Cumulative growing degree days

Australian climate data was either measured using monitoring equipment assembled as part of this project and placed in orchards in each of the growing regions, or obtained from the Climate and Consultancy Section of the NSW Regional Office of the Bureau of Meteorology. New Zealand climate data was obtained from the National Institute of Water and Atmosphere Research Ltd. Californian climate data was measured using monitoring equipment within the orchard.

Air temperatures were collected as, at a minimum, hourly means in all locations. These were used to calculate the growing degree-days (GDD) above 10°C (Tanner et al., 2004) for each month. For each recorded temperature measurement, the following equation was used to estimate contribution to GDD.

$$GDD_x = t\,(\theta - x) \qquad (1)$$

where: GDD = growing degree days
t = time interval between measurements (days)
θ = temperature (°C)
x = base temperature (10°C)

Note that for situations where the temperature at any time is below the base the GDD are 0, there was no reduction in GDD.

5.4.2 Density

Following the method of Tustin (2004), two sections of the cortex of the fruit midway between the epidermis and the cortex were removed from each fruit, one from each side of the fruit. Each sample was impaled on a fine needle and weighed (W_0). The sample volume was measured by weight of its displacement of water. The weight of water displaced (W_1) was recorded when the sample was weighed fully submerged on the pin with the sample holder mounted on the weighing pan. Fruit density ($kg.m^{-3}$) was calculated using the following equation:

$$Density = \frac{W_0}{W_1} \quad (2)$$

5.4.3 Flesh browning determination

Flesh browning data for the climatic analysis was from the same sources as described in Chapter 4. Fruit were assessed for flesh browning at 3 locations: the stem end, the middle and the calyx end of transverse sections of the fruit. Flesh browning was assessed as the percentage of fruit with symptoms, regardless of severity. For flesh browning incidence to be comparable across seasons, all flesh browning data reported is the incidence of FB 'Cripps Pink' apples that were stored in air at 0^oC for 7 months.

5.4.4 Statistical analysis

For incidence of flesh browning and fruit density, an analysis of variance was performed and least significant differences (5%) calculated using the general analysis of variance procedure in GenStat statistical software (9^{th} edition, version 9.1.0.147, Lawes Agricultural Trust, supplied by VSN International Ltd). Flesh browning incidence were transformed to angles ($Y = \sin^{-1} \sqrt{\%/100}$) for analysis and back-transformed to % for presentation. A simple linear regression was used to quantify the relationship between cumulative growing degree days and the incidence of flesh browning (Genstat statistical software).

5.5 Results

5.5.1 Seasonal accumulation of growing degree days

In Batlow (New South Wales) and the Huon Valley (Tasmania), cumulative GGD>10^{o}C were assessed in the 2004, 2005 and 2006 seasons. In both districts there was considerable variation in the accumulation of GDD>10^{o}C between the three seasons, however the variation was greater in the Huon Valley than in Batlow.

In Batlow, all 3 seasons had similar temperatures for the first 50 DAFB (Figure 5.1). Following this time, the 2004 season accumulated a higher number of GDD>10^{o}C indicating a warmer season than 2005 or 2006. From 50 DAFB, the 2005 season remained the coolest of the three seasons, accumulating the lowest number of GDD>10^{o}C. Following 150 DAFB, the 2004 and 2006 seasons accumulated similar GDD>10^{o}C indicating similar climatic conditions during this period in these two seasons.

In the Huon Valley (Figure 5.2), a greater variation in the accumulation of GDD>10^{o}C was observed between the seasons than was observed in Batlow. From the time of full bloom when temperature recording began, the 2004 season accumulated the lowest number of GDD>10^{o}C indicating that it was the coolest of the three seasons. The 2004 season was noticeably cooler in the first 50 DAFB than the 2005 and 2006 seasons. The 2005 and 2006 seasons accumulated similar GDD>10^{o}C for the period, however the 2006 season had a higher accumulation of GDD>10^{o}C indicating it was the warmest of the three seasons.

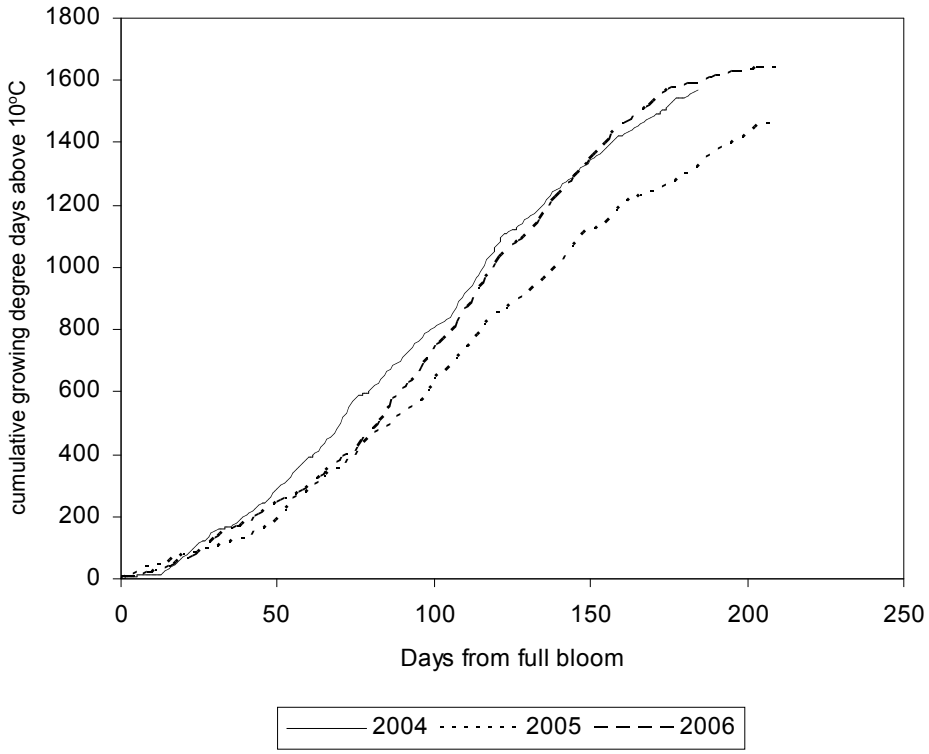

Figure 5.1 Cumulative growing degree days above 10°C from full bloom to optimal harvest in Batlow (New South Wales) in the 2004, 2005 and 2006 growing seasons.

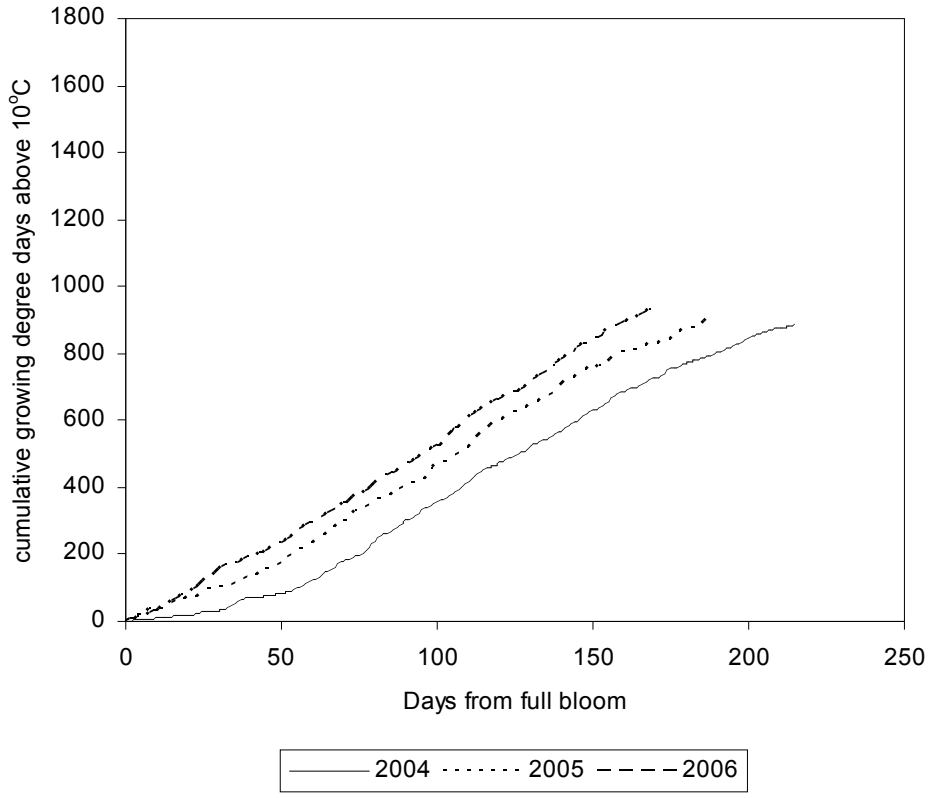

Figure 5.2 Cumulative growing degree days above 10°C from full bloom to optimal harvest in the Huon Valley (Tasmania) in the 2004, 2005 and 2006 growing seasons.

5.5.2 Relationship between GDD>10°C in the entire season (full bloom to harvest) and type of flesh browning

When the cumulative GDD>10°C for the entire season (from full bloom to harvest) from several growing districts in the 2004 season was compared to the type of flesh browning (RFB or DFB) that developed (Table 5.1) by Tanner et al. (2004) it was found that the climatic conditions determined the expression of symptoms. Cool growing districts, accumulating less than 1100 GDD>10°C, included the Huon Valley (Tasmania) and Nelson (New Zealand). These districts were found to exhibit symptoms of DFB only. Intermediate districts, accumulating between 1100 and 1400 GDD>10°C, included Hawke's Bay (New Zealand), the Yarra Valley (Victoria) and Manjimup (Western Australia). These districts were found to have only a low severity of symptoms and as such could not be definitively classified as being DFB or RFB through visual examination of the symptoms. Warm districts, accumulating between 1400 and 1700 GDD>10°C, included, Batlow (New South Wales) and the Goulburn Valley (Victoria). These districts were found to exhibit symptoms of RFB only. Hot districts, accumulating above 1700 GDD>10°C, were limited to a single district, California (United States of America). California did not develop symptoms of either DFB or RFB in this study (De Castro and Mitcham, 2004).

Table 5.1 Relationship between cumulative growing degree days (>10°C) between full bloom and optimal harvest date in the 2004 season and the type of flesh browning observed in the Huon Valley (Tasmania, Australia), Nelson (New Zealand), Hawkes Bay (New Zealand), Yarra Valley (Victoria, Australia), Manjimup (Western Australia, Australia), Batlow (New South Wales, Australia), Goulburn Valley (Victoria, Australia) and California (USA).

District	Cumulative growing degree days >10°C (2004 entire season)	Type of flesh browning
Huon Valley	888	Diffuse, CO_2 injury
Nelson	1030	Diffuse, CO_2 injury
Hawkes Bay	1102	Diffuse, Radial, CO_2 injury
Yarra Valley	1162	Diffuse, Radial, CO_2 injury
Manjimup	1405	Diffuse, Radial, CO_2 injury
Batlow	1567	Radial, CO_2 injury
Goulburn Valley	1668	Radial, CO_2 injury
California	1840	CO_2 injury

GDD and type of FB for non Australian regions (Nelson, Hawkes Bay and California) are adapted from (Tanner et al., 2004).

5.5.3 Relationship between specific climatic times, fruit density and the incidence of flesh browning

In Batlow (Table 5.2) and the Huon Valley (Table 5.3), the influence of the accumulation of GDD>10°C in the entire season (from full bloom to harvest), the

early season (first 50 DAFB) and the late season (last 60 DBH) were examined for their relationship to fruit density and the type and incidence of flesh browning that developed during storage. In Batlow (Table 5.2), the three seasons of data indicate that there was a trend of increasing fruit density with decreasing GDD>10^{o}C in the first 50 DAFB. The 2005 season had the coolest early season, accumulating 179 GDD>10^{o}C and this season also had the highest fruit density of the three seasons examined. The temperature in the last 60 DBH appeared to have no consistent relationship to the development of RFB. However, the entire season temperature had a close relationship to the incidence of RFB. The 2005 season had the coolest entire season, accumulating 1462 GDD>10^{o}C and had the highest incidence of fruit with RFB of the three seasons (57.5%). In contrast, the 2006 season was the warmest entire season, accumulating 1641 GDD>10^{o}C, and had the lowest incidence of fruit with RFB (10.0%).

In the Huon Valley (Tasmania), no clear relationship between temperatures during the first 50 DAFB and the density of the fruit at harvest was found (Table 5.3). The incidence of DFB in the Huon Valley was found to be associated with both the entire season and the early season accumulation of GDD>10^{o}C. The 2004 season had the coolest entire season (888 GDD>10^{o}C) and coolest first 50 DAFB (82 GDD>10^{o}C), this season had the highest incidence of fruit with DFB (95.4%). In contrast, the 2006 season had the warmest entire season (930 GDD>10^{o}C0 and warmest early season (230 GDD>10^{o}C), this season had the lowest incidence of fruit with DFB (8.1%).

Table 5.2 Relationship between cumulative growing degree days (>10^{o}C) for the entire season (full bloom to optimal harvest date), early season (full bloom to fifty days after full bloom) and late season (sixty days before optimal harvest to optimal harvest), fruit density and the incidence (%) of radial flesh browning (RFB) observed in Batlow (New South Wales)

	Cumulative growing degree days >10^{o}C			Fruit density	RFB
	Entire Season	Early Season	Late Season	(kg.m^{-3})	(%)
Batlow					
2004	1567	279	461	0.806	27.8
2005	1462	179	358	0.819	57.5
2006	1641	240	309	0.803	10.0
LSD				0.014	10.13
Significance				*	***

Incidence of radial flesh browning (RFB) percentage data were transformed to angles (Y = sin^{-1} √%/100) for analysis and back-transformed to % for presentation. Data is from optimally harvested, air stored at 0^{o}C for 7 months storage treatment only. Entire season is the period of time from full bloom to optimal harvest for long term storage, the early season is the first 50 days after full bloom and the late season is the last 60 days before harvest.
*, ***Significant at P<0.05 or 0.001 respectively

Table 5.3 Relationship between cumulative growing degree days (>10°C) for the entire season (full bloom to optimal harvest date), early season (full bloom to fifty days after full bloom) and late season (sixty days before optimal harvest to optimal harvest), fruit density and the incidence (%) of diffuse flesh browning (DFB) observed in the Huon Valley (Tasmania)

	Cumulative growing degree days >10°C			Fruit density	DFB
	Entire Season	Early Season	Late Season	($kg.m^{-3}$)	(%)
Huon Valley					
2004	888	82	232	0.830	95.4
2005	904	169	273	0.841	75.7
2006	930	230	242	0.840	8.1
LSD				0.037	9.98
Significance				ns	***

Incidence of diffuse flesh browning (DFB) percentage data were transformed to angles ($Y = \sin^{-1} \sqrt{\%}/100$) for analysis and back-transformed to % for presentation. Data is from optimally harvested, air stored at 0°C for 7 months storage treatment only. Entire season is the period of time from full bloom to optimal harvest for long term storage, the early season is the first 50 days after full bloom and the late season is the last 60 days before harvest.
***Significant at P<0.001

5.5.4 Relationship between GDD>10°C in the entire season (full bloom to harvest) and the incidence of flesh browning

As the accumulation of GDD>10°C for the entire season (from full bloom to harvest) had been found to have a close relationship to the incidence of fruit with RFB and DFB (following 7 months of air storage at 0°C), this relationship was examined by regression analysis, for RFB (Figure 5.3) and DFB (Figure 5.4).

For the development of RFB, data from optimally harvested 'Cripps Pink' apples were analysed separately from late harvested fruit due to the impact of harvest maturity on the development of RFB (Chapter 4). Optimally harvested fruit included RFB incidence data from the 2004, 2005 and 2006 seasons in Batlow (NSW) and from the 2004 season in California (United States of America), RFB incidence data from optimally harvested 'Cripps Pink' apples following 7 months of storage from the Goulburn Valley was not available and so was not included in the analysis. For optimally harvested fruit, a significant relationship (P<0.001) was found between the accumulation of GDD>10°C and the incidence of fruit with RFB, with an R^2 value of 0.997 for the variation in RFB observed in optimally harvested fruit from this data set (Figure 5.3).

For the analysis of late harvested fruit, RFB incidence data from the 2004, 2005 and 2006 seasons in Batlow (NSW) and from the 2004 season in the Goulburn Valley (Victoria) were included in the analysis. A similar significant relationship (P<0.05) was found between the accumulation of GDD>10°C and the incidence of fruit with RFB, with an R^2 value of 0.859 for the variation in RFB observed in late harvested

fruit from this data set (Figure 5.3). These analyses indicate that the accumulation of GDD>10^{o}C for the entire growing season has a significant impact on the incidence of RFB of 'Cripps Pink' apples.

For the development of DFB, a similar relationship between the accumulation of GDD>10^{o}C and the incidence of the disorder during storage was found (Figure 5.4). As only the Huon Valley (Tasmania) had DFB incidence data that could be compared across seasons using a regression analysis, data from optimally harvested (2004, 2005 and 2006 seasons) and late harvested (2004 and 2005 seasons) fruit were included in the regression. The relationship between the incidence of DFB and the accumulation of GDD>10^{o}C was significant ($P<0.05$), with an R^2 value of 0.952 for the variation in DFB observed in this data set (Figure 5.4). This analysis indicated that the accumulation of GDD>10^{o}C has a significant impact on the development of DFB of 'Cripps Pink' apples.

Interestingly, the incidence of DFB covered a much smaller range of GDD>10^{o}C than was found for RFB. The incidence of RFB varied from 0% at 1668 GDD>10^{o}C to 57.5% at 1462 GDD>10^{o}C (for optimally harvested fruit), covering a range of 206 GDD (Figure 5.3). However, the incidence of DFB varied from 8.1% at 930 GDD>10^{o}C to 95.4% at 888 GDD>10^{o}C (for optimally harvested fruit), covering a range of only 42 GDD>10^{o}C (Figure 5.4). This result suggests that the incidence of DFB has a higher sensitivity to the accumulation of GDD>10^{o}C than RFB does.

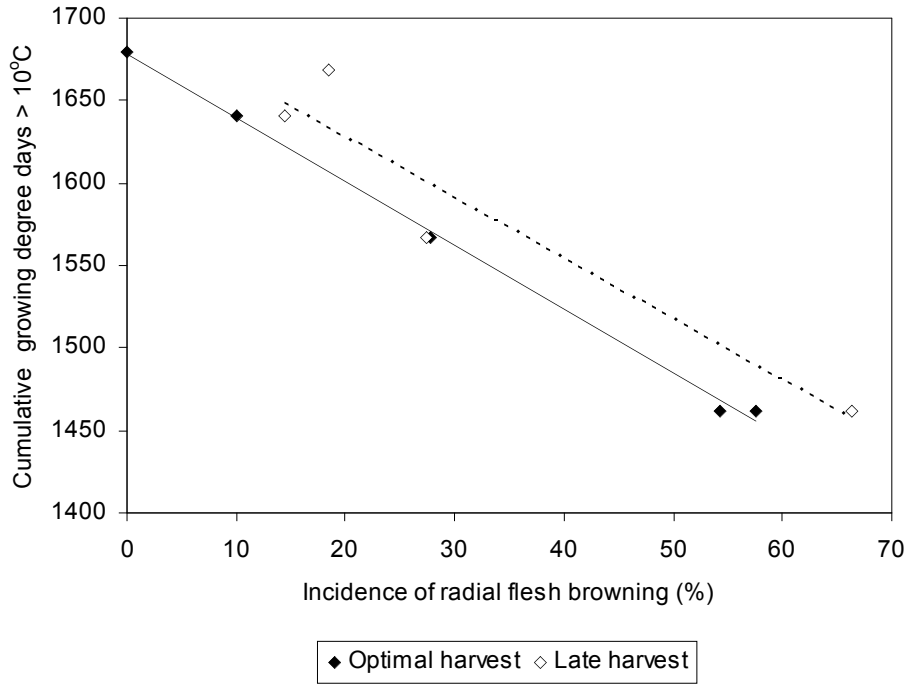

Figure 5.3 Relationship between cumulative growing degree days (>10°C) from full bloom to optimal harvest and the development of radial flesh browning (RFB) for optimal and late harvested 'Cripps Pink' apples. Optimal harvest includes data from Batlow (New South Wales) and California (USA), late harvest includes data from Batlow (New South Wales) and the Goulburn Valley (Victoria). Percentage incidence of RFB is from the air stored at 0°C for 7 months treatment only. Optimal Harvest R^2 =0.997, P<0.001. Late Harvest R^2=0.859, P<0.05. Californian climatic and incidence of RFB data adapted from (Tanner et al., 2004).

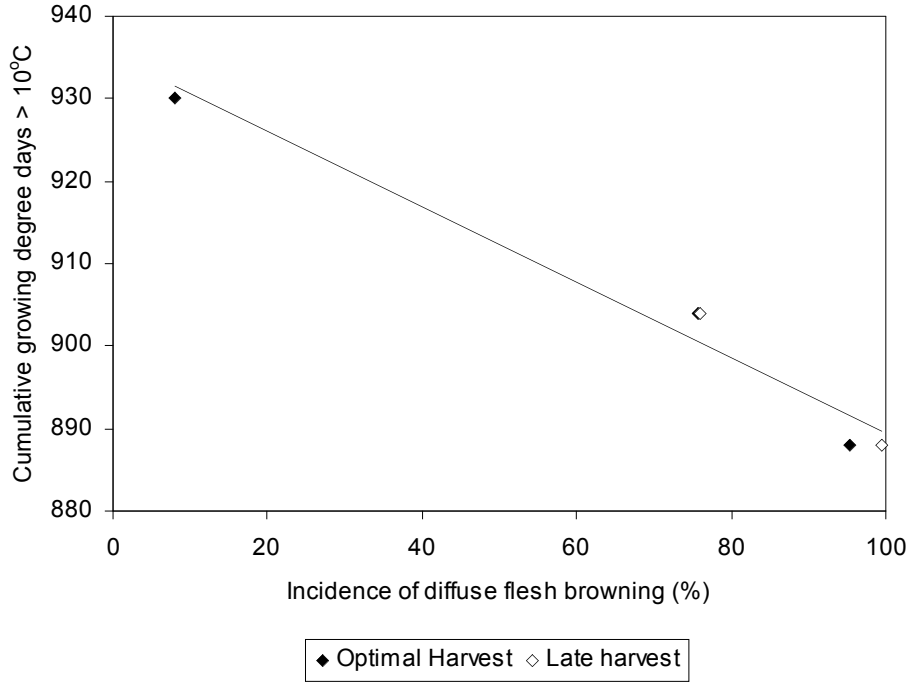

Figure 5.4 Relationship between cumulative growing degree days (>10°C) from full bloom to optimal harvest and the development of diffuse flesh browning of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in the 2004, 2005 and 2006 seasons. Data from Optimal and late harvest were combined for regression, R^2=0.952, P<0.05.

5.6 Discussion

The accumulation of GDD>10^{o}C during the entire season was found to determine the type of FB that developed during storage. The base temperature of 10^{o}C has been commonly used for the analysis of climatic data and how it relates to horticultural produce (Sharples, 1975; Tanner et al., 2004), however this temperature may be adapted for particular plant species or experimental procedures. In cool seasons or regions, accumulating less than 1100 GDD>10^{o}C, it is the DFB symptoms that consistently develop during storage. However, in warm seasons or regions, accumulating between 1400 and 1700 GDD>10^{o}C during the entire season, RFB was found to consistently develop during storage. In seasons or regions that had an intermediate accumulation of GDD>10^{o}C between 1100 and 1400 the expression of FB was inconsistent. Hot regions or seasons, accumulating greater then 1700 GDD>10^{o}C during the entire season did not develop any symptoms of FB during storage in this study. This indicates that regions and seasons can be classified based on climatic conditions during the entire season as being at risk of DFB, RFB or having a very low risk of developing FB during storage. As well as determining the type of FB that developed during storage, climatic conditions were also found to impact on the incidence of RFB and DFB occurring during storage in a given season.

5.6.1 Radial flesh browning

As discussed in Chapter 4, RFB is a senescent disorder that is aggravated by cool storage temperatures and CA storage conditions. Based on work by other researchers on similar disorders (DeEll, 2005; Lau, 1998; Sharples, 1975; Volz et al., 1998; Volz et al., 1999), RFB was hypothesised to be influenced by climatic conditions in the early and late season.

Cool temperatures during the first 50 DAFB can increase the period of cell division (Deene, 1960; Jackson, 2003), increasing the fruit density (Lau, 1998; Little and Holmes, 2000) and reducing gas diffusivity (Schotsmans et al., 2004), increasing the internal concentration of CO_2 (Lau, 1998). Consequently it seems likely that cool temperatures during the first 50 DAFB could increase the risk of developing RFB during storage. However, the relationship between the accumulation of GDD>10^{o}C during the first 50 DAFB was not found to be consistently related to increased fruit density at harvest (Table 5.2) in Batlow (New South Wales). However, a trend of increasing fruit density at harvest and the incidence of RFB was found (Table 5.2),

indicating that this climatic period may influence the development of RFB. More data is required in order to understand the impact that seasonal temperatures at this time have on fruit physiology and its susceptibility to RFB.

Climatic conditions during the late season have been found to influence fruit ripening and maturity (Blanpied and Little, 1991; Little and Holmes, 2000; Tromp, 1997), as well as the development of CI during long term storage (DeEll, 2005). As RFB is thought to be primarily a senescent disorder, increasing in incidence with increasing maturity at harvest and being exacerbated by low temperature storage (Chapter 4), it seemed likely that late season climatic conditions could influence the development of RFB. However, following three seasons of analysis, no clear relationship between the accumulation of GDD>10^{o}C during the last 60 DBH and the incidence of RFB during storage was found (Table 5.2).

Diurnal temperature variation in the 60 DBH have also been linked to apple maturity and colour development in other apple cultivars (Little and Holmes, 2000). As the 'Cripps Pink' apple is required by Australian supermarkets to have 45-50% red blush on the skin of the fruit (James et al., 2005), inadequate diurnal temperature variation during the last 60 DBH may delay the development of red blush and consequently postpone harvest, resulting in over mature fruit being placed in long term storage. An analysis of the diurnal temperature variation was completed by Tanner et al. (2004) who found no consistent relationship between diurnal temperature variation during the last 60 DBH and the development of red blush or on the incidence of RFB during storage.

While climatic conditions during the early and late season were found to have inconsistent effects on the risk of developing RFB during storage, the accumulation of GDD>10^{o}C during the entire growing season was found to be closely related to the incidence of RFB (Table 5.2 and Figure 5.3). Using only the accumulation of GDD>10^{o}C for the entire season, 99.76% of the variation in the incidence of RFB of optimally harvested fruit and 85.9% of the variation in late harvested fruit was accounted for (Figure 5.3). This analysis was restricted to the incidence of RFB of air stored fruit at 0^{o}C for a period of 7 months as this was the only storage treatment that could be compared across the three seasons. The entire season climate has also been found to be successful for determining the risk of developing CI in 'Cox's' apples (Sharples, 1975) as well as for determining the risk of high CO_2 injury in 'Braeburn' apples (Lau, 1998).

As discussed in Chapter 4, RFB is thought to be primarily the result of senescence, however the disorder is aggravated by the development of CI and high CO_2 injury. Consequently, climatic conditions that increase the susceptibility of fruit to CI or high CO_2 injury may also impact on the incidence of RFB observed during storage. Cool temperatures during growth and development have been shown to influence the structure and function of cell membranes (Lindberg et al., 2005), increasing the fruit's susceptibility to developing CI during storage (Chapter 4). As RFB has been shown to be significantly reduced by increasing the storage temperature from 0°C to 3°C (Chapter 4, Figures 4.9 and 4.10), it is likely that CI is a secondary cause of RFB. During the development of CI as well as during senescence, there is a progressive loss of membrane integrity resulting in a loss of membrane fluidity (Kays, 1991; Marangoni et al., 1996). Consequently, it is likely that cells of senescing fruit are more prone to CI, due to the additive impacts of senescence and CI on membrane fluidity.

With further seasons of analysis a more complex model could be developed for RFB. It is likely that the early and late season temperatures as well as conditions in the previous season when the flower buds are initiated could partially contribute to the risk of developing RFB during storage. However the current analysis, using only the entire season GDD>10°C, has a significant relationship to the development of RFB during storage and any additional factor added into this model may provide only a minor improvement. Although, if the model were developed further to include data from a range of districts then these factors may prove to be significant.

5.6.2 Diffuse flesh browning

As discussed in Chapter 4, DFB is a CI with high a high variation in incidence between seasons. Work by other researchers has indicated that the late season (DeEll, 2005) as well as the entire season (Martin, 1954; Sharples, 1975) could be climatic times that contribute towards the risk of developing CI, and consequently DFB during storage.

Examination of the accumulation of GDD>10°C during the last 60 DBH (Table 5.2) found no clear or consistent relationship with the incidence of DFB during storage. Similarly, analysis of the diurnal temperature variation during the last 60 DBH was not found to be consistently related to the development of red blush or to the

incidence of DFB (Tanner et al., 2004). It is likely however that as the current analysis only uses three complete seasons of temperature and DFB incidence data, that only the most direct relationships will be observed. The determination of the impact of late season climatic conditions on the development of DFB will require further seasons of research before a significant relationship can be established.

The accumulation of GDD>10^{o}C during the early season was not hypothesised to influence the development of DFB. Cool temperatures during the first 50 DAFB are typically involved in the development of disorders that occur as a result of the storage atmosphere rather than the storage temperature (Lau, 1998; Sharples, 1975). However, the early season accumulation of GDD>10^{o}C was found to be related to the incidence of DFB during storage (Table 5.2). This may have been due to the fact that the 3 seasons analysed in the Huon Valley were consistently different at each of the climatic times (Figure 5.2), consequently any relationship between specific climatic times and the development of DFB will be generalised for each climatic time within the same season. The low accumulation of GDD>10^{o}C was not consistently related to fruit density at harvest and the DFB disorder is not associated with increased CO_2 or reduced O_2 during storage (Chapter 4). However, cool climatic conditions during the first 50 DAFB have been shown to influence fruit ripening (Chapter 2). It is possible that the cool growing conditions experienced during the first 50 DAFB in the Huon Valley are responsible for the de-synchronisation of the ripening processes and possibly increasing the susceptibility of the fruit to injury during storage (Chapter 2).

Similarly as was found for RFB, the accumulation of GDD>10^{o}C showed a linear relationship with the incidence of DFB during storage (Figure 5.4). The entire season accumulation of GDD>10^{o}C was found to be the best predictor of the development of DFB during storage. Cool temperatures during growth have been shown to alter the lipid ratio in plant cell membranes as an adaptive change to withstand the cool growing conditions (Lindberg et al., 2005). However, the alteration to the lipid ratio was shown to decrease the concentration of sterols in the membrane (Lindberg et al., 2005), increasing the rigidity and reducing the fluidity of the membrane (Kays, 1991; Lindberg et al., 2005). As reducing the fluidity of the cell membrane increases the susceptibility of phase changes and lipid bi-layer separations occurring during storage (Kays, 1991; Marangoni et al., 1996), this increases the susceptibility to CI during storage. As a result, it is likely that cool temperatures during growth can increase the susceptibility of the fruit to CI during storage.

5.6.3 Climatic data for the prediction of developing flesh browning during storage

The type of FB that occurs during storage is determined by the accumulation of GDD>10^{o}C during the entire season. Districts can be categorised as having a risk of developing DFB (<1100 GDD>10^{o}C) or RFB (1400-1700 GDD>10^{o}C). In this study, not enough regions in the climatic range of 1100-1400 GDD>10^{o}C were analysed, consequently 'Cripps Pink' apples grown in this climatic range have not been definitively classified. This relationship between the climatic conditions and the expression of FB during storage could be used to predict the type of FB that will develop in future seasons.

As the storage conditions that prevent or control FB differ between RFB and DFB (Chapter 4), this is a valuable distinction to be made prior to placing fruit into long term storage. By knowing the type of FB that will occur during storage, decisions regarding the determination of optimal long term storage conditions can be made. Once the type of FB has been established, based on the entire season accumulation of GDD>10^{o}C, RFB and DFB then have different climatic risks that need to be assessed for predicting the incidence of the disorder in individual seasons.

For RFB, only the accumulation of GDD>10^{o}C for the entire season has presently been found to be related to the incidence of the disorder during storage. The incidence of RFB increases linearly with decreasing accumulation of GDD>10^{o}C for the entire season (Figure 5.3). Both the first 50 DAFB and the last 60 DBH have not been found to significantly or consistently relate to the incidence of RFB during storage.

For DFB, the accumulation of GDD>10^{o}C for the entire season and the early season were found to be related to the incidence of the disorder during storage. The incidence of DFB increased linearly with decreasing GDD>10^{o}C in both climatic periods. It is likely that the accumulation of GDD>10^{o}C during the entire season is the primary indicator of the incidence of DFB during storage, with the early season being an additive influence.

The climatic conditions during fruit growth and development can alter fruit biophysical properties, resulting in increased susceptibilities to injury during storage. Climatic conditions have been shown to have a close relationship to the development of FB

and to the incidence of both the RFB and DFB disorders during storage. With only three complete seasons of data it is premature to produce a model for the accurate prediction of RFB and DFB of 'Cripps Pink' apples during storage based on climatic conditions during growth. However, with further seasons of analysis, which will continue over subsequent seasons, such a model may be developed in the future. It is likely that the accumulation of GDD>10^{o}C for the entire season will be the primary indicator of the type of FB and the incidence of FB during storage. However, further seasons of analysis will be required to confirm this hypothesis. With future research it may also be found that adaptation of the calculation used to determine the accumulation of GDD could be modified to more accurately reflect the prediction of the development of the RFB and DFB disorders of 'Cripps Pink' apples developing during storage. With a deeper understanding of the physiological mechanism responsible for the link between the accumulation of GDD and the development of FB, a more accurate base temperature may be established. The impacts of the other climatic times as well as the diurnal temperature variation could also be taken into consideration in future predictive models.

5.7 Summary

- Radial flesh browning
  - ⇒ Occurs in regions or seasons accumulating greater than 1400 GDD>10^{o}C for the entire growing season from full bloom to harvest.
  - ⇒ Increases in incidence linearly with decreasing GDD>10^{o}C for the entire growing season from full bloom to harvest.

- Diffuse flesh browning
  - ⇒ Occurs in regions or seasons accumulating less than 1100 GDD>10^{o}C for the entire growing season from full bloom to harvest.
  - ⇒ Increases in incidence linearly with decreasing GDD>10^{o}C for the entire growing season from full bloom to harvest.

- CO_2 injury of 'Cripps Pink' apples was found to occur in fruit from any growing region when stored in CA storage. No consistent relationship between cool temperatures in the first 50 DAFB and the density of the fruit was found.

- Regions accumulating greater than 1700 GDD>10^{o}C during the entire season had a low risk of developing RFB

- Not enough data for regions accumulating between 1100-1400 GDD>10^{o}C was collected in this study and consequently the type of FB in these regions cannot be accurately classified.

5.8 References

Al-Hinai, Y.K.and T.R. Roper. 2004. Rootstock effects on growth, cell number, and cell size of 'Gala' apples. Journal of the American Society of Horticultural Science. 129: 37-41.

Anderson, J.L.and S.D. Seeley. 1993. Bloom delay in deciduous fruits. Horticultural Reviews. 15: 97-144.

Blanpied, G.D.and C.R. Little. 1991. Relationship among bloom dates, ethylene climacteric initiation dates, and maturity-related storage disorders of Jonathan apples grown in Australia. Postharvest Biology and Technology. 1: 3-10.

Campbell, J. 2002. Apple Varieties. New South Wales Department of Agriculture. Agfact: H4.1.12.

Clark, C.J.and D.M. Burmeister. 1999. Magnetic resonance imaging of browning development in 'Braeburn' apple during Controlled-Atmosphere storage under high CO_2. HortScience. 34: 915-919.

Cripps, J.E.L., L.A. Rishards, and A.M. Mairata. 1993. 'Pink Lady' apple. HortScience. 28: 1057.

Cutter, E.G. 1971. Part 2 Organs, p. 343. In: E.J.W. Barrington and A.J. Willis (eds.). Plant Anatomy: Experiment and Interpretation. Edward Arnold Publishers Ltd., London.

De Castro, E.and B. Mitcham. 2004. Controlled atmosphere induced internal browning in Californian Pink Lady^{TM} apples, p. 26-32. In: J. Jobling and H. James (eds.). Final Report HAL AP02009: Understanding the Flesh Browning disorder of Pink Lady^{TM} apples.

DeEll, J.R. 2005. Low risk of low-temperature storage disorders for 2005/06. Orchard Network. 9: 11-12.

Deene, M.P. 1960. The growth of apple fruitlets and the effect of early thinning on fruit development. Annals of Botany. 24: 397-406.

Denne, M. 1963. Fruit development and some tree factors affecting it. New Zealand Journal of Botany. 1: 265-294.

Dennis, F. 2003. Flowering, pollination and fruit set and development, p. 153-166. In: D. Ferree and I. Warrington (eds.). Apples: Botany, Production and Uses. CABI Publishing, UK, USA.

Elgar, H.J., D.M. Burmeister, and C.B. Watkins. 1998. Storage and handling effects on a CO_2-related internal browning disorder of 'Braeburn' apples. HortScience. 33: 719-722.

Elgar, H.J., C.B. Watkins, and N. Lallu. 1999. Harvest data and crop load effects on carbon dioxide-related storage injury of 'Braeburn' apple. HortScience. 34: 305-309.

Hurndall, R.and J. Fourie. 2003. The South African Pink Lady™ handbook. South African Pink Lady Association.

Jackson, J.E. 2003. Biology of apples and pears. Cambridge University Press, Melbourne.

James, H., G. Brown, E. Mitcham, D. Tanner, S. Tustin, I. Wilkinson, A. Zanella, and J. Jobling. 2005. Flesh browning in Pink Lady™ apples: research results have helped to change market specifications for blush colour which is an added bonus for growers. Acta Horticulturae. 687: 175-180.

Kays, S. 1991. Postharvest physiology of perishable plant products. Thomas Nelson Australia, Melbourne.

Lau, O.L. 1998. Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. Postharvest Biology and Technology. 14: 131-141.

Lindberg, S., A. Banas, and S. Stymne. 2005. Effects of different cultivation temperatures on plasma membrane ATPase activity and lipid composition of sugar beet roots. Plant Physiology and Biochemistry. 43.

Little, C.R.and R. Holmes. 2000. Storage technology for apples and pears. Department of Natural Resources and Economics, Victoria.

Marangoni, A.G., T. Palma, and D.W. Stanley. 1996. Membrane effects in postharvest physiology. Postharvest Biology and Technology. 7: 193-217.

Martin, D. 1954. Variation between apple fruits and its relation to keeping quality 3. between-season variation in relation to seasonal climate. Australian Journal of Agricultural Research. 5: 392-421.

Palmer, J.W., J.P. Prive, and D.S. Tustin. 2003. Temperature, p. 217-236. In: D. Ferree and I. Warrington (eds.). Apples. Botany, production and uses. CABI Publishing.

Park, Y.M. 1991. Seasonal changes in resistance to gas diffusion of 'McIntosh' apples in relation to development on lenticel structure. Journal of the Korean Society for Horticultural Science. 32: 329-334.

Reay, P.F. 1998. The effects of maturity on colour and concentration of pigments in the blush and shaded sides of 'Gala' apple fruit during cool storage. Journal of Horticultural Science and Biotechnology. 73: 841-845.

Schotsmans, W., B. Verlinden, J. Lammertyn, and B.M. Nicolai. 2004. The relationship between gas transport properties and the histology of apple. Journal of the Science of Food and Agriculture. 84: 1131-1140.

Sharples, R. 1975. Influence of climate on the storage quality of pome fruits. In: H.C. Pereira (ed.). Climate and the Orchard. Commonwealth Agricultural Bureaux, England.

Smock, R.M. 1977. Nomenclature for internal storage disorders of apples. HortScience. 12: 306-308.

Streif, J.and A.A. Saquet. 2003. Internal flesh browning of 'Elstar' apples as influenced by pre- and postharvest factors. Postharvest unlimited. p. 523-527.

Tanner, D., D.S. Tustin, J. Jobling, K. Phan-Thein, I. Wilkinson, G. Brown, B. Mitcham, and A. Zanella. 2004. Preharvest temperature as a predictor for Pink Lady™ flesh browning during storage, p. 125-158. In: J. Jobling and H. James (eds.). Final Report HAL AP02009: Understanding the flesh browning disorder of Pink Lady™ apples.

Tromp, J. 1997. Maturity of apple cv. Elstar as affected by temperature during a six-week period following bloom. Journal of Horticultural Science. 72: 811-819.

Tustin, D.S. 2004. The seasonal development and biophysical structure of Pink Lady™ apple fruit grown in Australia, New Zealand, USA and Italy, and the association to susceptibility to flesh browning, p. 36-40. In: J. Jobling and H. James (eds.). Final report HAL AP02009: Understanding the flesh browning disorder of Pink Lady™ apples.

Volz, R.K., W.V. Biasi, J.A. Grant, and E.J. Mitcham. 1998. Prediction of controlled atmosphere-induced flesh browning in 'Fuji' apple. Postharvest Biology and Technology. 15: 97-107.

Volz, R.K., M. Oliver, J. Stanley, C. Morgan, and H.J. Elgar. 1999. BBD Prediction. HortResearch report to ENZA (NZ) International. Report No 2000/147. 21 pages.

Warrington, I., T.A. Fulton, E.A. Halligan, and H.N. de Silva. 1999. Apple fruit growth and maturity are affected by early season temperatures. Journal of the American Society of Horticultural Science. 124: 468-477.

Watkins, C.B., J.F. Nock, S.A. Weis, S. Jayanty, and R. Beaudry. 2004. Storage temperature, diphenylamine, and pre-storage delay effects on soft scale, soggy breakdown and bitter pit of 'Honeycrisp' apples. Postharvest Biology and Technology. 32: 213-221.

West, C. 1930. Effect of climatic conditions upon the storage life of apples. Rep. Food Invest. Bd 1929: 52-54.

Wills, R., B. McGlasson, D. Graham, and D. Joyce. 1998. Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals. University of New South Wales Press Ltd, Sydney.

6

General Discussion and Conclusions

During the long term storage of apples, postharvest losses that result from the development of physiological storage disorders are often characterised by browning of the flesh. The specific pattern and location of the browning are often indicative of the cause of the disorder, but are generally cultivar specific.

As discussed in Chapter 3, browning is the result of oxidation. In order for the process of oxidation to occur, the cell must have ruptured in some way so as to have exposed its contents to O_2. This suggests that the basic physiology behind each description of browning of the flesh of apples has the same basic mechanism. The difference between the disorders is the process that resulted in the degradation of the cell.

While the storage conditions such as the temperature and composition of the atmosphere can interact to produce symptoms. It would appear that the primary processes resulting in cell degradation and the development of flesh browning in apples could fit into three categories:

1. Chilling injury induced membrane damage
2. High CO_2 or low O_2 induced metabolic degradation
3. Senescent related loss of membrane integrity

Any review of the literature surrounding storage disorders of apples is likely to encounter several inconsistencies surrounding their classification. With a body of literature that encompasses close to 100 years of research it is not surprising that such inconsistencies have developed. However, if the mechanism behind the cell degradation is identified, the conditions that resulted in the damage could be more accurately established, reducing the likelihood of conflicting definitions of storage disorders.

For example, chilling injury (CI) of apples has been subdivided into core browning, core flush, low temperature breakdown and internal breakdown. The mechanism of

all of these disorders is thought to be membrane damage resulting from storage at a temperature below the temperature threshold for membrane stability. The recommended primary control for each of these disorders is increasing the storage temperature to above the threshold level for damage. It is important to note that the threshold level for the development of symptoms is likely to be cultivar specific and may relate to cultivar traits in membrane composition.

Consequently, variation in the storage recommendations for the prevention of such disorders may be the result of cultivar characteristics and sensitivities rather than the subdivisions of CI. The names core browning, core flush, low temperature breakdown and internal breakdown are vaguely descriptive of the visual expression of symptoms. However (with the exception of low temperature breakdown) they don't give an indication of the physiological cause of the disorder. It might be more appropriate therefore to name these disorders as cultivar specific CI. For example, core flush of 'Granny Smith' would be renamed CI of 'Granny Smith' and internal browning of 'Delicious' renamed to CI of 'Delicious'.

This re-classification of internal storage disorders of apples could create more consistency in the identification of new disorders and would reduce the confusion that exists in the present naming system. However, this would require retrospectively re-classifying all the internal storage disorders of apples that are currently described and may present a difficulty for disorders that have yet to have their cause established. The proposed classification may also make it difficult to definitively classify disorders that are the result of the interaction of these three physiological causes and that don't have a clearly identifiable primary cause.

An example of how inconsistent and inappropriate the naming of disorders can be is the flesh browning disorder of 'Cripps Pink' apples. In preliminary research, variation in the expression of symptoms was observed. The types of flesh browning were named 'radial' and 'diffuse' based on their visual appearance rather than named based on their physiology which, at the point of naming had not been established. These names have been retained and used for describing the expression of symptoms in all subsequent work. However, the names radial and diffuse give no indication of the cause or the control of the two disorders.

One of the objectives of this work was to determine the causes of radial flesh browning (RFB) and diffuse flesh browning (DFB) and to classify each disorder

based on physiological factors and on storage behaviour. From a combination of microscopic examination (Chapter 3), storage experiments (Chapter 4) and climatic analysis (Chapter 5), RFB and DFB were found to be two distinct disorders occurring in contrasting climatic regions with distinct physiology and different storage recommendations.

The predisposition to 'Cripps Pink' apples developing DFB or RFB during storage was found to be determined by the accumulation of growing degree days (GDD) >10°C for the entire growing season (Chapter 5). In districts or seasons accumulating <1100 GDD>10°C there was a risk of developing DFB (Figure 6.1). On the other hand, fruit grown in districts or seasons accumulating >1400 GDD>10°C had a risk of developing RFB (Figure 6.1). For the intermediate climatic range (1100-1400 GDD>10°C) there is presently not enough data to indicate the type of FB that would be present. The accumulation of GDD>10°C was also found to be a major determinate of the incidence of both DFB (Chapter 5, Figure 5.4) and RFB (Chapter 5, Figure 5.3) developing during storage.

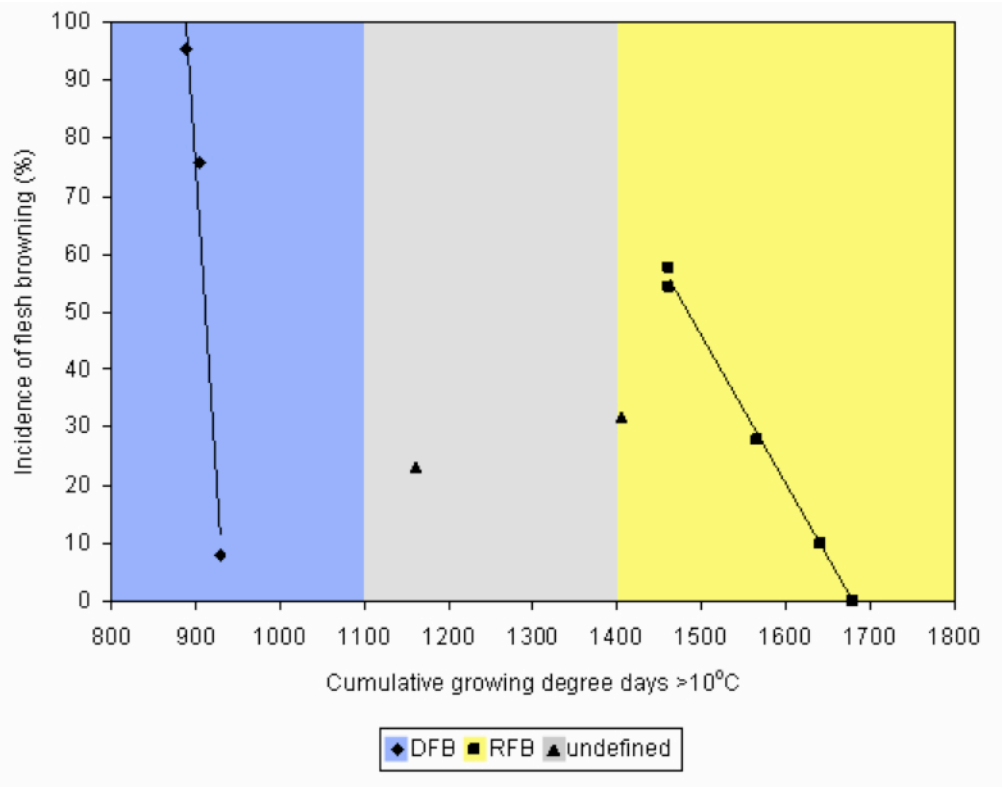

Figure 6.1 Incidence of DFB and RFB plotted against the cumulative growing degree days (GDD) >10°C for the entire growing season (from full bloom to optimal harvest for long term storage). Flesh browning incidence data is from optimally harvested 'Cripps Pink' apples stored at 0°C in air for 7 months.

Climatic conditions during fruit growth and development were also found to influence the period of greenlife, with cooler seasons and districts showing an extended period

of greenlife than those grown in warm seasons and districts (Chapter 2). It is hypothesised that the climatic conditions during this early period of fruit development are responsible for the synchronisation of the ripening processes within the fruit that occur proceeding harvest. It is also hypothesised that the de-synchronisation of the ethylene dependent and independent ripening processes may increase the susceptibility of the fruit to developing RFB and DFB during storage.

Diffuse flesh browning is characterised by browning of the cortex tissue of the fruit resulting from extensive cell collapse (Chapter 3, figure 3.5). Increasing the storage temperature from 0^{o}C to 3^{o}C was the only storage treatment found to have a considerable effect on reducing the incidence of DFB to within commercial threshold levels (Chapter 4). These results indicate that DFB is the direct result of CI. This indicates that DFB would be classified as a CI and therefore a more appropriate name would be CI of 'Cripps Pink' apples.

The pre and postharvest risk factors that result in the development of DFB during storage are shown in Figure 6.2. The primary risk factor in the development of DFB during storage is the accumulation of GDD>10^{o}C during the entire season. If the seasonal accumulation of GDD>10^{o}C was greater than 950 then the risk is classified as low and there will be no significant impact of any additive factors. However in a season accumulating less than 950 there is a moderate risk of developing DFB and the storage temperature will be an additive risk.

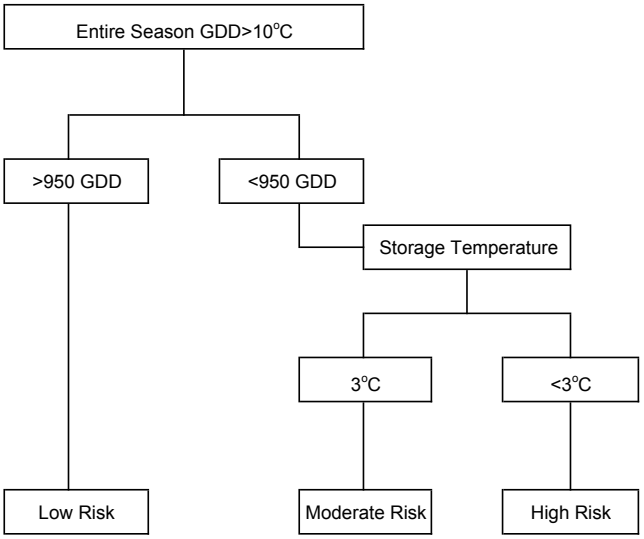

Figure 6.2 Risk factors for the development of diffuse flesh browning (DFB) of 'Cripps Pink' apples during storage.

In contrast, RFB is characterised as browning of the tissue adjacent to the vascular tissue of the fruit resulting from cell wall damage (Chapter 3, figure 3.3). The incidence of RFB during storage was influenced by a range of factors (Chapter 4) including fruit maturity at harvest, storage temperature and the level of CO_2 in the storage atmosphere. This indicates that RFB is the result of additive and interactive factors. Disorders that are affected by a range of postharvest factors are less easy to classify than those that have a direct cause. However, the primary cause of the RFB disorder is likely to be senescence, however as discussed in Chapter 3, senescence interacts with CI and high CO_2 injury. Consequently, storage temperature and the composition of the storage atmosphere are additive factors that exacerbate the symptoms of RFB. As the primary cause of RFB is likely to be senescence, this disorder could be classified as senescent breakdown of 'Cripps Pink' apples.

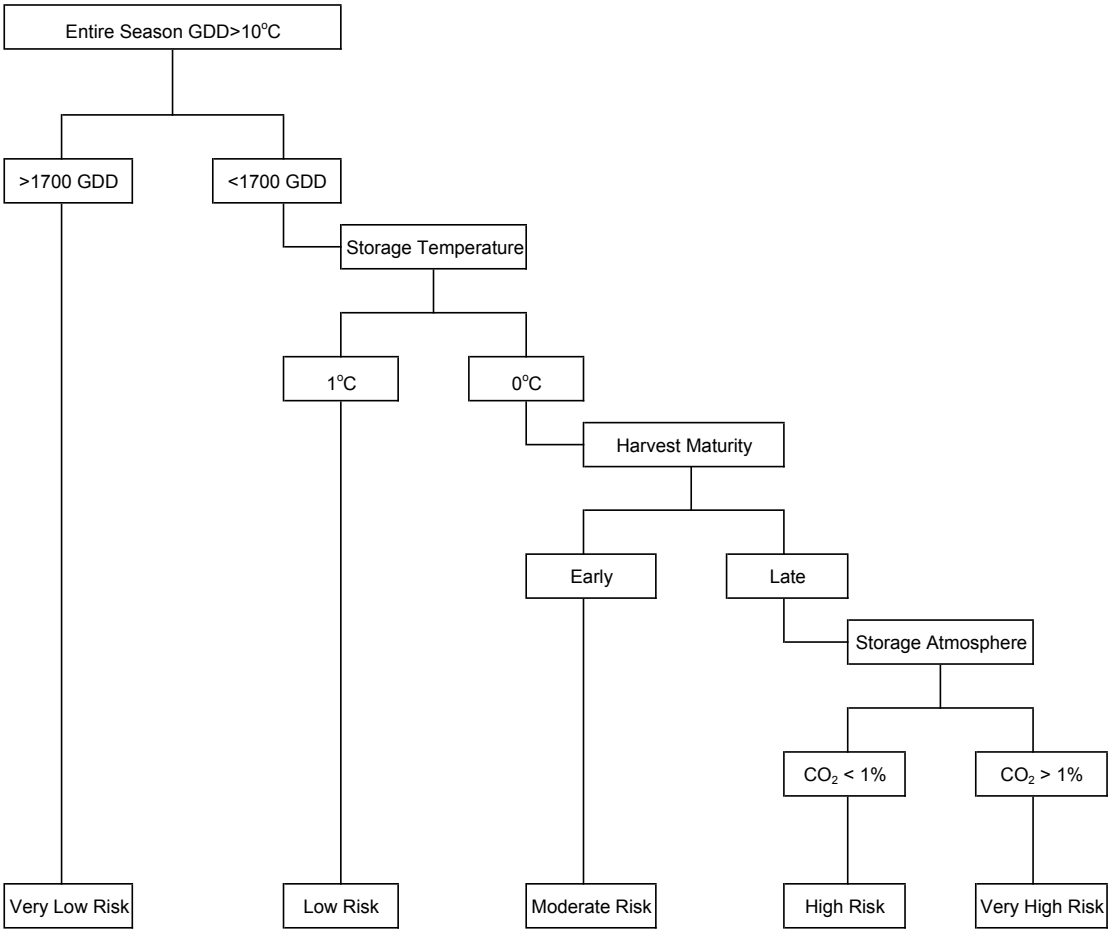

Figure 6.3 Risk factors for the development of radial flesh browning (RFB) of 'Cripps Pink' apples during storage.

The pre and postharvest risk factors that result in the development of RFB during storage are shown in Figure 6.3. The primary risk factor in the development of RFB during storage is the accumulation of GDD>10^{o}C during the entire season. If the seasonal accumulation of GDD>10^{o}C was greater than 1700 then the risk is classified as very low and there will be no significant impact of any further additive factors. However in a season accumulating less than 1700 there is a higher risk of developing RFB and the impact of additive risks needs to be taken into consideration. The second highest risk factor in the development of RFB is the storage temperature, storage at 1^{o}C will result in a low risk of developing RFB, however storage at 0^{o}C will increase the risk and the harvest maturity will be a further additive risk. The highest risk of 'Cripps Pink' apples developing RFB during storage occurs when each of the additive factors are involved. With the current three seasons of research, the impact of fruit nutrition, tree rootstock and crop load are have not been clearly established, however trends have been identified (Chapter 3) and future work may establish the relative impact of these factors on the risk of 'Cripps Pink' apples developing RFB during storage.

The risk associated with the development of DFB of 'Cripps Pink' apples was found to be strongly related to the storage temperature. The DFB disorder of 'Cripps Pink' apples has been reclassified as CI of 'Cripps Pink'. The strong impact of the seasonal climate on the development of the DFB disorder of 'Cripps Pink' apples suggests that there is a climatic range where the potential for the development of DFB during storage is high and results in reduced fruit quality and a dramatic reduction in the storage potential of the fruit.

The risks associated with the development of the RFB disorder of 'Cripps Pink' apples were found to be additive, interactive and complex. It is likely that RFB is the result of senescent breakdown, however is exacerbated by low storage temperature and modification of the storage atmosphere. The impact of climatic conditions indicates that there is an optimal climatic range where 'Cripps Pink' apples can be grown, where the potential for developing RFB is manageable without a substantial loss of fruit quality or storage potential. Despite the relative complexity of this disorder, the practical control strategies that have been identified in this work are comparatively straight forward.

The recommendations for the management of RFB and DFB are summarised in the following table.

Table 6.1 Recommendations for the optimal growth, harvest and storage of 'Cripps Pink' apples for the prevention of the diffuse flesh browning (DFB) and radial flesh browning (RFB) disorders.

	Diffuse flesh browning	Radial flesh browning
Classification	chilling injury	senescent breakdown
Climatic range	<1100 GDD	>1100 GDD[z]
Maturity	SPI[y] 3.5	SPI 3.5
Storage temperature	3°C[x]	1°C[w] or stepwise cooling[v]
Storage atmosphere	<1% CO_2	<1% CO_2
Orchard management	ensure calcium levels adequate	best commercial practice[u]

[z] Insufficient data in the climatic range of 1100-1400 growing degree days (GDD) >10°C has currently been collected, the type of flesh browning that develops in this range has not been determined however it is likely that the recommendations for RFB will be suitable as a guide.
[y] Starch pattern index (SPI) recommendation is based on the CTIFL 10 point scale.
[x] Storage at 3°C will prevent the development of DFB, however storage at 1°C will reduce symptoms. Storage at 3°C will reduce the period of storage time before the loss of quality occurs.
[w] Storage at 1°C was found to be successful for the prevention of RFB, however this was in a low risk season, in a high risk season, storage at a higher temperature may be required.
[v] Stepwise cooling recommendation is 2 weeks at 3°C followed by 2 weeks at 2°C then the remainder of the storage period at 1°C.
[u] Best commercial practice for the management of crop load and fruit nutrition are recommended.

With only three complete seasons, this research has identified and classified Australian regions that are susceptible to DFB and RFB and it has determined the pre and postharvest factors that are involved in the development of both of these disorders. This project has demonstrated how targeted research can lead to the physiological determination of a storage disorder in a relatively short timeframe. A physiological understanding of a disorder can lead to the accurate identification of risk factors allowing for the rapid establishment of commercial recommendations.