

Final Report

AP04008 Continuation of the Flesh Browning of Cripps Pink Project to Validate Recommendations (AP02009)

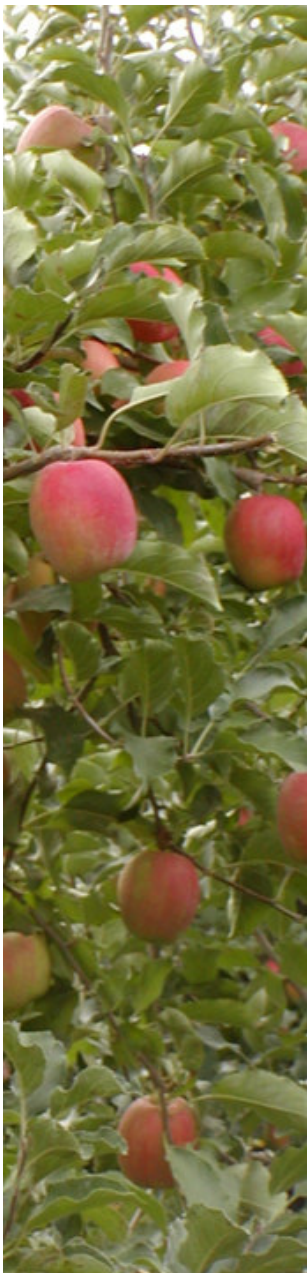
November 2007

Prepared By

Dr Jenny Jobling (Project Leader)

Project Team

Jenny Jobling (Project Leader)	Applied Horticultural Research Pty Ltd
Hannah James	University of Sydney
David Tanner	Food Science Australia
Ian Wilkinson	IHD Knoxfield
Gordon Brown	Scientific Horticulture (Tas)
Tony Portman	Agriculture WA
Stephen Morris	Sydney Postharvest Laboratory
Stuart Tustin	HortResearch NZ
Beth Mitcham	University of California, Davis USA
Angelo Zanella	Laimburg Research Station, Italy



Compiled by Jenny Jobling

Horticulture Australia Project:

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Media Summary

Flesh browning is an internal storage disorder of Pink Lady™ apples. Pink Lady™ is a premium quality apple and this disorder could erode the market confidence and in turn the market value of this variety. The disorder was first seen in 2000 in apples shipped from Australia to the UK for export.

The project involved international collaboration with 6 Institutes from USA, Italy, New Zealand and Australia. There were two projects funded by Horticulture Australia Ltd, Apple and Pear Australia levy funds and voluntary contributions to a value of \$1.1 million dollars. The projects ran from 2002 until 2007.

The research showed that are three types of flesh browning symptoms expressed by Pink Lady™ apples. They are called Diffuse Flesh Browning (DFB), Radial Flesh Browning (RFB) and CO₂ injury. The Diffuse type of FB (DFB) is a chilling injury, occurring in districts or seasons accumulating less than 1200 growing degree days (GDD) above 10°C between full bloom and harvest. Storing fruit at 3°C can reduce the incidence of DFB.

The Radial type of FB (RFB) is a disorder occurring in districts or seasons accumulating between 1200 and 1700 GDD above 10°C between full bloom and harvest. Storing fruit at 1°C can reduce the incidence of RFB. Harvest maturity, fruit calcium concentration and the level of CO₂ in the storage atmosphere are additive influences on the development of RFB. Seasons or districts accumulating more than 1700 GDD are not at risk for developing RFB or DFB. Fruit grown in all areas are susceptible to CO₂ injury in atmospheres containing high levels (around 3%) of CO₂.

The results show Pink Lady™ apples must be grown optimally for successful long term storage and includes a growing the fruit in a region where seasonal climate suits the variety.

In 2004 the research outcomes were shown to reduce the incidence of flesh browning by 20% which correlates to a massive 94% reduction in the UK rejection rate of Pink Lady™ apples. However it is likely that the final research results will reduce the incidence of flesh browning to below commercially problematic levels as the rate of grower uptake of the storage recommendations increases. On completion of the project a colour booklet will be prepared and available to all growers presenting the recommendation in a grower friendly format.

Technical Summary

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 1200 growing degree days (GDD) above 10°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°C can reduce the incidence of DFB.

The Radial type of FB (RFB) is a disorder occurring in districts or seasons accumulating between 1200 and 1700 GDD above 10°C between full bloom and harvest. In these climatic conditions, postharvest ethylene production is not delayed. Radial FB effects the cells surrounding the vascular tissue of the fruit and is characterised by damaged cell walls. Storing fruit at 1°C can reduce the incidence of RFB. Harvest maturity, fruit calcium concentration and the level of CO₂ in the storage atmosphere are additive influences on the development of RFB. Seasons or districts accumulating more than 1700 GDD are not at risk for developing RFB or DFB. Seasonal climatic conditions can provide a guide for predicting the risk of developing RFB and DFB during storage.

More work is required to improve the accuracy of the temperature modelling and to validate the storage protocols recommended. The storage protocols need to be validated for seasons when the seasonal risk of developing flesh browning is high.

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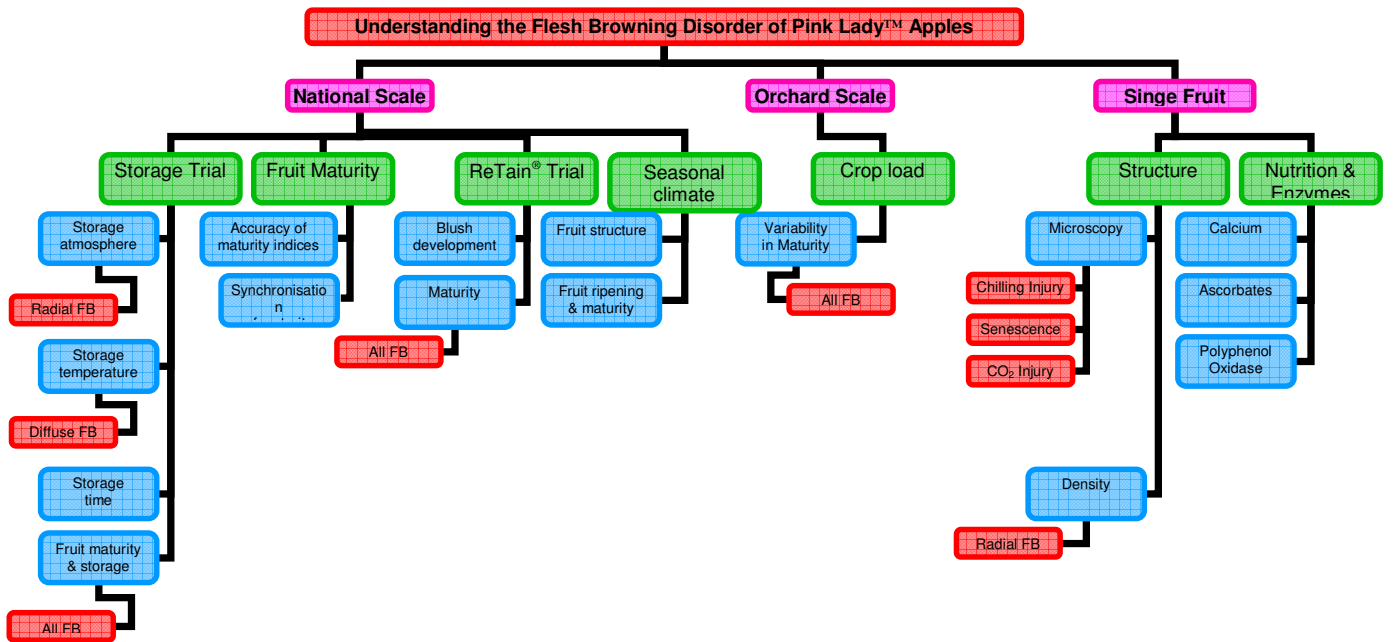
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Publications, Conference Presentations – Technology Transfer

1. Jobling, J, Brown, G, Mitcham, E, Tanner, D, Tustin, S, Wilkinson, I, Zanella, A (2004) Flesh browning of Pink Lady apples™: Why do symptoms occur? Results from an international collaborative study. *Act Hort.* 682:851 – 858.
2. 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.
3. 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? *Proceedings of the International Symposium on Harnessing the Potential of Horticulture in the Asian-Pacific Region. Acta Horticulturae.* 694, 399-403.
4. 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.
5. James, H., J. Jobling, and D. Tanner (2006). Investigating structural and physiological differences between radial and diffuse types of flesh browning of Cripps Pink apples. *Proceedings of the 27th International Horticultural Congress.* In Press.
6. Elena de Castro, Bill Biasi and Elizabeth Mitcham (2007). Quality of pink lady apples in relation to maturity at harvest, prestorage treatments, and controlled atmosphere during storage. *HORTSCIENCE* 42 (3): 605-610.
7. Elena de Castro, Bill Biasi, Elizabeth Mitcham, Stuart Tustin, David Tanner, and Jennifer Jobling (2007). Carbon Dioxide-induced Flesh Browning in Pink Lady Apples. *J. Amer. Soc. Hort. Sci.* 132: 713 - 719.
8. Hannah James (2007). Understanding the flesh browning disorder in ‘Cripps Pink’ apples. PhD Thesis, Faculty of Agriculture, Food and Natural Resources, University of Sydney, Sydney 2006 NSW, Australia.
9. The results have also been presented to the Australian Pink Lady Alliance (APLA) regularly at their AGM. Articles have been written for the industry magazines; *Tree Fruits*, the *Australian Fruit Grower* and *Good Fruit and Vegetable* magazine. The results were also regularly presented to the International Pink Lady Alliance AGM (IPLA) as well.

The industry adoption has been rapid as demonstrated by the reduced rejection rate of containers in the UK in recent years. Flesh browning has direct financial consequences and this has encouraged growers to trial and adopt the strategies outlined in this research.

Experimental Overview



General Discussion and Conclusions

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) $>10^{\circ}\text{C}$ for the entire growing season. In districts or seasons accumulating <1100 GDD $>10^{\circ}\text{C}$ there was a risk of developing DFB (Figure.1). On the other hand, fruit grown in districts or seasons accumulating >1400 GDD $>10^{\circ}\text{C}$ had a risk of developing RFB (Figure.1). For the intermediate climatic range (1100-1400 GDD $>10^{\circ}\text{C}$) there is presently not enough data to indicate the type of FB that would be present. The accumulation of GDD $>10^{\circ}\text{C}$ was also found to be a major determinate of the incidence of both Diffuse Flesh Browning (DFB) and Radial Flesh Browning (RFB) developing during storage.

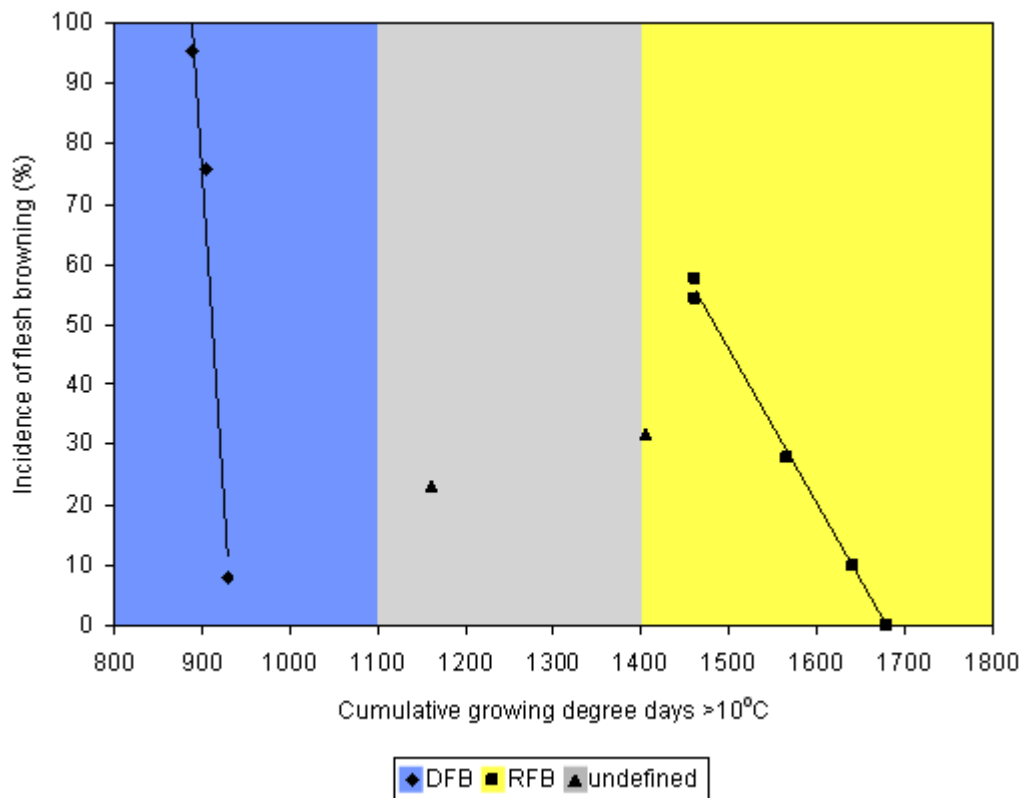


Figure.1 Incidence of DFB and RFB plotted against the cumulative growing degree days (GDD) $>10^{\circ}\text{C}$ for the entire growing season (from full bloom to optimal harvest for long term storage). Flesh browning incidence data is from optimally harvested ‘Cripps Pink’ apples stored at 0°C in air 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. 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 preceding 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. Increasing the storage temperature from 0°C to 3°C was the only storage treatment found to have a considerable effect on reducing the incidence of DFB to within commercial threshold levels. 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 2. The primary risk factor in the development of DFB during storage is the accumulation of $GDD > 10^{\circ}\text{C}$ during the entire season. If the seasonal accumulation of $GDD > 10^{\circ}\text{C}$ 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.

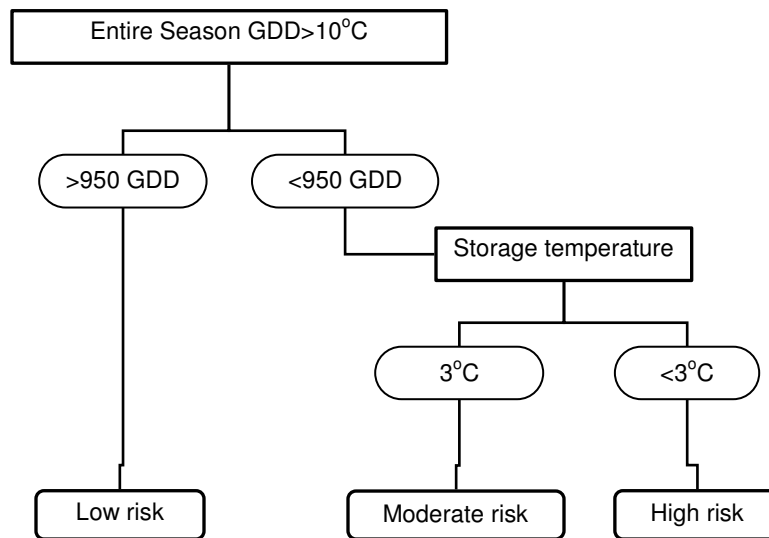


Figure 2 Risk factors for the development of diffuse flesh browning (DFB) of ‘Cripps Pink’ apples during storage.

In contrast, RFB is characterised as browning of the tissue adjacent to the vascular tissue of the fruit resulting from cell wall damage. The incidence of RFB during storage was influenced by a range of factors including fruit maturity at harvest, storage temperature and the level of CO₂ 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 senescence interacts with chilling injury and high CO₂ 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.

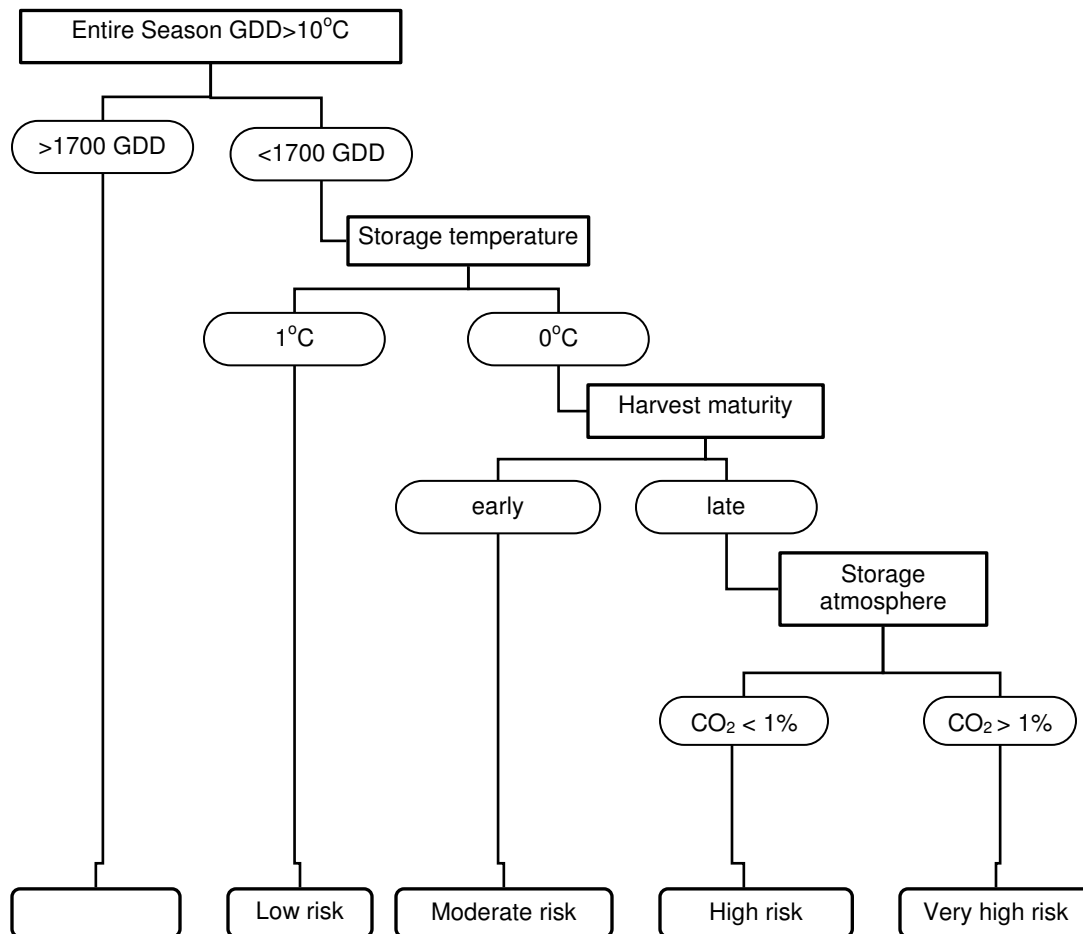


Figure 3. Risk factors for the development of radial flesh browning (RFB) of ‘Cripps Pink’ apples during storage.

The pre and postharvest risk factors that result in the development of RFB during storage are shown in Figure 3. The primary risk factor in the development of RFB during storage is the accumulation of $GDD > 10^{\circ}\text{C}$ during the entire season. If the seasonal accumulation of $GDD > 10^{\circ}\text{C}$ 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°C will result in a low risk of developing RFB, however storage at 0°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 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.

Recommendations

The recommendations for the management of RFB and DFB are summarised in the following table.

Table 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.

	<i>Diffuse flesh browning</i>	<i>Radial flesh browning</i>
<i>Classification</i>	chilling injury	senescent breakdown
<i>Climatic range</i>	<1100 GDD	>1100 GDD ^z
<i>Maturity</i>	SPI ^y 3.5	SPI 3.5
<i>Storage temperature</i>	3°C ^x	1°C ^w or stepwise cooling ^v
<i>Storage atmosphere</i>	<1% CO ₂	<1% CO ₂
<i>Orchard management</i>	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.

1. Summary of the Pink Lady™ Flesh Browning Work for 2002 to 2004 (HAL Project AP02009).

(See Milestone Report 6 AP02009 March 2005 for full experimental details and Review of Final Milestone Report AP02009 March 2005 p.131 of this report)

- In both seasons in both the Northern and Southern Hemispheres, fruit harvested at a late maturity (Ctfl 8.5) showed a higher incidence of flesh browning than early harvested fruit (Ctfl 3.5).
- Time in storage was found to increase the incidence of flesh browning of fruit grown in Australia.
- There are three types of flesh browning. One has a radial pattern of browning where the browning radiates out from the core around the vascular tissue, another has a diffuse pattern where the core is clear and the browning is under the skin in a scalloped pattern and the other shows pits and cavities which is typical of CO₂ injury.



1. Radial browning



2. Diffuse browning



3. CO₂ injury

- Diffuse browning is seen in cooler districts – Tasmania, South Tyrol Italy and Hawke's Bay. Radial browning is seen in warmer districts; California, USA, Mainland Australia and in Nelson in New Zealand. In some cases both types of symptoms can be seen in some districts depending on the season.
- Fruit from all regions are susceptible to CO₂ injury if the CO₂ level is high and the fruit are not pre-cooled prior to the establishment of CA.
- The structural development of radial and diffuse symptoms was compared and found to be significantly different. The pattern of radial flesh browning suggested that gas permeability may be involved in this type of browning. However, the pattern of diffuse browning indicated that this type of browning may be the result of a chilling injury.
- In California, the incidence of radial flesh browning was shown to increase with greater CO₂ and with lower O₂ concentrations. In Italy it was found that in comparison to reference CA (O₂: 1.8%, CO₂: <1.3%), increasing O₂ or CO₂ levels increased the incidence of diffuse flesh browning. In contrast, the Australian trials found that the storage atmosphere was not a major

contributing factor to the development of either radial or diffuse flesh browning, however the fruit appeared to be sensitive to CO₂ levels during storage.

- The assessment of fruit maturity in the 2003/04 season showed significant variation between two growing districts in Australia, Batlow and Tasmania. Tasmania showed the longest period of greenlife and is characterised by the coolest climate. Despite showing the longest period of greenlife, Tasmania shows the highest incidence of diffuse flesh browning.
- In California in the 2003 season, 1-MCP was found to eliminate scald and retain firmness and green background colour, however it was not found to reduce the incidence of flesh browning. In Italy, the addition of 1-MCP to CA was not found to have a beneficial effect on flesh browning.
- In California in the 2003 season, the use of DPA (2200 ppm) was found to completely inhibit the appearance of flesh browning. In Italy, the use of DPA (600, 900, 1200 ppm) had an inconsistent effect.
- In Italy it was found that reducing the relative humidity from 97-99% to 88%, the incidence of flesh browning was increased.
- Tree crop load studies indicated that late harvested fruit from low crop load trees are at an increased risk of all types of flesh browning compared to fruit harvested at optimum maturity and commercial crop load.
- Stepwise cooling (first week at 5°C, second week at 4°C, third week at 3°C, after than 2.5°C) in combination with CA (O₂: 1.8%, CO₂: <1.3%) was found in Italy to have the highest efficiency on the reduction of diffuse flesh browning after either six or eight months of storage. A low incidence of any type of flesh browning in the 2003/04 season resulted in no significant effect of stepwise cooling in the Australian trials.
- Work has been done compiling the seasonal weather data and linking that to the incidence of flesh browning in each district. This work focused on key periods in the growing season, the first 50 days after full bloom, the diurnal difference in temperature around harvest and the overall seasonal growing degree days. There is a link between a low number of growing degree days above 10°C (cooler growing season) and an increased risk of diffuse flesh browning.

Research areas followed up in HAL Project AP04008

1. Maturity and greenlife study – understand the difference between warm and cold districts, effect of seasonal climate on greenlife

Experiment to address this issues in this report is:

The determination of maturity and the physiology of ripening of ‘Cripps Pink’ apples grown in contrasting climatic conditions.....17

Browning in Pink Lady™ apples: Research results have helped to change market specifications for blush colour which is an added bonus for growers.....63

Influence of harvest maturity and storage on the incidence of flesh browning in ‘Cripps Pink’ apples in New Zealand..... 130

2. Determine if the radial and diffuse symptoms are induced by different causes – induce radial with high CO₂ and diffuse with low temperature. Also look at fruit structural differences.

Experiment to address this issues in this report is:

Investigating structural and physiological differences between Radial and Diffuse types of Flesh Browning in Cripps Pink apples.....76

Biochemical Factors Associated with a CO₂-Induced Flesh Browning Disorder of Pink Lady™ Apples99

Carbon Dioxide-Induced Flesh Browning in Pink Lady™ Apples..... 116

Influence of harvest maturity and storage on the incidence of flesh browning in ‘Cripps Pink’ apples in New Zealand130

3. Define the climatic differences for the sites in trial and develop a predictive climate model – looking at the first 50 days after full bloom, whole season averages, diurnal differences at harvest, mean temperatures at harvest and the time until the onset to dormancy as possible key climatic indicators.

Experiment to address this issues in this report is:

Climatic conditions during growth relate to risk of Pink Lady™ apples developing flesh browning during storage.....69

Seasonal fruit development of ‘Cripps Pink’ apples in New Zealand.....139

Summary Climatic Data from Food Science Australia..... 141

4. Determine if manipulating harvest with ReTain[®] is a potential tool in a high risk year – Treat with ReTain[®] and have a small storage trial to assess treatment effect.

Experiment to address this issues in this report is:

Effects of fruit maturity at harvest and ReTain[®] treatment on incidence and severity of flesh browning of ‘Cripps Pink’ apple during storage..136

2. The determination of maturity and the physiology of ripening of ‘Cripps Pink’ apples grown in contrasting climatic conditions

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.

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.

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.

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).

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 avocados, 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 through 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 CO₂ and reducing the concentration of O₂ in the storage atmosphere has been shown to reduce the sensitivity of apples to ethylene (Beaudry, 1999; Gorny and Kader, 1996).

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.

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.

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.

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.

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.

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 (Hurdall 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%.

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.

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 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).

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 hydrolysed 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 an 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.

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 CO₂ 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.

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.

Variability in maturity

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

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).

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 processes 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.

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.

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.

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.

Materials and methods

Fruit sources

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.

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.

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.

Maturity and quality

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°C, the injector was 130°C and the detector was 200°C.

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.

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).

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.

Ripening evaluation

The remaining 30 fruit per harvest per maturity were stored at 20°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 1 □L.kg⁻¹hr⁻¹. The number of days from harvest until the fruit reached a rate of ethylene production of 1 □L.kg⁻¹hr⁻¹ was defined as the greenlife of the fruit.

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.

Results

Maturity

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 2.2), 2005 (Table 2.3) and 2006 (Table 2.4).

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 2.3).

Flesh firmness decreased significantly ($P < 0.001$) with successive harvests in the 2005 (Table 2.2) and 2006 seasons (Table 2.4). 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 2.2 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 (\square 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 2.3 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 (\square 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 2.4 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 (\square 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)

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 2.5 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 ($\square\text{L.L}^{-1}$)
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 2.6 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 ($\square\text{L.L}^{-1}$)
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)

Ripening

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°C. With each successive harvest, the fruit had a higher respiration rate indicating further advanced maturity.

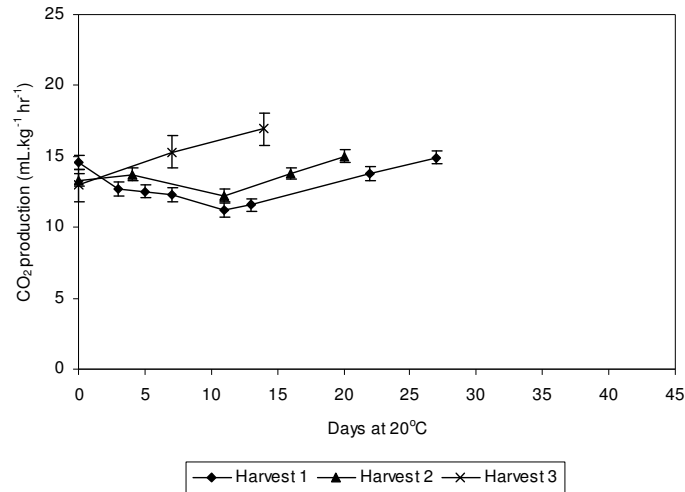


Figure 2.2 Respiration rate ($\text{mL.kg}^{-1}.\text{hr}^{-1}$) of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2004. Harvest 1 (07/04/2004) starch pattern index: 1.4, Harvest 2 (15/04/2004) starch pattern index: 3.1, Harvest 3 (21/04/2004) starch pattern index 4.8. Bars represent standard error.

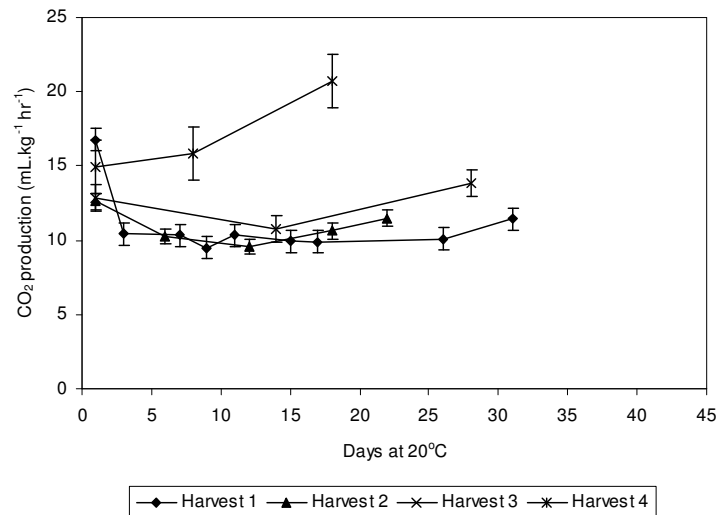


Figure 2.3 Respiration rate ($\text{mL.kg}^{-1}.\text{hr}^{-1}$) of 'Cripps Pink' apples grown in the Huon Valley (Tasmania) in 2004. Harvest 1 (01/04/2004) starch pattern index: 1.8, Harvest 2 (07/04/2004) starch pattern index: 4.3, Harvest 3 (14/04/2004) starch pattern index 5.6, Harvest 4 (26/04/2004) starch pattern index 8.1 Bars represent standard error.

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 $1 \mu\text{L}\cdot\text{kg}^{-1}\text{ hr}^{-1}$, 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.

Batlow

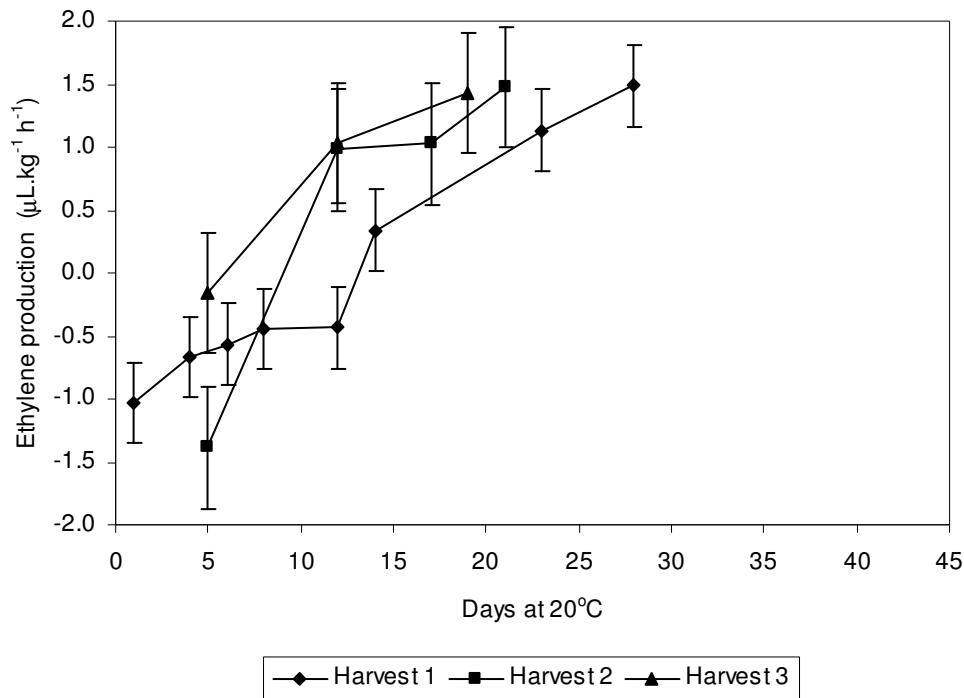


Figure 2.4 Ethylene production at 20°C of ‘Cripps Pink’ apples grown in Batlow (New South Wales) in 2004. Harvest 1 (07/04/2004) starch pattern index: 1.4, Harvest 2 (15/04/2004) starch pattern index: 3.1, Harvest 3 (21/04/2004) starch pattern index 4.8. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 1.31

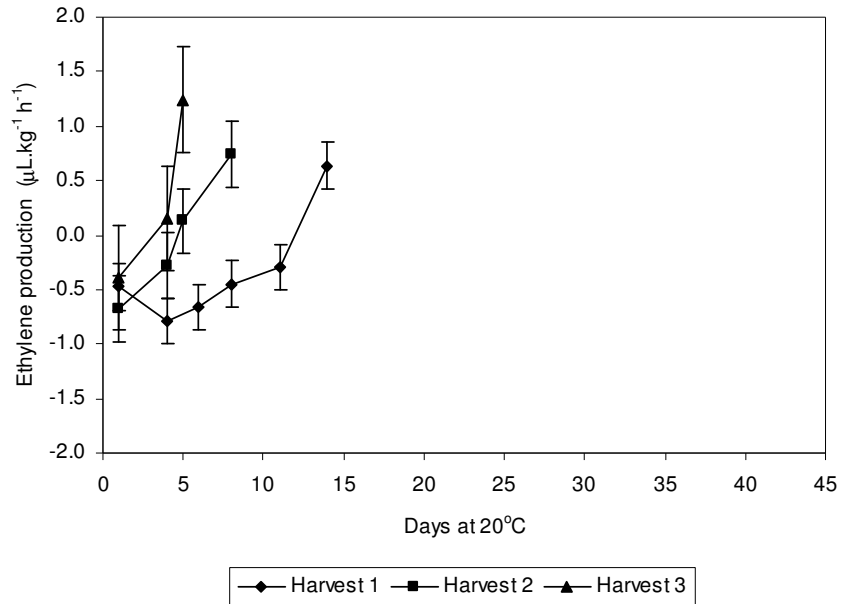


Figure 2.5 Ethylene production at 20°C of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2005. Harvest 1 (21/04/2005) starch pattern index: 4.3, Harvest 2 (28/04/2005) starch pattern index: 5.4, Harvest 3 (04/05/2005) starch pattern index 5.4. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 0.86

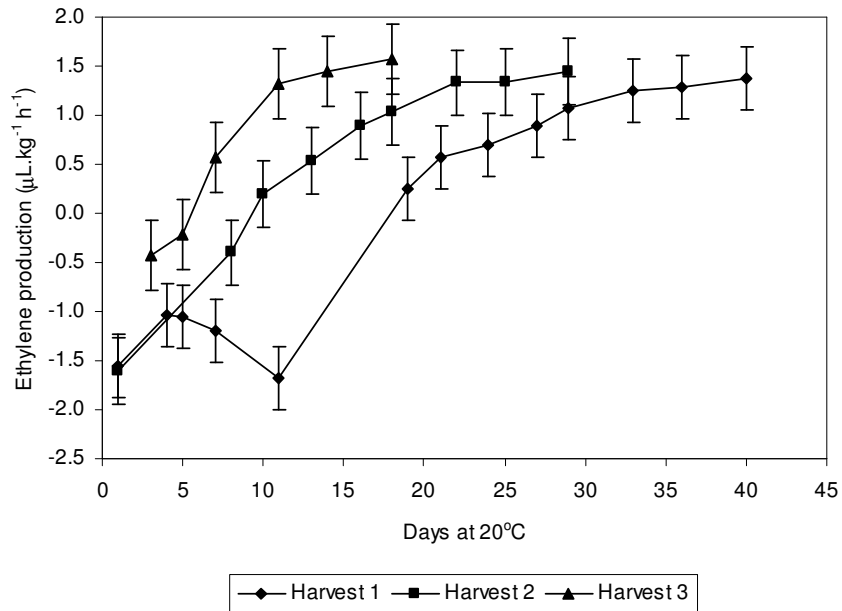


Figure 2.6 Ethylene production at 20°C of 'Cripps Pink' apples grown in Batlow (New South Wales) in 2006. Harvest 1 (30/03/2006) starch pattern index: 1.0, Harvest 2 (10/04/2006) starch pattern index: 3.5, Harvest 3 (21/04/2006) starch pattern index 5.5. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 0.95

The Huon Valley

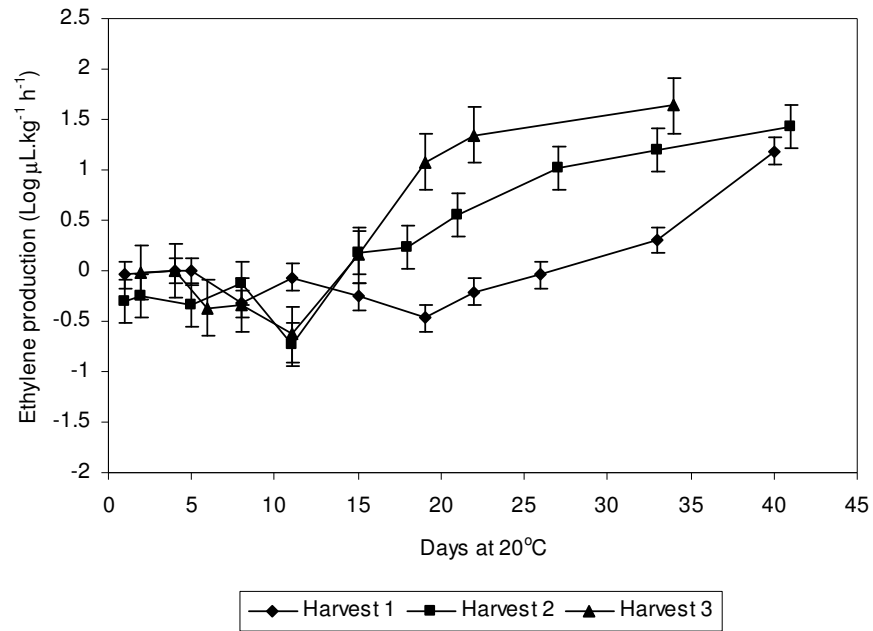


Figure 2.7 Ethylene production at 20°C of ‘Cripps Pink’ apples grown in the Huon Valley (Tasmania) in 2004. Harvest 1 (01/04/2004) starch pattern index: 1.8, Harvest 2 (07/04/2004) starch pattern index: 4.3, Harvest 3 (14/04/2004) starch pattern index 5.6, Harvest 4 (26/04/2004) starch pattern index 8.1. Bars represent standard errors, for comparison between harvests and times, LSD (5%): 0.94.

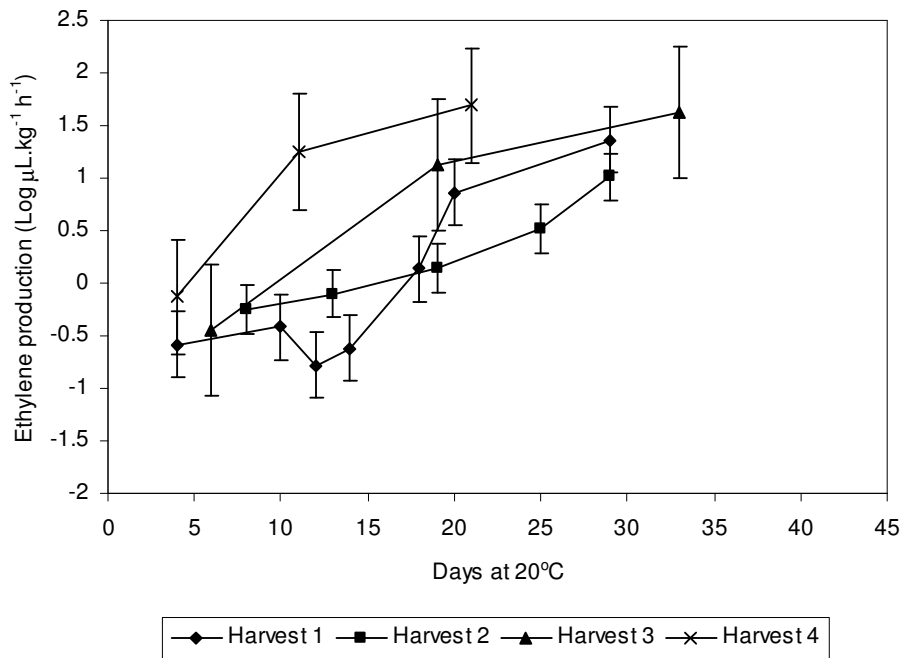


Figure 2.8 Ethylene production at 20°C of ‘Cripps Pink’ apples grown in the Huon Valley (Tasmania) in 2005. Harvest 1 (31/03/2005) starch pattern index: 2.6, Harvest 2 (06/04/2005) starch pattern index: 7.9, Harvest 3 (13/04/2005) starch pattern index 8.7. Bars represent standard errors, for comparison between harvests and days, LSD (5%): 0.60

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 2.7). 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 2.7 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 = $-1.69 \times \text{SPI} + 16.57$	$P < 0.001$	32.9
Batlow + Huon Valley	2004 + 2005 + 2006	greenlife = $-0.874 \times \text{SPI} - 4.72 \times \text{BG colour} + 31.48$	$P < 0.001$	42.9
Huon Valley	2004 + 2005	greenlife = $-1.609 \times \text{SPI} + 23.54$	ns	31.8
Huon Valley	2004 + 2005	greenlife = $-1.368 \times \text{SPI} - 7.18 \times \text{BG colour} + 50.58$	$P = 0.002$	94.1
Huon Valley	2004	greenlife = $-2.092 \times \text{SPI} + 22.07$	$P = 0.036$	89.3
Huon Valley	2005	greenlife = $-2.166 \times \text{SPI} + 32.24$	ns	97.1
Batlow	2004 + 2005 + 2006	greenlife = $-2.474 \times \text{SPI} + 18.41$	$P < 0.001$	67.8
Batlow	2004 + 2005 + 2006	greenlife = $-2.771 \times \text{SPI} - 0.552 \times \text{FF} + 25.56$	$P < 0.001$	68.3
Batlow	2004	greenlife = $-2.274 \times \text{SPI} + 16.81$	$P < 0.001$	69.5
Batlow	2005	greenlife = $-5.826 \times \text{SPI} + 35.53$	$P < 0.001$	83.6
Batlow	2005	greenlife = $-5.257 \times \text{SPI} - 7.73 \times \text{IEC} + 33.66$	$P < 0.001$	84.2
Batlow	2006	greenlife = $-2.47 \times \text{SPI} + 19.02$	$P < 0.001$	64.5

Background (BG) colour: Ctifl colour chart for background colour of 'Cripps Pink' apples

Flesh firmness (FF): N

Internal ethylene concentration (IEC): $\mu\text{L.L}^{-1}$

Starch pattern index (SPI): Ctifl 10 point scale

Greenlife: number of days at 20°C until rate of ethylene production reaches $1 \mu\text{L.kg}^{-1} \text{hr}^{-1}$

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 2.10). 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 2.10), 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 2.11) occurred in the 2005 season ($R^2=0.921$). In the Huon Valley (Figure 2.12), 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 2.11), whereas fruit from the Huon Valley had a greenlife of between 15 and 25 days (Figure 2.12). 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)

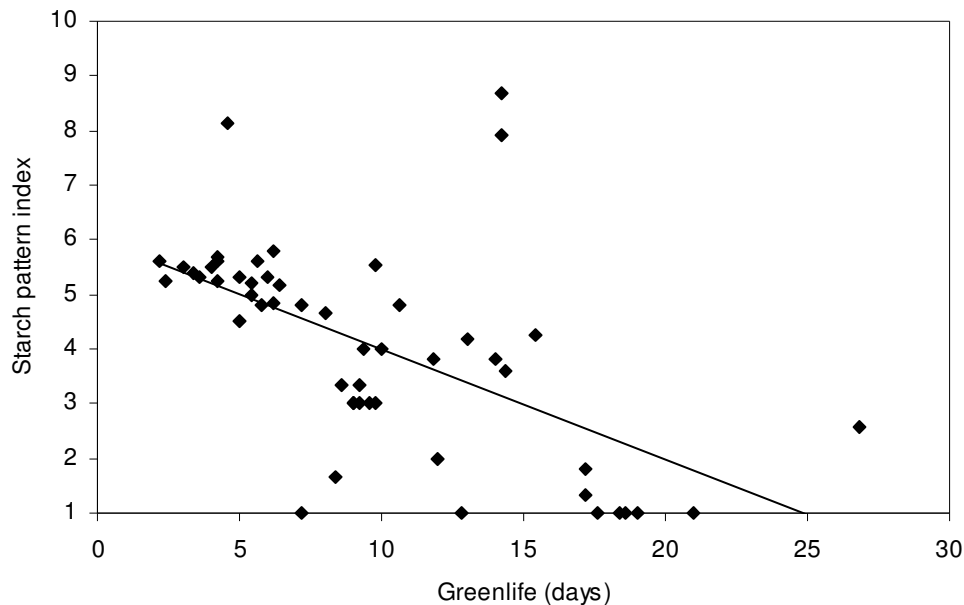


Figure 2.9 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from Batlow (New South Wales), 2004, 2005 and 2006 seasons and the Huon Valley (Tasmania) 2004 and 2005 seasons combined. $R^2 = 0.585$, $P < 0.001$

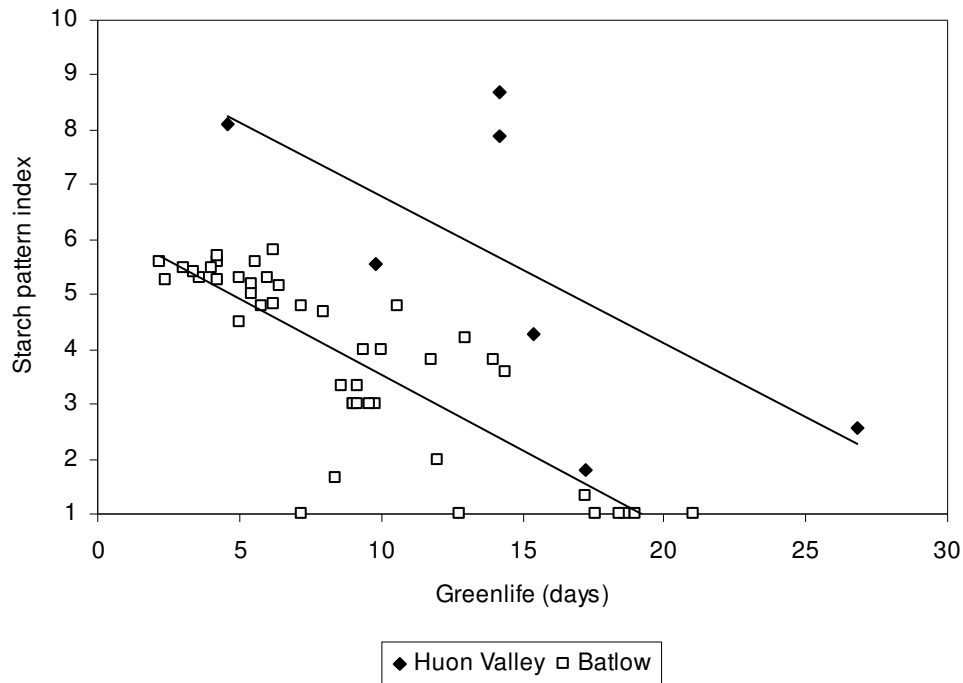


Figure 2.10 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from Batlow (New South Wales), 2004, 2005 and 2006 seasons combined and the Huon Valley (Tasmania) 2004 and 2005 seasons combined. Batlow $R^2=0.683$, $P<0.001$, Huon Valley $R^2=0.657$, ns

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 2.8).

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 2.8 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, 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): $\mu\text{L.L}^{-1}$

Starch pattern index (SPI): Ctifl 10 point scale

Greenlife: number of days at 20°C until rate of ethylene production reaches $1\ \mu\text{L.kg}^{-1}\ \text{hr}^{-1}$

Significance: **** $P < 0.001$

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 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): $\mu\text{L.L}^{-1}$

Starch pattern index (SPI): Ctifl 10 point scale

Greenlife: number of days at 20°C until rate of ethylene production reaches $1 \mu\text{L.kg}^{-1} \text{hr}^{-1}$

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 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): $\mu\text{L.L}^{-1}$

Starch pattern index (SPI): Ctifl 10 point scale

Greenlife: number of days at 20°C until rate of ethylene production reaches $1 \mu\text{L.kg}^{-1} \text{hr}^{-1}$

Significance: ****, ***, ** represent $P < 0.001$, < 0.02 , < 0.05 respectively

Table 2.11 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): $\mu\text{L.L}^{-1}$

Starch pattern index (SPI): Ctifl 10 point scale

Greenlife: number of days at 20°C until rate of ethylene production reaches $1\ \mu\text{L.kg}^{-1}\ \text{hr}^{-1}$

Significance: **** P<0.001

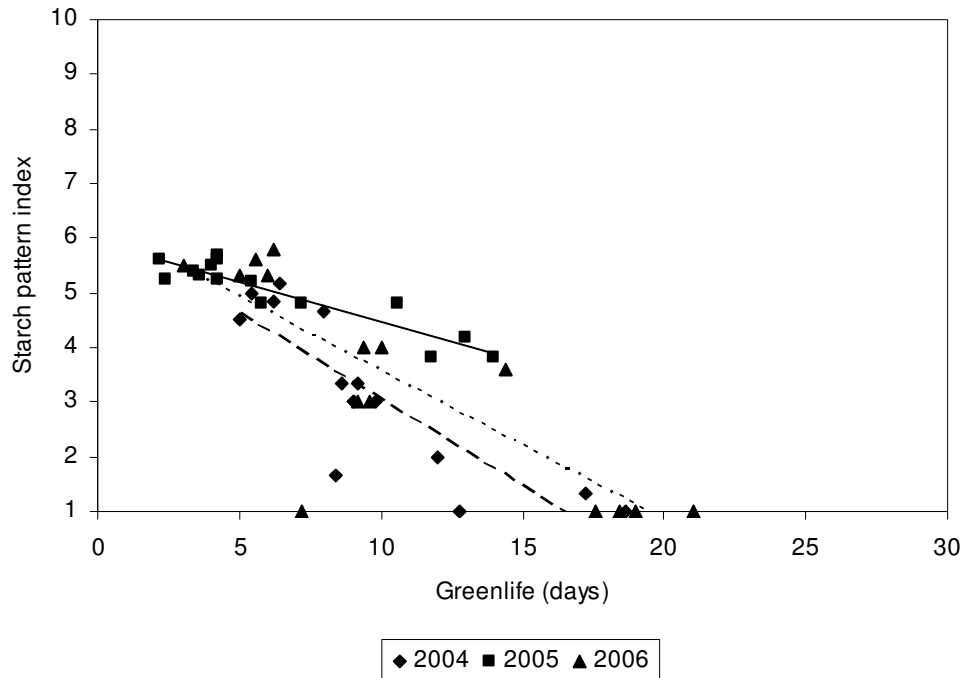


Figure 2.11 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from Batlow (New South Wales), 2004, 2005 and 2006 seasons. 2004 $R^2=0.695$, $P<0.001$. 2005 $R^2=0.842$, $P<0.001$. 2006 $R^2=0.645$, $P<0.001$.

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 2.7). 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 2.7).

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 2.12 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): $\mu\text{L.L}^{-1}$

Starch pattern index (SPI): Ctifl 10 point scale

Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu\text{L.kg}^{-1}\text{hr}^{-1}$

Significance: ** P<0.05

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 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): $\mu\text{L.L}^{-1}$

Starch pattern index (SPI): Ctifl 10 point scale

Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu\text{L.kg}^{-1}\text{hr}^{-1}$

Significance: **, * represent P<0.05, <0.1 respectively

Table 2.14 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): $\mu\text{L.L}^{-1}$
 Starch pattern index (SPI): Ctifl 10 point scale
 Greenlife: number of days at 20°C until rate of ethylene production reaches $1\mu\text{L.kg}^{-1}\text{hr}^{-1}$
 Significance: * P<0.1

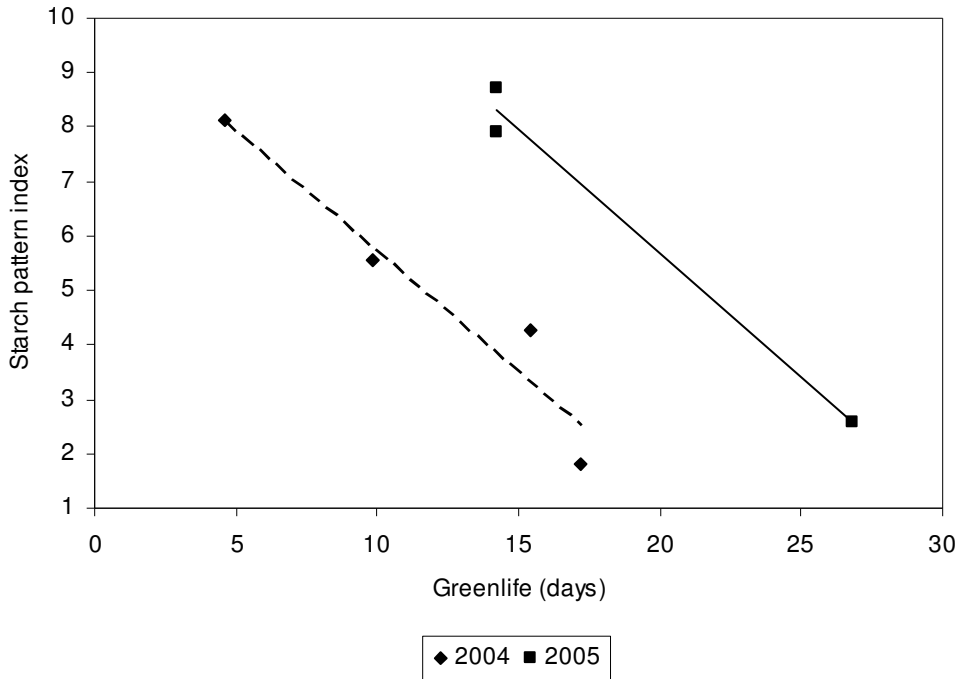


Figure 2.12 Days of greenlife against starch pattern index for 'Cripps Pink' apples, data from the Huon Valley (Tasmania), 2004 and 2005 seasons. 2004 $R^2=0.0964$, $P=0.036$. 2005 $R^2=0.993$, ns

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, greenlife 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 CO₂ 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 $0.1 \text{ ul.kg}^{-1}\text{hr}^{-1}$ (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 $1 \text{ ul.kg}^{-1}\text{hr}^{-1}$ of ethylene (Blanpied and Little, 1991). In these experiments, greenlife was determined as the number of days to an ethylene production rate of $1 \text{ ul.kg}^{-1}\text{hr}^{-1}$ 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 2.10). 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°C). 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 and it has been shown that the climate affects 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°C) during this period can delay the onset of ripening in 'Elstar' apples compared to those grown at 22°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 processes 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. 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 2.11). 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 2.9). In the 2005 season, neither BG colour nor FF were equally correlated with the SPI (Table 2.10) 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.

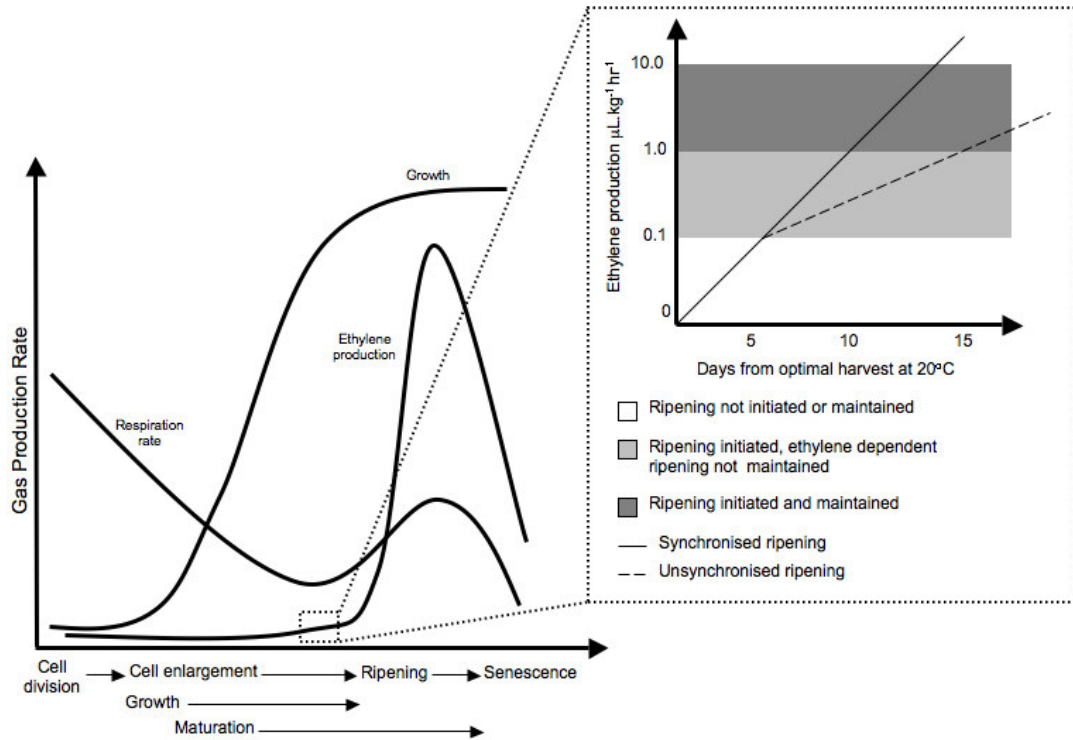


Figure 2.13 Relationship between fruit growth and development, respiration rate and ethylene production (adapted from Little and Holmes, 2000). Inset graph of days from optimal harvest at 20°C (greenlife) indicating hypothesised patterns of synchronised and unsynchronised ripening based on data collected 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%), the season with the highest degree of ripening synchronization. The incidence of RFB was highest in the 2005 season (57.50%) the season with no synchronisation. The 2004 season had an intermediate incidence of RFB (27.78%) 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°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.

Summary

- Maturity
 - ⇒ IEC, rate of ethylene production and CO₂ 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 ($1\mu\text{L}\cdot\text{kg}^{-1}\text{hr}^{-1}$) 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.

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3. Browning in Pink Lady™ apples: Research results have helped to change market specifications for blush colour which is an added bonus for growers.

H James^{1,8*}, G Brown², E Mitcham³, D Tanner⁴, S Tustin⁵, I Wilkinson⁶, A Zanella⁷, J Jobling¹

¹University of Sydney, Sydney, Australia; ²Scientific Horticulture, Tasmania, Australia; ³University of California, Davis, USA; ⁴Food Science Australia, North Ryde, Sydney, Australia; ⁵HortResearch, Hawke's Bay, New Zealand; ⁶Primary Industries Research, Knoxfield, Victoria, Australia; ⁷Research Centre for Agriculture and Forestry, Laimburg, Italy; ⁸Sydney Postharvest Laboratory, North Ryde, Sydney, Australia

[*hjam2402@mail.usyd.edu.au](mailto:hjam2402@mail.usyd.edu.au)

Keywords: Pink Lady™ apple, flesh browning, maturity, storage

Abstract

The flesh browning disorder of Pink Lady™ apples is sporadic in nature and appears to be the manifestation of a complex combination of preharvest and postharvest factors. Two types of browning symptoms are currently known which may be correlated to growing and storage conditions. In this study fruit from four growing districts in Australia were harvested at two maturities (Ctfl 3.5 and 8.5) and stored at 0°C in air or a combination of air + 1% CO₂. This paper presents the results of the first removal, after four months of storage. The results confirm that fruit maturity at harvest is a key factor predisposing Pink Lady™ fruit to both types of flesh browning during storage and support the recent change made by Australian supermarkets to reduce the percentage blush specification for the cultivar.

INTRODUCTION

The Cripps Pink apple cultivar arose out of the Western Australian apple breeding program and was released to the industry for commercial evaluation in 1986 (Cripps et al., 1993). The Cripps Pink apple cultivar is marketed as the Pink Lady™ apple, a trademark owned by Apple and Pear Australia (APAL). The Pink Lady™ apple has been marketed on a worldwide scale resulting in a unique market identity. In order to be marketed as a Pink Lady™ apple in export markets, the Cripps Pink apple must meet strict quality criteria including a minimum percentage of red blush. A royalty of \$US1 per carton of apples covers licensing and marketing costs for the brand. The variety's image is at risk of being damaged as a result of the flesh browning disorder that has been a problem for both domestic and export markets since 2001.

The disorder is sporadic in nature, not occurring in every season or in every district. Such an unpredictable and intermittent disorder may undermine the established trade confidence in the variety and cause downward price pressure. The disorder is observed in both air and controlled atmosphere storage and appears to be the result of a combination of factors that have been implicated in other storage disorders of apples (Clark and Burmeister, 1999; DeEll and Prange, 1998).

A number of postharvest browning disorders of other apple cultivars helped to establish the research parameters for investigating the flesh browning disorder of Pink Lady™ apples. Two types of postharvest browning disorder of Pink Lady™ apples have currently been

described. The first, radial browning, appears to be associated with CO₂ injury during storage. High internal CO₂ and low O₂ have been implicated in other browning disorders of apples such as Braeburn Browning disorder (Clark and Burmeister, 1999). CA related browning of 'Elstar' (Streif and Saquet, 2003) and 'Fuji' (Grant et al., 1996) apples was also found to be related to CO₂ in the storage atmosphere. A second form of browning, diffuse browning, has been observed in both air and CA storage and is likely to be the result of chilling injury. Similar chilling injuries have been observed in other apple cultivars; 'Cortland' apples have been found to be sensitive to low temperatures in storage but relatively insensitive to increased CO₂ in the storage atmosphere (DeEll and Prange, 1998).

An international team of researchers from four countries in both the northern and southern hemispheres contribute to this project. Our preliminary results show that Pink Lady™ apples are sensitive to high CO₂ levels in storage and that late harvested fruit are more susceptible to the disorder. The objectives of this work were assess the effect of the growing district on the incidence and severity of flesh browning, to develop a more accurate method for the assessment of flesh browning and to confirm the relationship between maturity and CO₂ levels and the incidence and severity of flesh browning in Pink Lady™ apples. An unexpected outcome was the change in blush colour specifications by the major supermarket chains in Australia in response to the results from this project. This outcome is important for growers as they no longer have to push the variety to its physiological limits.

MATERIALS AND METHODS

Fruit were sourced from four apple growing districts in Australia, Batlow (New South Wales), Goulburn Valley (Victoria), Huon Valley (Tasmania) and Manjimup (Western Australia). The fruit were harvested at two maturities, starch pattern indexes 3.5 and 8.5 (CTIFL 10 point starch pattern index scale), fruit from Manjimup were harvested at a starch pattern index of 3.5 only. Each district included five orchard blocks. Half of the fruit from each district were double waxed with a commercial apple wax using a commercial packing line before being placed in storage to induce flesh browning (Jobling et al., 2004). Fruit were stored in 60 L drums in a randomised block design under an atmosphere of air, or air + 1% CO₂ on a flow through system with a flow rate of 200 mL per minute and stored at 0°C. There were four replicates per maturity, wax, and atmosphere combination with fifty fruit per replicate (ten fruit per orchard block). Fruit were assessed for browning after four and seven months storage. Browning was assessed at three locations: the stem, the middle and the blossom end of transverse sections of the fruit. Assessments were for incidence (the percentage of fruit with flesh browning, irrespective of the severity) and severity based on a 1 to 5 scale where 1 = none and 5 = severe. Assessment of incidence and severity was performed visually and if any browning was present the sections were placed on a flatbed scanner and an image was acquired. This image was then assessed for the actual percentage of browning using Starchanalyser software (v0.9 copyright 2004, Food Science Australia). Eight hundred fruit were assessed for each maturity at the end of each storage period per region. The fruit was held at 20°C for seven days before assessing the fruit for internal flesh browning.

RESULTS AND DISCUSSION

Preliminary results from 2003 using fruit from only one district, Batlow (New South Wales), had shown that double waxing can induce radial browning (Jobling et al., 2004). The symptoms were more severe in later harvested, longer stored fruit (Jobling et al., 2004). This

season, worked aimed to determine if waxing could be used to establish district susceptibility to flesh browning. The technique would also help to establish whether the two types of symptoms, radial and diffuse browning, were the result of similar or different factors. After four months of storage in 2004, a significant difference in the incidence of flesh browning was found between the four districts with Tasmania showing the highest incidence (94.87 %) of flesh browning for all treatments (fig. 1). It is likely that seasonal conditions predispose Pink Lady™ apples to flesh browning as climatic conditions are known to affect the biophysical properties of fruit such as cellular structure, tissue density, air content, gas permeability and wax cuticle properties (Stanley et al., 2000). Fruit density was found to be significantly higher in Goulburn Valley (0.830 g cm^{-3}) and Tasmania (0.830 g cm^{-3}) than in Western Australia (0.812 g cm^{-3}) and Batlow (0.806 g cm^{-3}) (fig. 2). However the correlation between district incidence of flesh browning and fruit density was not significant this season. This distinguishes flesh browning in Pink Lady™ apples from the Braeburn Browning disorder which are related to fruit density (Elgar et al., 1999).

Double waxing the fruit induced symptoms of radial flesh browning in 2003 (Jobling et al., 2004) however the effect was not as clear after four months of storage in 2004. Double waxing the fruit induced radial flesh browning in fruit sourced from Goulburn Valley (unwaxed: 5.45 %, waxed: 7.14 % of fruit) however the treatment did not induce the diffuse symptoms of flesh browning observed in fruit sourced from Tasmania (unwaxed: 97.28 %, waxed: 92.33. % of fruit). The wax treatment was not significant for fruit from Batlow or Western Australia, both of these districts exhibit symptoms of radial flesh browning. Radial symptoms of flesh browning are similar to those reported for vascular breakdown, while diffuse symptoms appear to be related to chilling injury (Jobling et al., 2004).

The structural development of radial and diffuse symptoms of flesh browning was found to be significantly different. Radial browning shows a high mean area of browning at the stem end of the fruit which decreases towards the calyx end. Radial browning has been shown to be influenced by increasing CO_2 and decreasing O_2 in the storage atmosphere (Jobling et al., 2004). The development of browning indicates that gas permeability, which may be lowest at the stem end of the fruit, may be involved in the development of this type of browning. In contrast, diffuse browning was highest at the stem and calyx ends with a relatively low area of browning in the middle section of the fruit. This pattern of browning is more likely to be the result a chilling injury (Jobling et al., 2004).

After four months of storage in 2004 the incidence of radial browning was relatively low (13.20 % of fruit) and there was no significant factor predisposing fruit to flesh browning during storage. However, fruit harvested at a later maturity tended to have a higher mean incidence of browning (fig. 3). This finding confirms the results from Jobling et al., 2004 where maturity was a key factor after four months storage and other treatment effects, such as storage atmosphere, only became significant after increased storage time. The incidence of diffuse browning (94.87 % of fruit) was significantly higher than that of radial browning (13.20 % of fruit) after four months of storage. The mean percentage area of browning was significantly higher for later harvested fruit in Tasmania (Maturity 1: 40.54 %, Maturity 2: 46.76 % affected area of browning) (fig. 4) indicating that maturity is a key factor in the development of both types of browning. Fruit maturity at harvest has been shown to influence flesh browning disorders in other apple varieties with advanced fruit maturity resulting in a higher incidence and severity of browning (Brown et al., 2003; Grant et al., 1996; Toivonen et al., 2003; Volz et al., 1998).

Fruit maturity at harvest is of particular significance for Pink Lady™ apples as major Australian retailers set the specifications for red blush at 60% which often led to fruit being

harvested over mature, increasing the risk of the development of flesh browning during storage and negating all the positive qualities of this cultivar. As a result of the findings of the close link between fruit maturity at harvest and flesh browning, retailers have reduced the percentage of red blush specifications to 45-50% which is an important difference for growers. The results from 2004 confirm the relationship between maturity at harvest and the development of flesh browning during storage, validating the change in blush specifications. Growers are now able to provide fruit with optimum storage quality as well as reducing the risk of flesh browning developing in storage, restoring the integrity of this cultivar.

CONCLUSION

The results from 2004 validate the change in market specifications for Pink Lady™ apples. The risk of both types of symptoms of browning is increased with late maturity. The ideal blush specification for Pink Lady™ apples is 40-50%. Higher specifications often force growers to leave fruit on the tree well past the optimal harvest time resulting in fruit that do not store well, have a reduced shelf life after removal from storage and are at an increased risk of developing flesh browning. This research project is an example of how good collaborative research can make a significant difference to an industry.

ACKNOWLEDGEMENTS

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Figures

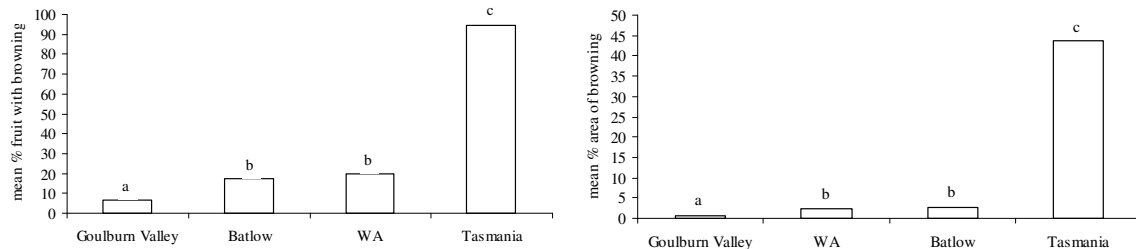


Fig. 1. Mean percentage of fruit with flesh browning and mean percentage area of fruit affected by flesh browning for Goulburn Valley (Victoria), Batlow (New South Wales), Western Australia and Tasmania after four months of storage, 2004. Treatments followed by a different letter are significantly different at a 5% level of significance.

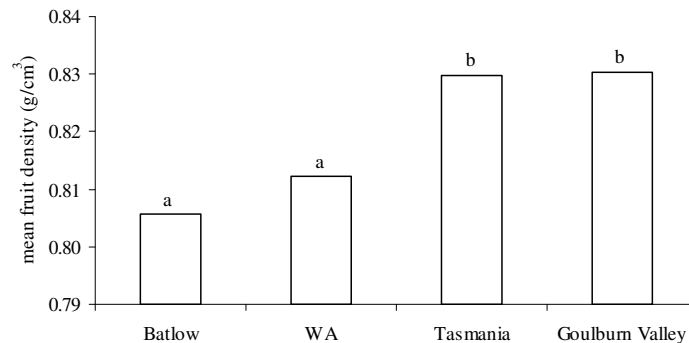


Fig. 2. Mean fruit density (g/cm³) at harvest of fruit from Goulburn Valley (Victoria), Batlow (New South Wales), Western Australia and Tasmania. Treatments followed by a different letter are significantly different at a 5% level of significance.

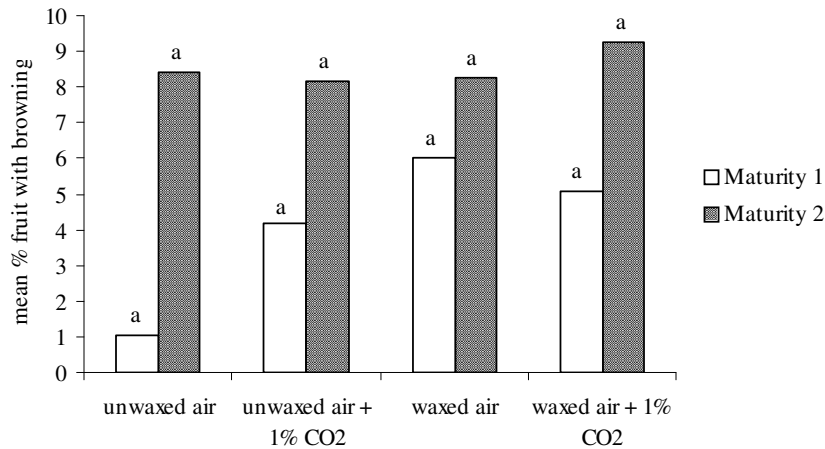


Fig. 3. Mean percentage of fruit affected by browning for fruit grown in Goulburn Valley, after four months of storage, 2004. Maturity 1 SPI: 3.5, maturity 2 SPI: 8.5. Treatments followed by a different letter are significantly different at a 5% level of significance.

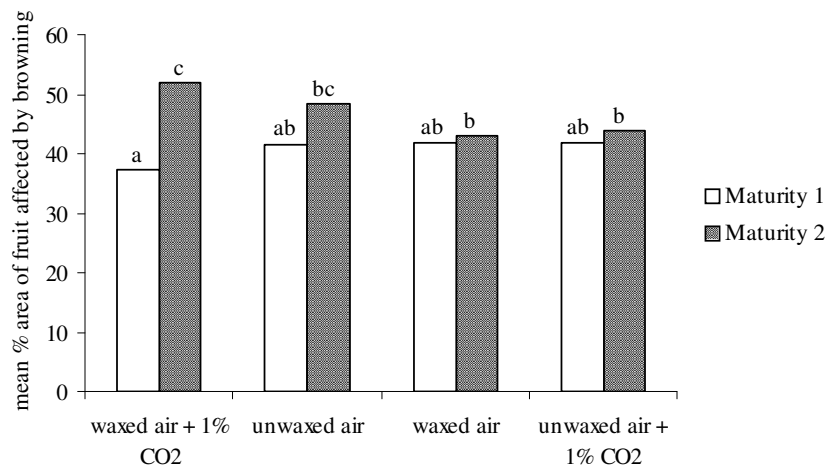


Fig. 4. Mean percentage of fruit area affected by browning for fruit with diffuse symptoms of flesh browning (Tasmania), after four months of storage, 2004. Maturity 1 SPI:3.5, maturity 2 SPI:8.5. Treatments followed by a different letter are significantly different at a 5% level of significance.

4. Climatic conditions during growth relate to risk of Pink Lady™ apples developing flesh browning during storage

H. James^{1,2*}, J. Jobling², D. Tanner³, S. Tustin⁴, I. Wilkinson⁵

¹Sydney Postharvest Laboratory, North Ryde, Sydney, Australia;

²University of Sydney, Sydney, Australia; [*hjam2402@mail.usyd.edu.au](mailto:hjam2402@mail.usyd.edu.au)

³Food Science Australia, North Ryde, Sydney, Australia;

⁴HortResearch, Hawke's Bay Research Centre, New Zealand;

⁵Primary Industries Research, Knoxfield, Victoria, Australia

Keywords: Pink Lady™ apple, flesh browning, storage, climate

Abstract

Climatic conditions during two distinct periods of the growth and development of Pink Lady™ apples may be associated with the development of two expressions of flesh browning (FB) symptoms. The expression of symptoms has been linked to certain seasonal and storage conditions, growing district and several other factors have been implicated in the development of the disorder over the last two seasons. The variation between districts could be related to climatic conditions, for example low temperatures during the 50 days after full bloom growth period are speculated to increase the risk of CO₂ injury during storage as the cooler growing conditions result in more dense fruit with reduced gas diffusivity. Such fruit may accumulate high internal levels of CO₂ resulting in a predisposition to develop CO₂ related FB known as radial FB. Pink Lady™ apples grown in districts experiencing <1200 cumulative growing degree days from full bloom to harvest are at an increased risk of diffuse FB. Another climatic risk is if there is a low diurnal temperature variation during the 60 days prior to harvest maturity which is associated with reduced blush colour development. This situation results in growers delaying harvest in order to maximise the development of blush in order to meet strict quality guidelines designed for marketing of the cultivar under the trademark. Such fruit have an increased risk of developing both types of FB. Our results also show that the risk of developing both types of FB is higher with advanced fruit maturity at harvest. The atmospheric conditions and length of time of storage have also been shown to influence the severity of the disorder.

INTRODUCTION

The Cripps Pink apple cultivar was bred in the Western Australian apple breeding program and was released to the industry for commercial evaluation in 1986 (Cripps et al., 1993). The apple originated from a cross between Lady Williams and Golden Delicious cultivars with the aim of combining the sweet fruit of the Golden Delicious with the firm, long storing fruit of Lady Williams (Cripps et al, 1993). The Cripps Pink apple cultivar is marketed as the Pink Lady™ apple in export markets, a trademark owned by Apple and Pear Australia (APAL). The Pink Lady™ apple has been marketed on a worldwide scale resulting in a unique market identity. In order to be marketed as a Pink Lady™ apple in export markets, the Cripps Pink apple must meet strict quality criteria including a minimum percentage of red blush. The variety's

unique image is currently at risk of being damaged as a result of the flesh browning (FB) disorder that has been a problem for both domestic and export markets since 2001.

A number of postharvest browning disorders of other apple cultivars helped to establish the research parameters for investigating the FB disorder of Pink Lady™ apples. The FB disorder of Pink Lady™ apples is sporadic in nature, not occurring in every season or in every district. Such variation in incidence may increasingly undermine the established trade confidence in the variety and result in a lack of confidence in the cultivar. Two types of postharvest browning disorder of Pink Lady™ apples have currently been described, both of which have been observed in air and controlled atmosphere (CA) storage. The first, radial FB (RFB), appears to be associated with senescent breakdown aggregated by high CO₂ levels during storage. High internal CO₂ and low O₂ have been implicated in other browning disorders of apples such as Braeburn browning disorder (BBD) (Clark and Burmeister, 1999). CA related browning of 'Elstar' (Streif and Saquet, 2003) and 'Fuji' (Grant et al., 1996) apples was also found to be related to CO₂ in the storage atmosphere. A second form of browning, diffuse FB (DFB), is likely to be the result of chilling injury. Similar chilling injuries have been observed in other apple cultivars; 'Cortland' apples have been found to be sensitive to low temperatures in storage but relatively insensitive to increased CO₂ in the storage atmosphere (DeEll and Prange, 1998).

The Pink Lady™ apple is a bi-coloured apple which matures late in the season (Cripps et al., 1993). The problem some growers have had is that the fruit is slow to develop optimum red blush colour. Warm days (above 20°C) and cool nights (below 10°C) are required for optimal blush development (Little and Holmes, 2000). Without this diurnal temperature difference, growers are often forced by commercial pressure to wait for colour development which has meant that the fruit is often picked over-mature. Fruit maturity at harvest has previously been implicated in both RFB and DFB of Pink Lady™ apples (James et al., 2005; Jobling et al., 2004)

Other climatic factors have been identified which may lead to the risk of Pink Lady™ apples developing flesh browning. Low temperatures during fruit development have been found to increase the period of cell division resulting in denser fruit at harvest (Little and Holmes, 2000). Lau (1998) suggests that the relatively high fruit density of 'Braeburn' apples is indicative of high resistance to gas diffusion, and increases the risk of internal injury under CA conditions. Lau (1998) also found that the development of BBD was associated with cool growing seasons, which accumulated less than 1300 degree-days above 10°C. This finding is supported by observations that BBD is more prevalent in southern or colder regions, and in colder districts or higher altitudes within a region (Elgar et al., 1999).

Previous results show that Pink Lady™ apples are sensitive to high CO₂ levels in storage and that late harvested fruit are more susceptible to the disorder (James et al., 2005; Jobling et al., 2004). The objectives of this work were to assess the effect of growing district on the incidence and severity of FB and to assess the climatic factors of the districts which may predispose fruit to FB during storage. The expression of symptoms of FB of Pink Lady™ apples is related to number of pre- and postharvest factors, this paper focuses on the risk associated with climatic conditions during growth and development which predispose fruit to FB under certain storage conditions. An international team of researchers from four countries in both the northern and southern hemispheres contribute to this project.

MATERIALS AND METHODS

Induction and assessment of types of browning

Fruit were sourced from four apple growing districts in Australia, Batlow (New South Wales), Goulburn Valley (Victoria), Huon Valley (Tasmania) and Manjimup (Western Australia). The fruit were harvested at two maturities (starch pattern indices 3.5 and 8.5 on the CTIFL 10 point starch pattern index scale; fruit from Manjimup were harvested at a starch pattern index of 3.5 only). Each district included five orchard blocks. Half of 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 to induce flesh browning (Jobling et al., 2004). Fruit were stored in 60 L drums in a randomised block design under an atmosphere of air, or air + 1% CO₂ in a flow through system with a flow rate of 200 mL per minute and stored at 0°C. There were four replicates per maturity, wax, and atmosphere combination with fifty fruit per replicate (ten fruit per orchard block). Fruit were assessed for browning after four and seven months of storage. Browning was assessed at three locations: the stem end, the middle and the blossom end of transverse sections of the fruit. Assessments were for incidence (the percentage of fruit with flesh browning, irrespective of the severity) and severity based on a 1 to 5 scale where 1 = none and 5 = severe. Assessment of incidence and severity was performed visually and if any browning was present the sections were placed on a flatbed scanner and an image was acquired. This image was then assessed for the actual percentage of browning using Starchanalyser software (v0.9 copyright 2004, Food Science Australia). Eight hundred fruit were assessed for internal flesh browning for each maturity at the end of each storage period per region, after being held for 7 days at 20°C.

Climatic risk

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. 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 for each month. For each recorded temperature measurement, the following equation was used to estimate contribution to GDD.

$$DD_x = t (\theta - x) \quad (1)$$

where:

DD	=	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.

RESULTS AND DISCUSSION

Preliminary results from 2003 using fruit from only one district, Batlow (New South Wales), had shown that double waxing fruit prior to storage can induce RFB (Jobling et al., 2004). The symptoms were found to be more severe in later harvested, longer stored fruit (Jobling et al., 2004). In the 2004 season, work aimed to determine if waxing could be used to

establish a risk of developing FB based on district climatic observations and to help establish whether the two types of symptoms, RFB and DFB, were the result of similar or different factors. After seven months of storage in 2004, a significant difference in the incidence of FB was found between the four districts assessed with Tasmania showing the highest incidence (93.2%) of FB for all treatments (Fig. 1).

Double waxing the fruit induced symptoms of RFB in 2003 (Jobling et al., 2004); however the effect was not as clear after four or seven months of storage in 2004 (Table 1). Double waxing the fruit induced RFB in fruit sourced from Western Australia (unwaxed: 28.4%, waxed: 41.1% of fruit); however, the treatment did not significantly induce the development of DFB symptoms observed in fruit sourced from Tasmania (unwaxed: 95.6%, waxed: 92.8% of fruit). The structural development of RFB and DFB symptoms was found to be significantly different (Table 2). RFB shows a high mean area of browning at the stem end of the fruit which decreases towards the calyx end (Table 2). RFB has been shown to be influenced by increasing CO₂ and decreasing O₂ in the storage atmosphere (Jobling et al., 2004). This development of browning indicates that gas permeability, which was found to be lowest at the stem end of the fruit, may be involved in the development of this type of FB. Symptoms of RFB are similar to those reported for vascular breakdown (Meheriuk et al., 1994). In contrast, DFB was highest at the stem and calyx ends with a relatively low area of browning in the middle section of the fruit (Table 2). This pattern of browning is more likely to be the result of chilling injury. Storing fruit which are susceptible to DFB at higher temperatures (2.5°C) has been found to decrease the incidence of DFB (Zanella, 2004) further indicating that this type of FB is the result of a chilling injury.

It is likely that district seasonal conditions predispose Pink Lady™ apples to FB as climatic conditions during fruit growth and development are known to affect the biophysical properties of fruit such as cellular structure, tissue density, air content, gas permeability and wax cuticle properties (Stanley et al., 2000). Three key climatic times have been identified as result in predisposing Pink Lady™ apples to FB during storage.

The first key temperature time focuses on the period of time from full bloom to fifty days after full bloom. Low temperatures during this time extend the period of cell division which results in denser fruit at harvest (Little and Holmes, 2000). Such fruit accumulate elevated levels of internal CO₂ during CA storage and consequently have an increased risk of developing RFB (Lau, 1998). In the 2003/04 season, the GDD during the first 50 days after full bloom was not found to be consistently related to fruit density or RFB (data not shown). This distinguishes the FB disorder in Pink Lady™ apples from BBD which is related to fruit density (Elgar et al., 1999). Climatic conditions during early season development may only partially contribute to a more complex situation in which climatic conditions during other key periods of growth and development may also contribute to the potential risk of FB, as has been described by Volz et al, (2000; 1999) for the prediction of BBD.

The second key temperature time examines the effect of diurnal temperature differences during the period of time from sixty days prior to harvest to harvest. Diurnal temperature differences are essential during this period for the development of red blush (Little and Holmes, 2000). In order to meet market specifications, Pink Lady™ apples must have 45-50% of red blush (James et al., 2004). In seasons where there is a low diurnal temperature difference leading up to harvest, growers tend to delay harvest in order to maximise the potential for colour development. As a consequence, fruit are often harvested over-mature and are at an increased risk of developing both types of FB which have been linked to fruit maturity at harvest (James et

al., 2004; Jobling et al., 2004). In the 2003/04 season, the Goulburn Valley had the highest diurnal temperature difference (data not shown) and the lowest incidence of RFB of the districts assessed (Fig. 1). This data supports the hypothesis that increased diurnal temperature differences in the 60 days prior to harvest may be associated with a decreased risk of RFB, however the results from the other districts assessed were inconclusive (data not shown).

The third key temperature time assesses the effect of the number of GDD for the entire growing season, from full bloom to harvest. Lau (1998) assessed this period of time and found that fruit grown in districts experiencing less than 1200 GDD were at an increased risk of developing BBD. In the 2003/04 season it was found that Tasmania, a district prone to developing DFB during storage, received 807 GDD. In contrast, Western Australia, Batlow and the Goulburn Valley, districts which develop RFB during storage, accumulated 1405, 1556 and 1668 GDD respectively. These results agree with those of Sharples (1975) and Johnson and Ridout (1998) who found that cool growing seasons were associated with an increased risk of chilling injury and low temperature breakdown during storage. Examination of further seasons and districts indicates that the GDD accumulated can be used to determine the type of FB that will develop during storage. Climate analysis indicates that seasons or districts accumulating below 1200 GDD develop DFB while seasons accumulating over 1200 GDD were found to develop RFB.

CONCLUSION

Results from previous studies have highlighted postharvest effects on the development of FB in Pink Lady™ such as storage time, harvest maturity and the storage atmosphere (James et al., 2005; Jobling et al., 2004). Results from this study indicate that DFB occurs in districts accumulating below 1200 GDD and the risk of RFB occurs in seasons or districts accumulating over 1200 GDD. The influences of the GDD during the early season and the cumulative diurnal temperature variation in the late season have not been found to consistently relate to either RFB or DFB. It must be noted that the short duration of this project means that there still remains a need to examine in more detail the degree of influence that each of the key climatic times exerts. This is particularly so in relation to variability in seasonal conditions. Climate modelling in combination with a deeper physiological understanding of the mechanisms of the two types of FB may provide a useful technique for predicting the relative district seasonal risk of Pink Lady™ apples developing FB during storage.

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Tables

Table 1. Mean percentage of fruit with flesh browning for Goulburn Valley, Batlow, Western Australia and Tasmania after four and seven months of storage, 2004. Treatments followed by a different letter are significantly different at a 5% level of significance.

	4 months		7 months	
	unwaxed	waxed	unwaxed	waxed
Batlow	15.9 fg	18.6 ef	24.9 cdef	30.1 cd
Goulburn Valley	5.9 g	6.8 g	22.6 cdef	33.1 c
Tasmania	95.6 a	92.8 a	95 a	95.7 a
Western Australia	19.5 ef	20.5 def	28.4 cde	41.1 b

Table 2. Mean percentage area of fruit tissue affected by flesh browning at stem, middle and calyx positions of the fruit for radial and diffuse types of flesh browning. Factors followed by a different letter are significantly different at a 5% level of significance.

	Radial FB	Diffuse FB
Stem	4.69 d	46.84 a
Middle	4.07 e	41.84 c
Calyx	2.34 f	43.09 b

Figures

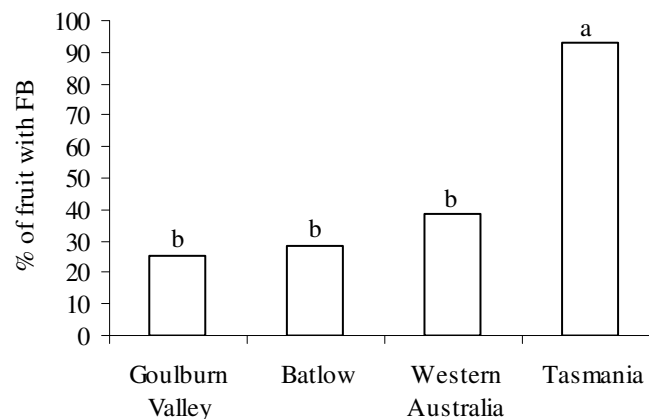


Fig. 1. Mean percentage of fruit with flesh browning for Goulburn Valley (1668 GDD), Batlow (1556 GDD), Western Australia (1495 GDD) and Tasmania (807 GDD) after seven months of storage, 2004. Treatments followed by a different letter are significantly different at a 5% level of significance.

5. Investigating structural and physiological differences between Radial and Diffuse types of Flesh Browning in Cripps Pink apples

Hannah James^{1,2*}, Jenny Jobling¹ and David Tanner³

¹*Faculty of Agriculture, University of Sydney, Sydney, NSW, 2006, Australia*

²*Sydney Postharvest Laboratory, North Ryde, Sydney, NSW, 2113, Australia*

³*Food Science Australia, Sydney, NSW, 2113, Australia*

*Corresponding author: hjam2402@mail.usyd.edu.au

Key words: Pink Lady, Apple, Chilling Injury, Storage Disorder, Cripps Pink, Flesh Browning, Scanning Electron Microscopy

Abstract

The Flesh Browning (FB) disorder of Cripps Pink apples can be divided into two distinct disorders, Radial and Diffuse FB, based on a number of structural and physiological differences. The type of FB observed is strongly influenced by climatic conditions during fruit growth and development. The Incidence and severity of symptoms of both types of FB during storage are influenced by a combination of pre and postharvest factors. The growing degree days (GDD) accumulated from full bloom to harvest determines the type of FB observed during storage. A high risk of developing Diffuse FB occurs in seasons or districts experiencing <1200 GDD above 10°C from full bloom to harvest. It is suggested that cool growing seasons desynchronise fruit ripening processes and result in fruit that are susceptible to chilling injury during storage. A high risk of developing Radial FB occurs in seasons with low GDD accumulation during the first 50 days after full bloom which have been found to be related to fruit density and gas diffusivity. The GDD during the 60 days prior to harvest maturity relates to a risk of developing chilling injury during storage and affects both types of FB. Postharvest factors which influence the development of Radial FB include storage at low temperatures, 0°C and concentration of CO₂ in the storage atmosphere. The primary postharvest factor influencing incidence and severity of both types of FB is storage temperature. Fruit stored at 3°C showed a significantly lower incidence of both types of FB than those stored at 0°C. Scanning electron microscopic analysis was carried out to determine structural differences between the two expressions of FB.

INTRODUCTION

Flesh Browning (FB) in Cripps Pink apples has become a significant economic problem for both domestic and export markets in Australia since the disorder was first recognized in 2001. The FB disorder in Cripps Pink apples has been investigated since 2003 and a range of similar postharvest browning disorders in other apple cultivars have facilitated the identification of possible causes. Three types of FB in Cripps Pink apples have currently been identified which can be related to a number of pre and postharvest conditions (James et al., 2005a; James et al., 2005b; James et al., 2005c; Jobling et al., 2004).

The first, CO₂ injury, appears to be similar to CO₂ injury in other apple cultivars. This type of FB is characterized by the appearance of defined areas of brown tissue throughout the fruit cortex with the appearance of lens shaped pits and cavities occurring with increasing severity of symptoms. CO₂ injury in Cripps Pink apples is aggravated by controlled atmosphere (CA) conditions with increasing levels of CO₂ and decreasing levels of O₂ in the storage

atmosphere (James et al., 2005b; Jobling et al., 2004). The effect of CA conditions on the incidence of CO₂ injury has been previously observed in other apple cultivars (Argenta et al., 2000; Johnson et al., 1998; Park and Lee, 1992). Due to the similarity between the CO₂ injury observed in Cripps Pink apples to that observed in other apple cultivars, this paper will primarily focus on the other 2 expressions of FB.

The second type of FB, Radial FB (RFB), appears to be similar to a type of senescent or vascular breakdown described in other apple cultivars (Meheriuk et al., 1994) and is aggravated by the storage temperature and CA conditions. High internal CO₂ and low O₂ have been implicated in similar browning disorders such as Braeburn Browning Disorder (BBD) (Clark and Burmeister, 1999). CA related browning of 'Elstar' (Streif and Saquet, 2003) and 'Fuji' (Grant et al., 1996) apples has also been related to CO₂ in the storage atmosphere. RFB is characterized by brown discoloration associated with the vascular tissue throughout the fruit cortex.

The third form of FB, Diffuse FB (DFB), is likely to be the direct result of a chilling injury. Similar chilling injuries have been observed in other apple cultivars; 'Cortland' apples have been found to be sensitive to low temperatures in storage but relatively insensitive to increased CO₂ in the storage atmosphere (DeEll and Prange, 1998). DFB is characterized by brown discoloured and damaged tissue throughout the fruit cortex tissue and in contrast to RFB, the vascular tissue was found to remain relatively unaffected.

Climatic conditions during fruit growth and development have been related to the occurrence of similar storage disorders of apples (DeEll, 2005; Elgar et al., 1999; Johnson and Ridout, 1998; Lau, 1998). Seasonal climatic conditions have been found to influence the development of the three types of FB in Cripps Pink apples (James et al., 2005c). Climatic conditions experienced during specific periods of fruit growth and development of Cripps Pink apples may be used to determine the risk of developing FB in a district or season.

The first climatic risk period is during the first 50 days from full bloom. Low temperatures during this time period have been shown to increase the period of cell division, resulting in fruit with an increased density and consequently reduced gas diffusivity (Lau, 1998). Lau (1998) also indicates that apple cultivars with a relatively high cell density, such as the Braeburn cultivar, are more susceptible to internal injury under CA conditions. This climatic period is related to the risk of developing CA related RFB in Cripps Pink apples (James et al., 2005c).

Climatic conditions during the period from full bloom to harvest have also been related to the development of storage disorders of apples (Lau, 1998). The Growing Degree Days (GDD) above 10°C accumulated during this time may be used to determine the risk of developing the type of FB encounter in a district or season.

The third climatic risk period is the last 60 days prior to harvest. The GDD during the this climatic risk period has been implicated in the risk of the development of chilling injuries in other apple cultivars (DeEll, 2005; Johnson and Ridout, 1998; Sharples, 1975). Seasons or districts with a low GDD during the 60 days prior to harvest have been shown to be more susceptible to developing chilling injuries during storage (DeEll, 2005; Johnson and Ridout, 1998; Sharples, 1975). As both RFB and DFB are associated with cool storage temperatures, this climatic period is speculated to be related to the development of both types of FB.

Previous work has shown that the FB disorder in Cripps Pink apples is influenced by the climate of the growing district as well as by the composition of the storage atmosphere and the fruit maturity at harvest (James et al., 2005a; James et al., 2005b; James et al., 2005c; Jobling et al., 2004). One of the objectives of this work was to use data from multiple seasons and storage

trials to establish risk factors associated with the development of FB. The secondary objective was to establish the structural differences between the cellular damage observed between RFB and DFB.

MATERIALS AND METHODS

Induction and assessment of types of browning

Fruit were sourced from two apple growing districts in Australia; Batlow, New South Wales (35°31'S 148°09'E) and the Huon Valley, Tasmania (43°16'S 146°92'E). The fruit were harvested at two maturities (starch pattern indices 3.5 and 8.5 on the CTIFL 10 point starch pattern index scale). Half of 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 to induce flesh browning (Jobling et al., 2004). Fruit were stored in a randomised block design at either 0 or 3°C. There were four replicates per maturity, wax, and temperature combination with 25 fruit per replicate. Eight hundred fruit were assessed for internal flesh browning for each maturity per region (a total of 3200 fruit). Fruit were assessed for browning after being held at 20°C for 7 days (James et al., 2005c).

Climatic risk

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. Air temperatures were collected as, at a minimum, hourly means in all locations. These were used to calculate the GDD above 10°C for each month (James et al., 2005c).

Microscopy

Sections of apple tissue were taken from apples sourced from Batlow (New South Wales) and the Huon Valley (Tasmania). Apples had been stored for 9 months at 0°C in air. Sections of vascular and cortex tissue from undamaged fruit and fruit affected by radial and diffuse types of flesh browning were analysed. For SEM, apples were fixed with a 25 g.l⁻¹ glutaraldehyde solution (0.1M phosphate buffer, pH 7.2) for 1 hr, postfixed with 10 g.l⁻¹ OsO₄ 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 100 kV.

RESULTS AND DISCUSSION

Previous results have shown that the incidence and severity of the symptoms of FB in Cripps Pink apples are dependent on the climatic conditions of the growing location (James et al., 2005c). The results showed that district seasonal conditions can be related to the development of FB during storage. Climatic conditions during fruit growth and development are known to affect the biophysical properties of fruit such as cellular structure, tissue density, air content, gas permeability and wax cuticle properties (Stanley et al., 2000). For the 2005 season, fruit were sourced from 2 growing districts in Australia, one with a known susceptibility to RFB (Batlow, NSW mean GDD: 1557) and the other with a known susceptibility to DFB (the Huon Valley, Tasmania mean GDD: 908) to further establish that the 2 types of FB could be defined

by specific climatic conditions during growth. As had previously been found, fruit sourced from the Huon Valley in 2005 consistently showed symptoms of DFB and those sourced from Batlow consistently showed symptoms of RFB confirming that the susceptibility to each type of FB is district dependent.

The climatic conditions of each district were assessed during 3 key periods of fruit growth and development to establish the relative risk that each period provided to the development of FB during storage. The differentiation of climatic conditions on their influence on the development of each disorder allows for the classification of a season or district as having a risk for a specific type of FB during storage. It is likely that each climatic period during fruit growth and development may only partially contribute to a more complex situation in which interactions between climatic conditions during other key periods of fruit growth and development may also contribute to the potential risk of FB, as has been described by Volz et al (2000; 1999) for the prediction of BBD.

Previously reported results from a single season illustrated a clear relationship between the GDD from full bloom to harvest and the type of FB symptoms observed (James et al., 2005c). Districts or seasons experiencing less than 1200 GDD have been found to develop symptoms of DFB (James et al., 2005c). In the Huon Valley, the 2004 and 2005 growing seasons accumulated 888.89 and 904.07 GDD respectively (Table 1) classifying this district as being at risk of developing DFB. In contrast, districts or seasons experiencing greater than 1200 GDD have been found to develop symptoms of RFB (James et al., 2005c). In Batlow, the 2004 and 2005 growing seasons accumulated 1567.21 and 1462.88 GDD respectively (Table 1) classifying this district as being at risk of developing RFB.

Seasonal comparison of the accumulation of GDD from full bloom to harvest and the incidence of DFB and RFB indicated that seasons with reduced GDD had an increased incidence of FB. The Huon Valley accumulated fewer GDD in the 2004 season than in the 2005 season and associated with the reduced GDD was a higher mean incidence of DFB (Table 1). Similarly, seasonal comparison of the mean incidence of RFB and the GDD from full bloom to harvest in Batlow showed that the season with the higher incidence of RFB was associated with the reduced accumulation of GDD (Table 1). Cool growing conditions may alter cellular metabolism, reduce skin and tissue diffusivity and/or increase susceptibility to elevated CO₂ and low O₂ (Lau, 1998). Cool growing seasons have increased the risks of core browning in 'Cox's Orange Pippin' and 'McIntosh' and of low temperature breakdown in 'Bramley's Seedling', 'Cox's Orange Pippin', 'Jonathan' and 'Yellow Newton' apples (Lau, 1998).

The risk factors may also relate to the climatic regulation of specific growth phases. For example the climatic period during the first 50 days after full bloom is the period of cell division and has been associated with the propensity to develop RFB during storage (James et al., 2005c). Cooler temperatures during this time have been found to extend the period of cell division which results in denser fruit at harvest (Little and Holmes, 2000). Such fruit have been found to accumulate elevated levels of internal CO₂ during CA storage (Lau, 1998) and consequently have an increased risk of developing RFB. In comparison to the 2004 season, Batlow experienced a cooler period of growth during this period in 2005 and accumulated only 178.83 GDD (Table 1). Associated with this cooler period of growth in the early season was an increased fruit density (Table 1) and an increased incidence of RFB (2004: 27.8%, 2005: 57.5%). No clear trend has been identified for fruit density and DFB.

Another important phase is the climatic period during the last 60 days before harvest which has been linked both to the risk of the harvest of over mature fruit and to the risk of the

development of chilling injury during storage. Maturity at harvest has been shown to influence RFB and DFB with later harvested fruit having a higher incidence of FB (James et al., 2005b). The GDD during this period have been associated with the development of chilling injury during storage where seasons or districts with cool temperatures were found to be at increased risk (DeEll, 2005). In comparison to the 2004 season, the Huon Valley accumulated a higher number of GDD during this period in the 2005 season (2004: 232.07 GDD, 2005: 272.76 GDD). Associated with the warmer season was a lower incidence of DFB (2004: 94.5%, 2005: 75.6%). Similarly in Batlow, the season with the higher GDD during the last 60 days before harvest was associated with a lower incidence of RFB during storage, suggesting that RFB is also associated with chilling injury.

Due to the indication that both types of FB were related, at least in part, to chilling injury, in the 2005 season fruit from the Huon Valley and Batlow were stored at 0°C and 3°C to determine the influence of storage temperature on the development of both RFB and DFB. After 7 months, storage at 3°C was found to significantly reduce the incidence of both RFB and DFB (Table 2). The effect of increasing the storage temperature was found to be most effective on fruit sourced from the Huon Valley where the incidence of DFB for fruit of early maturity and stored at 0°C was reduced from 75.7% to 1.5%. This data confirms that DFB is a chilling injury and indicates the significance of the GDD during the last 60 days before harvest on the development on this type of FB. In Batlow, the incidence of RFB was also significantly reduced from 57.5% to 30.7% however the incidence of RFB at 3°C remained higher than the commercial threshold level of 2%. This data indicates that RFB is strongly influenced by storage temperature, however is also related to other climatic, orchard and storage conditions. One risk factor that may predispose fruit to developing FB during storage is the physical structure of the fruit.

Scanning Electron Microscopy (SEM) has been used to determine the cellular damage associated with each type of FB. It was found, in support of earlier visual observation, that RFB was associated with the vascular tissue (Figure 1) while DFB was not located near the vascular tissue but found throughout the fruit cortex (Figure 2). The type of damage observed was also seen to be different for the different types of FB. In fruit affected by DFB, the areas of brown tissue were observed by SEM to be extensive cell collapse (Figure 2b) while in fruit affected by RFB, the damage was observed as fractured cell walls (Figure 1b). Membrane damage and cell collapse have been observed in relation to the development of chilling injuries in other horticultural crops (Luza et al., 1992; Marangoni et al., 1996; Sharom et al., 1994) further supporting the definition of DFB as a chilling injury. The damaged observed by SEM in association with RFB however, could not definitively be classified as no previous published studies using SEM on apples have reported symptoms similar to the RFB seen in Cripps Pink apples.

CONCLUSION

The results from this study show that the structural and physiological differences between RFB and DFB. This research confirms that DFB is a chilling injury and indicates that RFB is strongly influenced by storage temperature but is also influenced by an as yet undefined combination of pre and postharvest conditions. The short duration of this project means that it is difficult to draw clear conclusions on the effect of the climatic conditions on the development or expression of FB, particularly in relation to RFB. The SEM images show distinctive structural

differences in the type of damage for RFB and DFB, this is a clear indication that the disorders are unique despite having similar risk factors.

ACKNOWLEDGEMENTS

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Tables

Table 1.

Growing Degree Days >10°C for Tasmania and Batlow districts and fruit density. Numbers followed by a different letter are significantly different at a 5% level of significance.

Season	Tasmania		Batlow	
	2004	2005	2004	2005
Growing Degree Days >10°C				
Entire season	888.89	904.07	1567.21	1462.88
Early Season	81.67	169.03	279.20	178.83
Late Season	232.07	272.76	461.05	357.60
Density (kg.m ⁻³)	#### a	#### a	#### ab	#### b

Table 2.

Incidence of flesh browning for Tasmania and Batlow districts. Fruit stored in air at either 0°C or 3°C, M1: SPI 3.5, M2: SPI 8.5. Significance is restricted to each column and numbers followed by a different letter are significantly different at a 5% level of significance.

Maturity	Wax	Temperature	Incidence of Flesh Browning			
			Tasmania		Batlow	
M1	unwaxed	0°C	75.7	a	57.5	bc
M1	waxed	0°C	69.5	ab	53.3	c
M2	unwaxed	0°C	59.6	b	66.3	b
M2	waxed	0°C	73.5	a	85.7	a
M1	unwaxed	3°C	1.5	cd	30.7	d
M1	waxed	3°C	0.5	d	35.2	d
M2	unwaxed	3°C	3.5	cd	33.5	d
M2	waxed	3°C	8	c	32.1	d

Figures

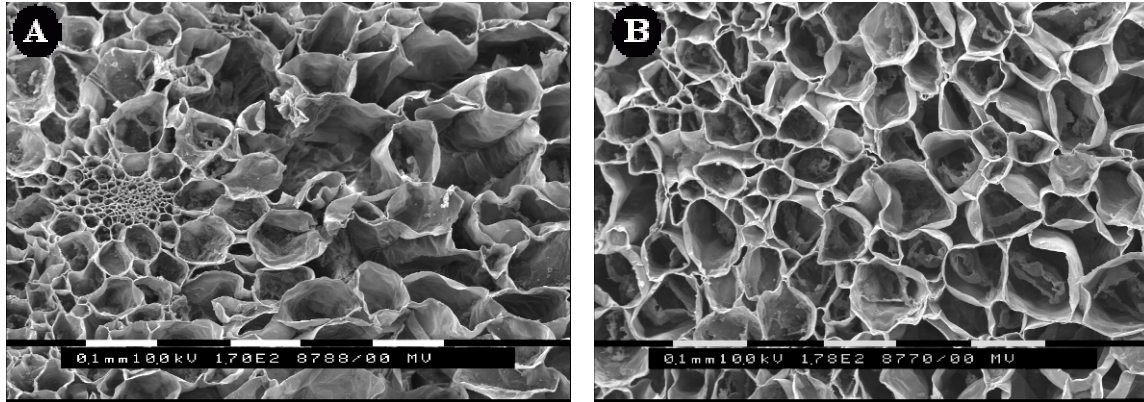


Figure 1. Ultrastructure of apple tissue from fruit affected with Radial flesh browning **A.** Vascular tissue **B.** Cortex tissue

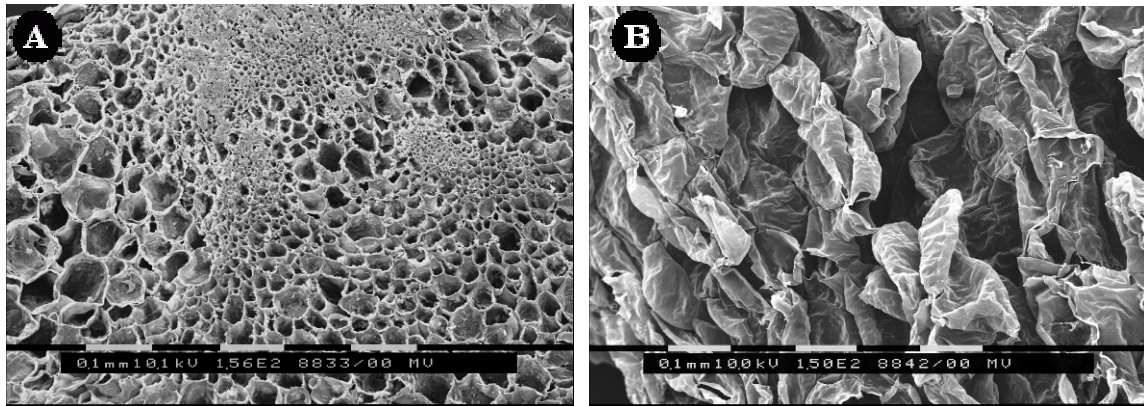


Figure 2. Ultrastructure of apple tissue from fruit affected with Diffuse flesh browning **A.** Vascular tissue **B.** Cortex tissue

6. Climatic Conditions validated from the 2007 storage season,

Sydney Postharvest Laboratory
 Michael Forbes-Smith & Stephen Morris
 12 December 2007

Background

Incidence of internal flesh browning in Cripps Pink apples (Pink Lady™) during storage has caused considerable economic losses both domestically and internationally. Radial and diffuse types of flesh browning develop in Pink Lady™ apples. Seasonal, orchard and storage factors have been shown to influence Pink Lady™ apples to develop flesh browning.

The aim of this study was to provide further data to improve and validate recommendations for the management of radial and diffuse flesh browning in Pink Lady™ apples, in particular climatic conditions (grower degree days) during fruit growth, and storage temperature.

Methods

Orchard details

Cripps Pink apples were sourced from the following two properties in 2007:

Batlow – The fruit were harvested from the same commercial orchard at Batlow (35°31'S 148°09'E) used in the 2004, 2005 and 2006 seasons, the trees were eight years old and grown and managed using current commercial practices.

Huon Valley – The fruit were harvested from a commercial orchard in the Huon Valley (43°16'S 146°92'E) within four kilometres of the orchard used in the 2004 and 2005 seasons. The trees were grown and managed using current commercial practices. The trees were on MM106 rootstocks with M9 interstems for vigour control.

Maturity and quality evaluation

Maturity and quality characteristics of fruit were measured at harvest (*ie.* directly after arrival to SPL), after 4 and 7 months storage at 0, 1 and 3°C in air for apples from Huon Valley, and after 4 and 7 months storage at 0, 1 and step-wised cooled (2 weeks at 3°C, 2 weeks at 2°C, thereon at 1°C) in air for apples from Batlow. Maturity and quality assessments included:

- starch point index (SPI), as measured on the CTIFL 10 point SPI scale.
- internal ethylene concentration (IEC), using GC/FID
- flesh firmness, using a drill-press mounted Effegi penetrometer fitted with an 11mm tip
- background colour, using the Ctifl Pink Lady™ colour chart
- skin greasiness (rating scale: 1=not greasy, 2=slightly greasy, 3=very greasy)

Twenty fruit were assessed for each district, temperature and storage combination. Assessments were carried out on the day that the fruit were removed from storage permitting 3hrs for the fruit to warm to 20°C (10 fruit) and after 7 days at 20°C (10 fruit).

Flesh browning determination

Radial flesh browning (RFB) and diffuse flesh browning (DFB) were assessed at 3 positions in the fruit - stem end, middle and calyx end of transverse sections of the fruit. Flesh browning was measured as the percentage of fruit with symptoms, regardless of severity. 125 fruit were assessed for each district, temperature and storage combination. A further 125 fruit were cut open to determine possible incidence of browning at harvest. All samples were held at 20°C for 7 days before evaluation.

Statistical analysis

Data were analysed by analysis of variance using SIMSTAT statistical software package. When F ratios indicated a significant effect, means were compared using the Waller-Duncan k-ratio LSD rule. The k-ratio LSD for the test of significance was calculated at k-ratio of 100:1, which is generally considered to take the place of the 5% level of significance. Flesh browning incidence percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation. Internal ethylene concentration data were transformed to common logarithm for analysis and back-transformed to linear scale for reporting.

Results

Fruit maturity at harvest

Starch content, indicated by the SPI, is considered to be the most valuable index of harvest maturity in Pink Lady™ apples. In samples of fruit at harvest from Batlow, the SPI mean score was about 4, which is close to the intended 3.5 SPI. The apples from the Huon Valley were harvested at a more mature stage with an SPI mean score of 6.5 (Table 1), this is more mature than the intended 3.5 SPI but lower than the 8.5 SPI used as the higher level of maturity in previous results.

Quality assessment	Apples (mean score)			
	Batlow		Huon Valley	
	At harvest	7d@20°C	At harvest	7d@20°C
SPI (Ctfl 10 point)	4.0	6.8	6.5	9.2
Flesh firmness (N)	88.5	82.0	95.8	105.5
Background colour (Ctfl)	4.7	4.4	5.5	6.3
IEC (µl/L)	0.00	0.01	0.62	63.76
Skin greasiness	1.0	1.0	1.0	2.0

Table 1 Maturation parameters of Pink Lady™ apples harvested at Batlow and Huon Valley, 2007.

Fruit from Batlow also showed no signs of internal ethylene production, which suggests the fruit were pre-climacteric. Even after 7 days at 20°C (to simulate transport conditions and retail marketing), only 5% of the fruit produced ethylene and at very low concentrations (mean IEC of fruit: 0.01µl/L). However, apples from the Huon Valley showed higher levels of internal ethylene (mean IECs at harvest and after 7 days at 20°C were 0.62 and 63.8µl/L, respectively), indicating the fruit were within the climacteric phase and maturing rapidly. In addition, the background skin colour of apples at harvest from Huon Valley (mean SPI score of 5.5) was less

green than fruit at harvest from Batlow (mean SPI score: 4.7), suggesting apples from the Huon Valley were from a late harvest and suited for the fresh market.

Skin from apples from both growing areas at harvest were not greasy (mean score:1.0), although skin greasiness of apples from the Huon Valley increased to a mean score of 2.0, after 7 days at 20°C. Mean flesh firmness for fruit at harvest from Huon Valley was 95.8N, slightly higher than fruit at harvest from Batlow (mean score: 88.5N).

Fruit quality and storage

Flesh firmness, skin greasiness, IEC and background colour are important quality measurements to determine market acceptability of Pink Lady™ apples. Flesh firmness decreased with storage time for apples grown in Batlow (Fig. 1) and the Huon Valley (Fig. 2). In Batlow apples, storage temperatures (0, 1 and stepwise cooling to 1°C) had little influence on fruit firmness, whereas apples from the Huon Valley stored at 3°C tended to be firmer than those stored at 0 or 1°C for 4 and 7 months. Apples held at 20°C for 7 days following storage for 4 or 7 months caused a slight decrease in firmness, depending on the storage temperature.

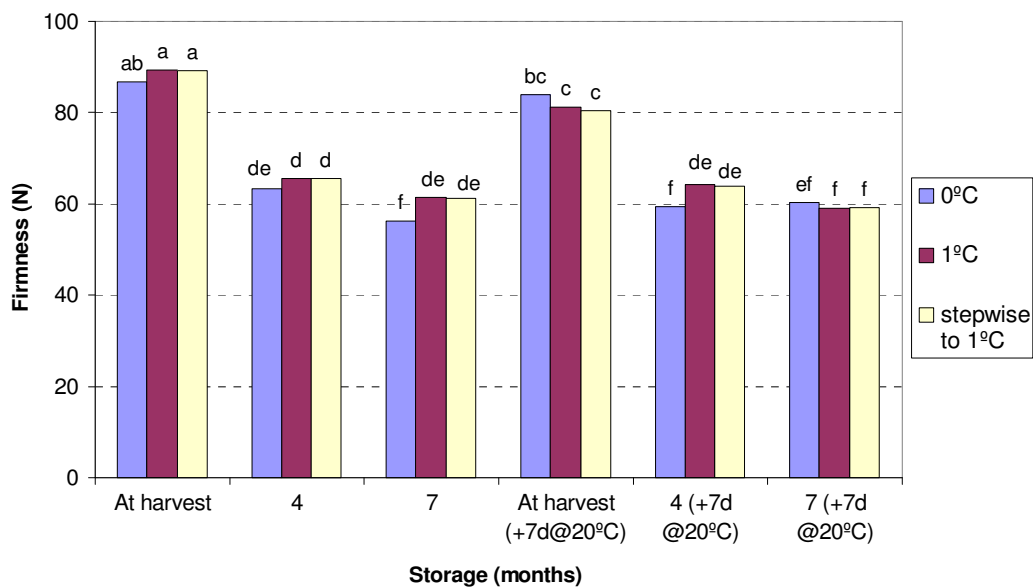


Fig. 1 Effects of storage temperature and time in air on the flesh firmness (N) of Pink Lady™ apples grown in Batlow NSW. Columns represented by a different letter are significantly different at k-ratio LSD at k=100 or approximately 5% level of significance

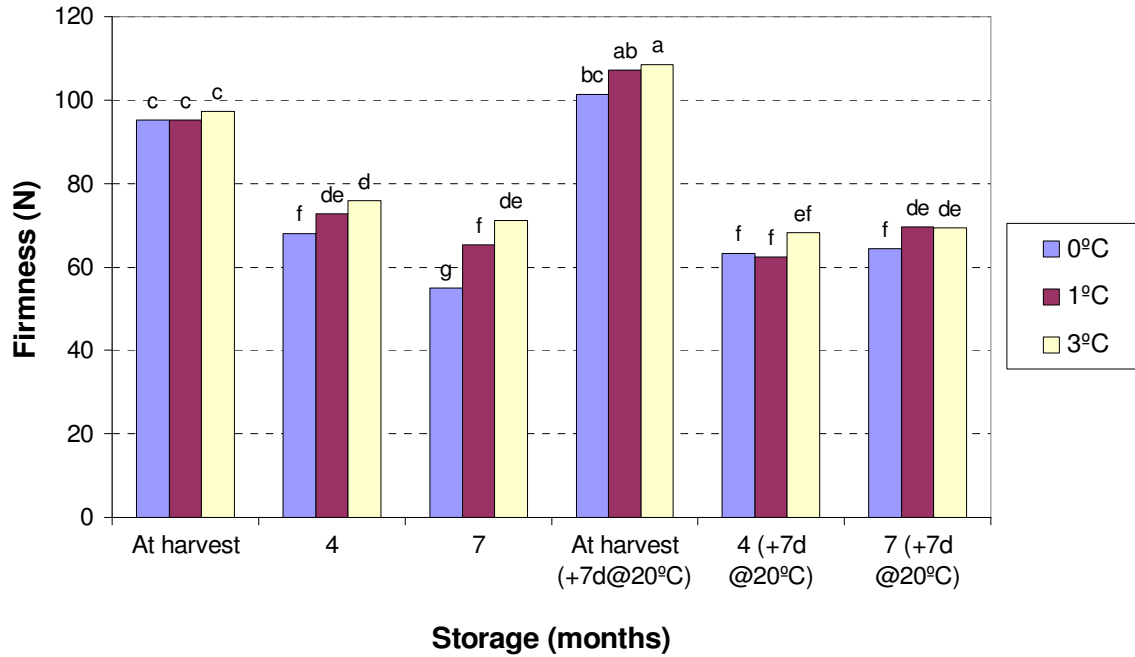


Fig. 2 Effects of storage temperature and time in air on the flesh firmness (N) of Pink Lady™ apples grown in the Huon Valley Tasmania. Columns represented by a different letter are significantly different at k-ratio LSD at k=100 or approximately 5% level of significance

Skin greasiness increased slightly with storage time for apples grown in Batlow (Fig. 3) and the Huon Valley (Fig. 4). Storage temperature had no significant effect on the level of skin greasiness in apples from Batlow, either stored for 4 or 7 months. The apples from the Huon Valley apples had a significantly higher level of greasiness for fruit stored at 3 °C for 4 months than fruit stored at 0 or 1 °C , however after all 7 months fruit from all storage temperatures had similar levels of greasiness.

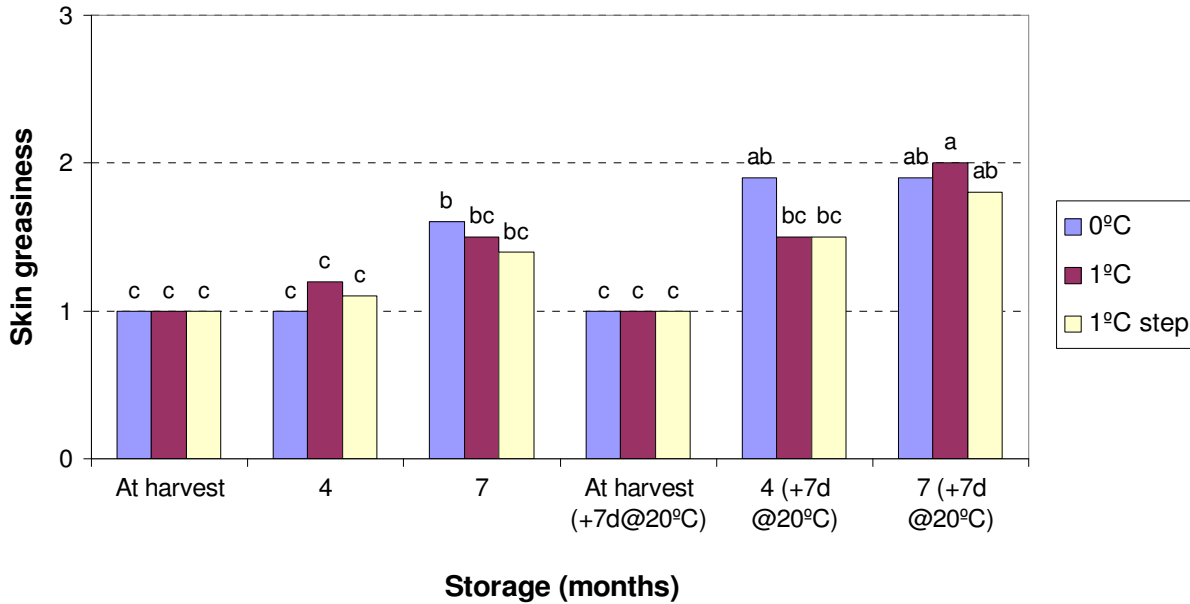


Fig. 3 Effects of storage temperature and time in air on skin greasiness (rating scale: 1=not greasy, 2=slightly greasy, 3=very greasy) of Pink Lady™ apples grown in Batlow NSW. Columns represented by a different letter are significantly different at k-ratio LSD at k=100 or approximately 5% level of significance.

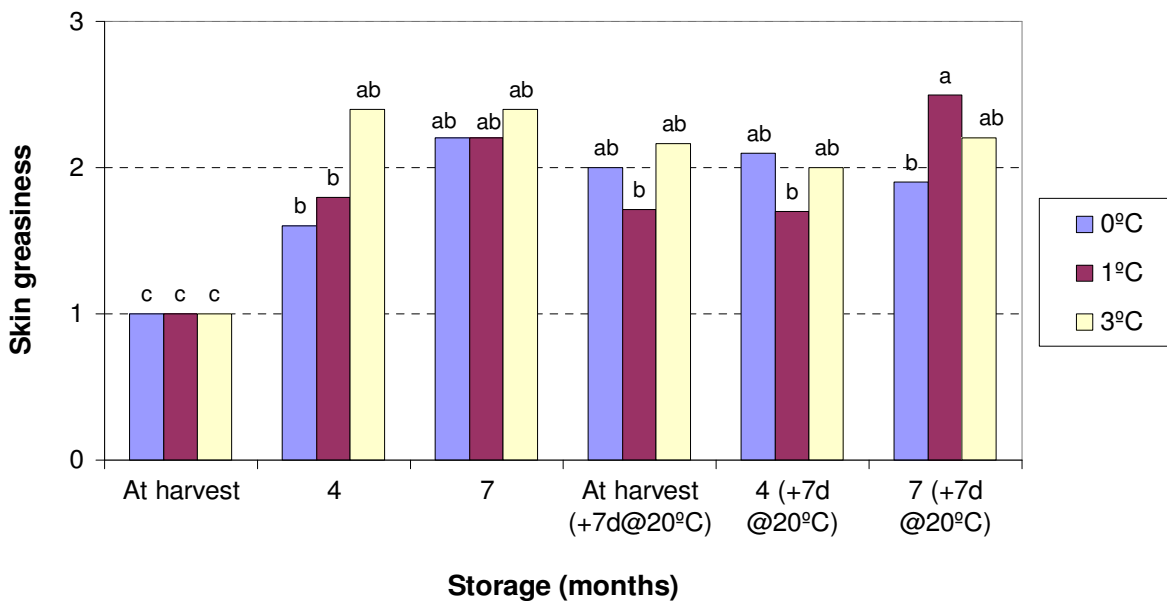


Fig. 4 Effects of storage temperature and time in air on skin greasiness (rating scale: 1=not greasy, 2=slightly greasy, 3=very greasy) of Pink Lady™ apples grown in the Huon Valley Tasmania. Columns represented by a different letter are significantly different at k-ratio LSD at k=100 or approximately 5% level of significance

Internal ethylene concentrations in apples from Batlow and the Huon Valley increased rapidly from harvest to 4 months storage (Table 2), with little further increase in IEC after 7 months storage. The only significant difference was an increase in Batlow apples stored at 0°C, which showed significantly higher levels of internal ethylene (mean IEC at 0°C for 7 months storage: 155.47 µl/L).

Storage duration (months)	Storage temperature (°C)	Internal Ethylene Concentration (µl/L)			
		Batlow		Huon Valley	
		Day 1	Day 7	Day 1	Day 7
At harvest	0	0 d	0.03 d	0.67 d	92.33 c
	1	0 d	0 d	0.51 d	47.33 c
	step-cooled 1°C 3	0 d	0 d	0.68 d	51.62 c
4	0	76.75 c	124.73 b	168.64 b	354.87 ab
	1	54.63 c	133.62 ab	184.41 b	300.43 ab
	step-cooled 1°C 3	72.60 c	95.84 bc	198.13 ab	416.73 a
7	0	155.47 ab	201.14 a	229.69 ab	365.97 ab
	1	79.10 c	139.80 b	259.25 ab	289.35 ab
	step-cooled 1°C 3	109.54 bc	166.23 ab	200.88 b	408.98 ab

Table 2 Effects of storage temperature and duration on the internal ethylene concentration of Pink Lady™ apples grown in Batlow and the Huon Valley following up to 7 months storage in air. Values represented by a different letter are significantly different at k-ratio LSD at k=100 within each district (column). Fruit were removed from storage and assessed allowing 3h to warm to 20°C (Day 1) and after 7 days at 20°C (Day 7).

Storage temperature had an inconsistent effect on background colour of apples, although apples became gradually less green with increasing storage time (Figs. 5 and 6).

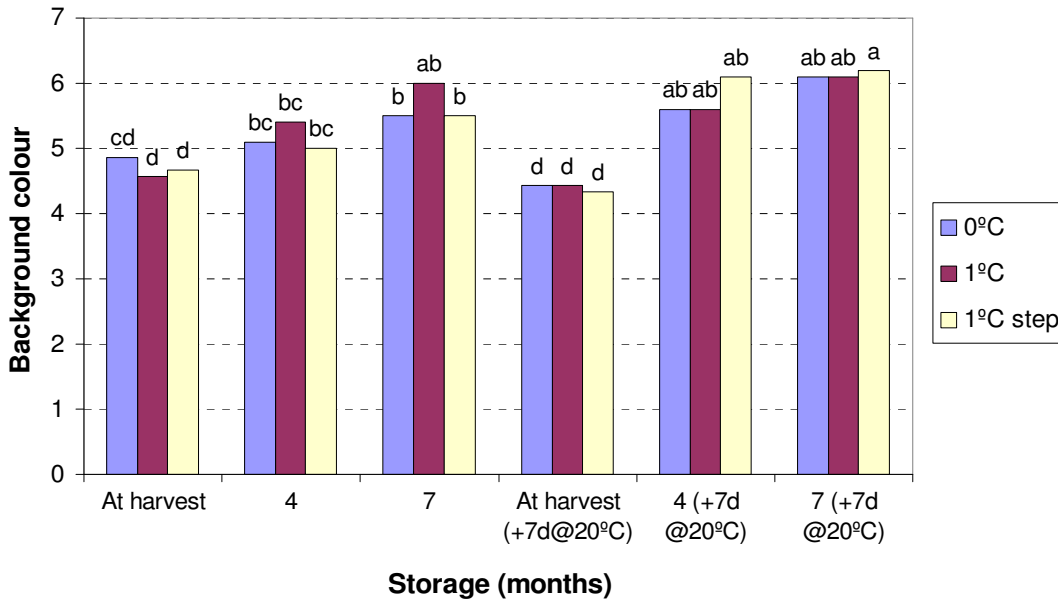


Fig. 5 Effects of storage temperature and time in air on background colour (rating scale: Ctifl Pink Lady™ colour chart) of Pink Lady™ apples grown in Batlow NSW. Columns represented by a different letter are significantly different at k-ratio LSD at k=100 or approximately 5% level of significance

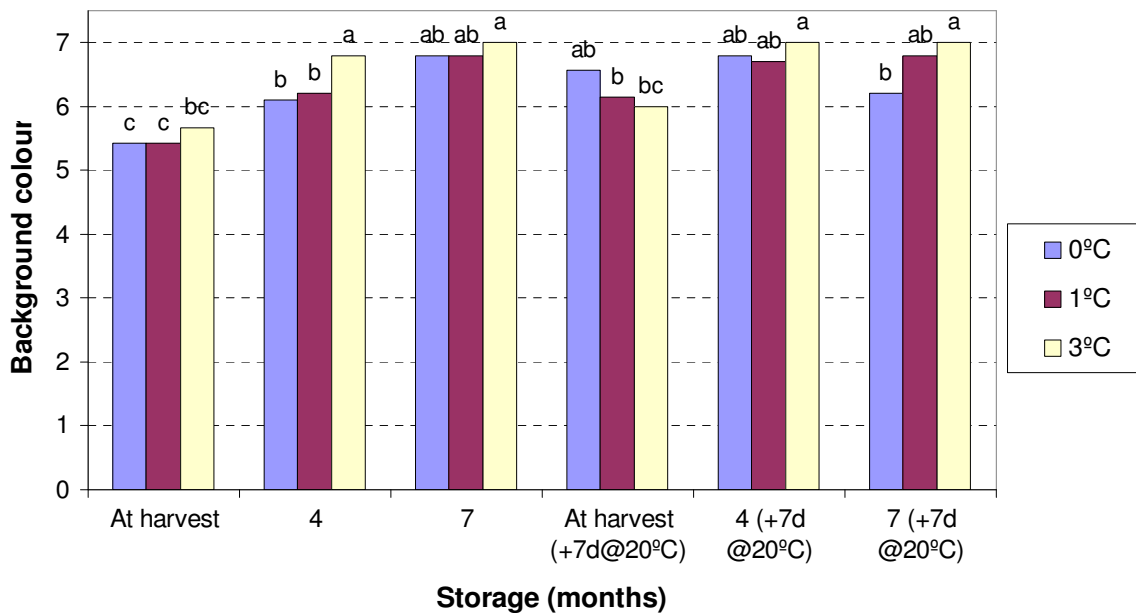


Fig. 6 Effects of storage temperature and time in air on background colour (rating scale: Ctifl Pink Lady™ colour chart) of Pink Lady™ apples grown in the Huon Valley Tasmania. Columns represented by a different letter are significantly different at k-ratio LSD at k=100 or approximately 5% level of significance

Flesh browning

At harvest, no radial or diffuse flesh browning symptoms were detected in any Pink Lady™ apples grown in either Batlow or the Huon Valley (Fig. 5). Apples from Batlow, stored at 0, 1 and step-wise 1°C, and apples from the Huon Valley, stored at 0, 1 and 3°C, were further assessed for RFB and RDB after 4 and 7 months.

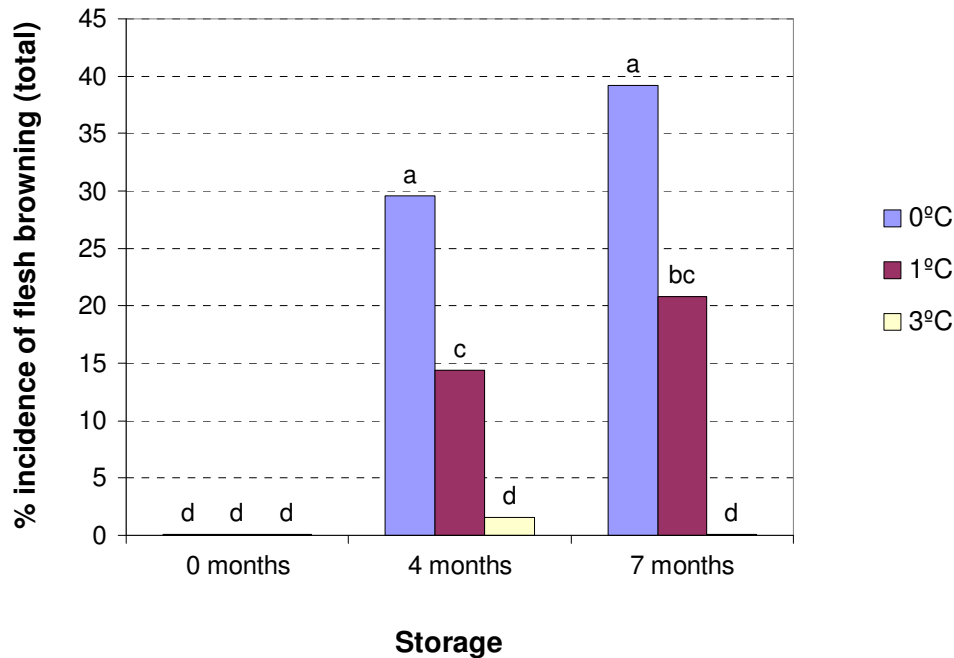


Fig. 5 Effect of storage temperature on total flesh browning in Pink Lady™ apples from the Huon Valley after 4 and 7 months storage. Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation. Columns represented by a different letter are significantly different at k-ratio LSD at k=100 or approximately 5% level of significance

Apples from Batlow showed no incidence of flesh browning at any temperature or storage time. However, apples from the Huon Valley showed high levels of total flesh browning when stored at 0°C (Fig. 5), with levels increasing slightly from 4 to 7 months. Increasing storage temperatures to 1 °C effectively halved levels of flesh browning, however, increasing storage temperature to 3°C, reduced flesh browning to non-significant levels.

Radial flesh browning: It should be noted that while reference pictures used in previous years for Radial Flesh Browning and Diffuse Flesh Browning were used in all these assessments, it is very difficult to distinguish symptoms of less severe RFB from less severe DFB. Apples from Batlow showed no incidence of RFB at any storage time or temperature. However, in apples from the Huon Valley, the incidence of RFB after 4 months storage at 0°C was 8%, which doubled to 16% following 7 months at 0°C storage (Fig. 6). RFB was also occurred in fruit stored at 1°C, but to a

lesser extent. For example, incidence of RFB in fruit stored at 1°C for 4 and 7 months was 4 and 7.2%, respectively; no RFB was detected in any apples stored at 3°C, regardless of storage time.

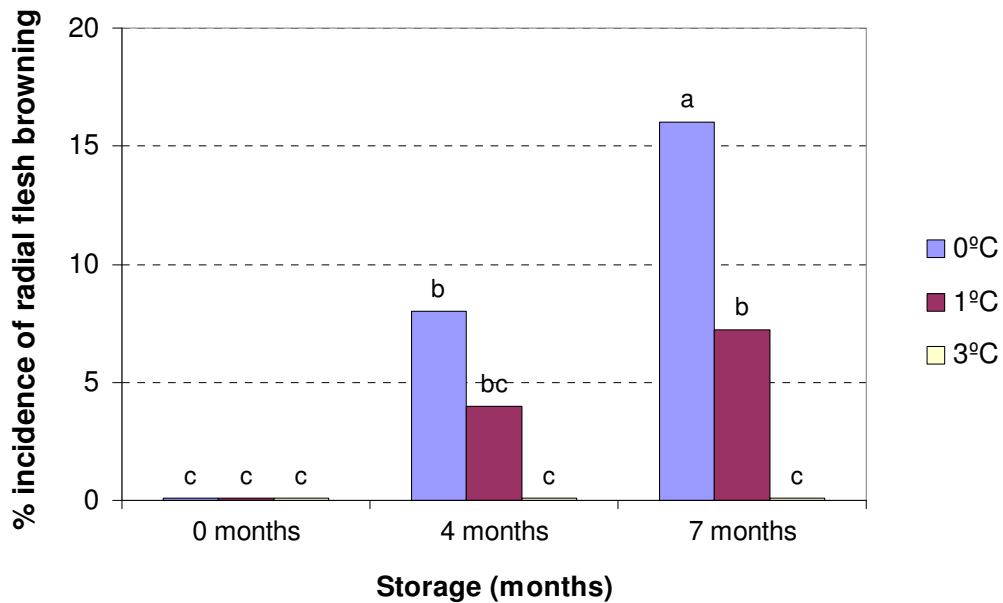


Fig. 6 Effect of storage temperature on radial flesh browning in Pink Lady™ apples from the Huon Valley after 4 and 7 months storage. Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation. Columns represented by a different letter are significantly different at k-ratio LSD at $k=100$ or approximately 5% level of significance

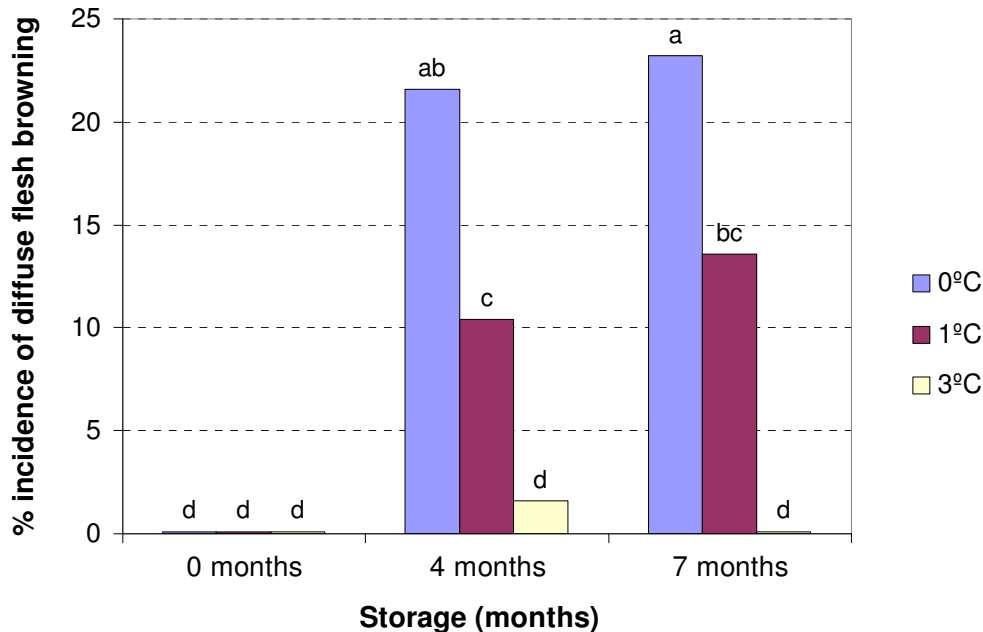


Fig. 7 Effect of storage temperature on diffuse flesh browning in Pink Lady™ apples from the Huon Valley after 4 and 7 months storage. Percentage data were transformed to angles ($Y = \sin^{-1}\sqrt{\%/100}$) for analysis and back-transformed to % for presentation. Columns represented by a different letter are significantly different at k-ratio LSD at $k=100$ or approximately 5% level of significance.

Diffuse flesh browning: No DFB was observed in apples from Batlow for any storage temperature or duration. In contrast, not only was DFB present in apples from the Huon Valley but at a considerably higher incidence than RFB (Fig. 7) (see comment about RFB & DFB above). For example, incidence of DFB in apples stored at 0°C for 4 and 7 months was 21.6 and 23.2%, respectively. Warmer storage temperatures considerably decreased the incidence of DFB, especially storage at 3°C, which reduced development of DFB in fruit after 4 and 7 months to non-significant levels.

Growing Degree Days

In the 2006/2007 growing season there was no readily available weather record for the farmers properties. However, reasonably accurate estimates of GDD >10°C can be determined from nearby Bureau of Meteorology (BOM) Weather Stations. This was done using daily maximum and minimum via the well established formula $GDD > 10C = (Maximum + Minimum)/2 - 10$. The totals for each day were summed over the season from flowering to harvest to give GDD for the season.

For Huon Valley there was a BOM weather station in the centre of the valley (Grove (Comparison) Site 094069, Latitude 42.98 °S Longitude 147.08 °E, Elevation: 63 m), this was located within 8 km of the orchard used this year and at a very similar elevation. However, for Batlow unfortunately the nearest BOM weather station is a Tumbarumba which is 30 km SSE of Batlow (Tumbarumba Post Office, Site 072043, Latitude 35.78 °S, Longitude 148.01 °E,

Elevation: 645 m). This elevation is similar to the lower orchards in the Batlow area. There are well established protocols for estimating temperatures by extrapolation from nearby weather stations (Stahl, Moore, Floyer, Asplin and McKendry 2006). By using historical data for four weather stations in the Batlow area (Tumut Plains (Homesdale), Green Hills State Forest, Kunama and Bago (Pilot Hill)) and comparing to historical data from Tumbarumba, correction factors based on proximity and elevation differences can be determined. For the weather stations in the Batlow area relative to Tumbarumba, this was found to be 6.0 °C decrease in temperature for each 1000m increase in elevation. Since the Batlow district is unusual in that there is a wide range of elevations between orchards (from below 550m to 1000m), two GDD calculations were made for low altitude orchards and high altitude orchards.

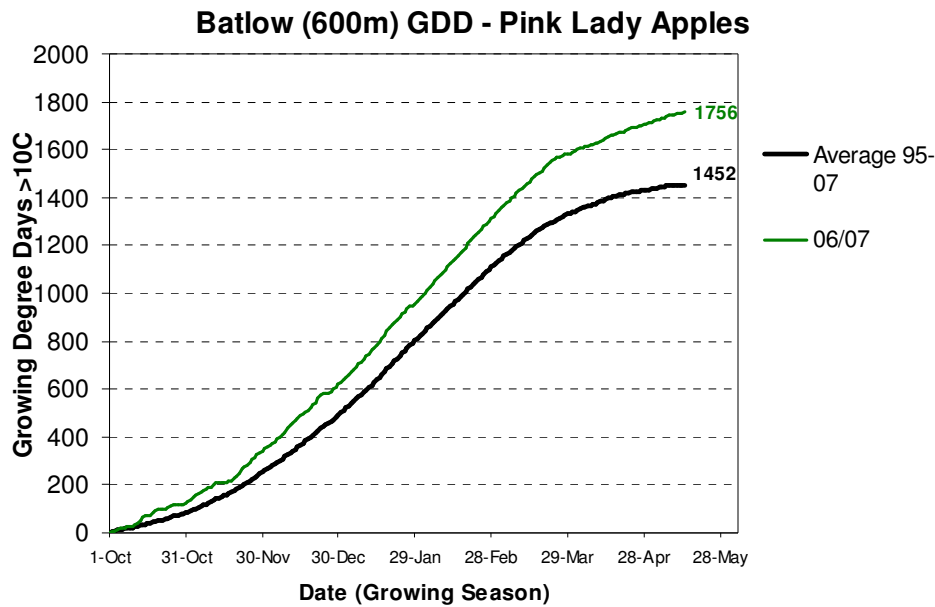


Fig. 8 Growing degree days > 10°C accumulation for orchards at 600m elevation in the Batlow area calculated by interpolation from nearby weather stations.

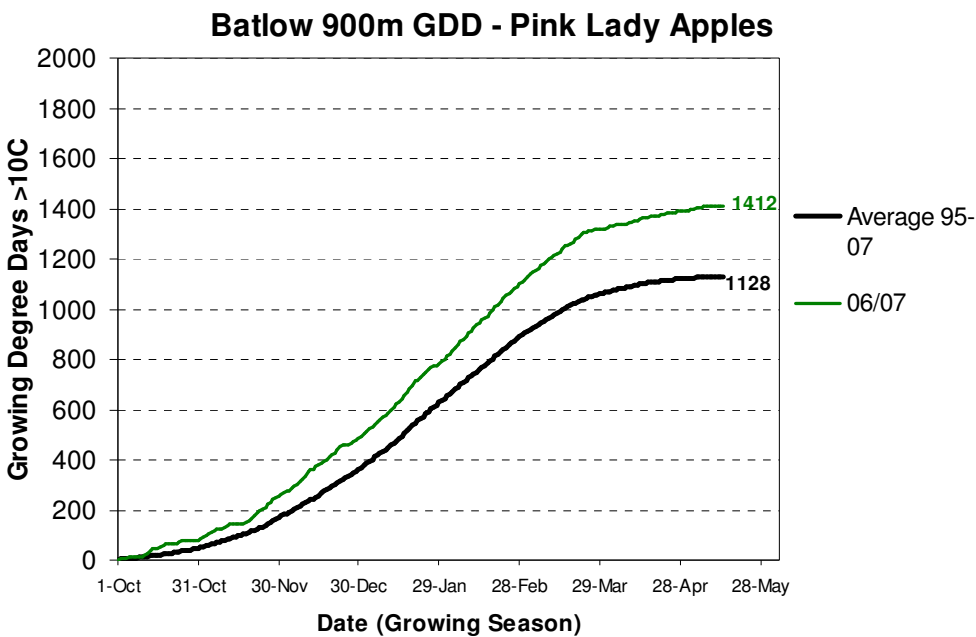


Fig. 9 Growing degree days > 10°C accumulation for orchards at 900m elevation in the Batlow area calculated by interpolation from nearby weather stations.

The orchard at Batlow used in previous years and this year is closest in elevation of 600m. The GDD for 2006/2007 at 600m elevation of 1756 was the highest of the last 12 years (ranged from 1149 to 1756, with an average of 1452). This means that the GDD being above 1700 are above the range for either RFB or DFB. Further, the development of GDD through the season was consistent, with no extended cool periods after flowering or before harvest that may cause subsequent problems. Consequently, the lack of any flesh browning is consistent with previous hypothesis. In an average year at 1450 GDD, one would expect slight levels of flesh browning, and this being more so in cooler years.

Apple orchards at the 900m elevation had significantly less GDD and in the warmest year of 2006/07 some flesh browning would be expected without the use of any alleviation measures. Further, in an average year with 1128 GDD flesh browning would be expected to be significant. The range of GDD for the last 12 years was from 817 to 1412, and during the cold years flesh browning would be expected to be a major problem for high altitude apple orchards in the Batlow area.

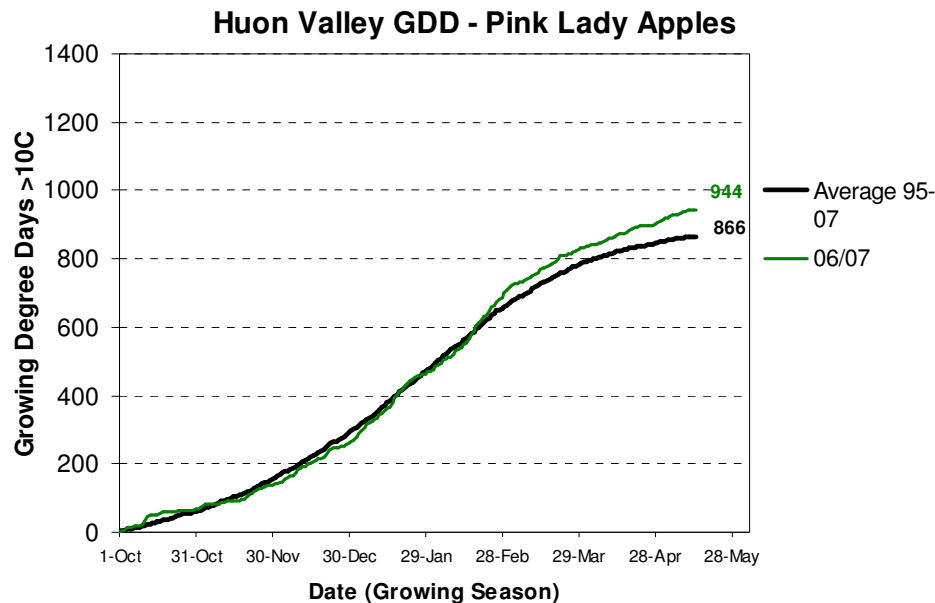


Fig. 10 Growing degree days > 10°C accumulation for orchards in the Huon Valley.

The GDD for Huon Valley of 944 was slightly above the average for the last twelve years of 866. Based on previous experience Huon Valley is a region that is very susceptible to flesh browning and consequently moderate to high levels of flesh browning would be expected based on this GDD. For the early part of the season GDD were slightly lower than average, suggesting some increased risk of higher fruit density and possible carbon dioxide related flesh browning. The range of GDD for the last twelve years is from 665 to 1011, this suggests that in cold years (such as 1995/96) flesh browning would be extreme, but in warmer years (such as 2000/01) the problem would be much less severe.

Discussion

It seems clear that development of flesh browning (FB) in Pink Lady™ is due to various interactions between growth and postharvest situations. While preharvest factors (*eg.* climate) have been demonstrated to induce and initiate development of FB in Pink Lady™ apples, storage conditions (*eg.* temperature) can influence and worsen symptoms of FB. Flesh browning is essentially made up of radial flesh browning (RFB), which is browning of the vascular areas of the fruit and diffuse flesh browning (DFB), identified by browning of the cortex tissue with minimal or no damage of the vascular tissue.

In this study, storage at 0°C was demonstrated to promote the incidence of flesh browning (both radial and diffuse) in apples from the Huon Valley in Tasmania. However, with slightly warmer storage conditions of 3°C, the incidence of flesh browning was essentially reduced to zero, with little or no loss to apple quality.

The Huon Valley is a district that can have quite cool growing conditions *ie.* <1700 growing degree-days <10°C, which favours the development of Pink Lady™ flesh browning, especially RFB, but more especially growing conditions below 1100 GDD which favours the more severe DFB. As the Huon Valley had less than 944GDD in 2007, Pink Lady™ apples were therefore

prone to flesh browning. Subsequently, all possible postharvest measures including warmer storage (eg. 3°C) should be used to minimise flesh browning in Pink Lady™ apples grown in the Huon Valley. Measure that would alleviate flesh browning in the Huon Valley besides storing at 3°C, would include harvesting at earlier maturities of 3.5 SPI or less, ensuring carbon dioxide levels in the CA are below 1%, storing for shorter periods, maximising calcium levels in the fruit and use of senescence retardants such as DPA.

In contrast, apples grown in Batlow, NSW showed no incidence of flesh browning during storage, which coincides with a very warm growing season in 2007 of 1756 GDD. Subsequently, flesh browning is unlikely to occur in Pink Lady™ apples from Batlow, even when very cold storage regimes (0°C) are used. Caution would recommend use of some flesh browning alleviation measures in the Batlow district, especially for higher elevation orchards and in cooler years.

Fruit maturity at harvest is also believed to be a key factor for determining the risk of flesh browning in Pink Lady™ apples, where fruit harvested at a late stage of maturity tend to show higher incidence of flesh browning than early harvested fruit during storage. Batlow apples, which showed no flesh browning, were harvested at a earlier maturity (see Table 1 for harvest maturity assessments) than apples from the Huon Valley, which showed considerable flesh browning when stored at 0°C. In practice, these apples from the Huon Valley would have been preferred for the fresh market after harvest.

Concluding remarks

- Growing degree-days were close to average for Huon Valley apples (944), therefore suggesting that this should be a moderate to severe flesh browning year. Storage at 3°C was found to be a very effective measure to prevent flesh browning in Huon Valley apples, with minimal change in quality over 7 months compared to storage at 0°C. All possible flesh browning minimisation treatments should be used routinely for Pink Lady™ apples from the Huon Valley
- Growing degree-days for Batlow apples in 2007 was 1756, which is above the 1700 upper limit for flesh browning being observed. This is consistent with no flesh browning being detected in any apples from Batlow even those stored at 0°C for 7 months

References

Stahl K., Moore R.D., Floyer J.A., Asplin M.G. and McKendry I.G. (2006) Comparison of approaches for spatial interpolation of daily air temperature in a large region with complex topography and highly variable station density. *Agricultural and Forest Meteorology*, 139 (3-4), Pages 224-236

7. Biochemical Factors Associated with a CO₂-Induced Flesh Browning Disorder of Pink Lady™ Apples

Elena de Castro, Jennifer Jobling and Elizabeth Mitcham^a

^a *Department of Plant Sciences, University of California, Davis, CA.*

Abstract

The underlying biochemical factors associated with the CO₂-induced internal flesh browning (FB) disorder of Pink Lady™ apples are poorly understood. To investigate this disorder, Pink Lady™ apples were stored in air or controlled atmosphere (CA) with 1.5% O₂ and 5% CO₂ at 0.5°C for 2 and 4 months in 2004 and 2005. Following CA storage, fruit were separated into two categories, damaged (FB) and undamaged and tissue from each of the categories were studied separately. Cell viability studies revealed that the cells were dead in the brown tissue in damaged apples. All healthy tissue contained viable cells. The damaged tissue in apples with FB also showed a decrease in ascorbic acid and an increase in dehydroascorbic acid during the first two months of storage in CA. Undamaged tissue of CA-stored apples retained the a higher concentration of ascorbic acid after 2 months in storage. The level of hydrogen peroxide (H₂O₂) also increased significantly in all tissue from CA-stored apples. In addition, concentrations of H₂O₂ were significantly lower in diphenylamine (DPA) treated apples. Treatment with DPA inhibited FB completely compared to untreated apples. The results also showed that the polyphenol oxidase (PPO) activity of damaged apples was higher than in undamaged apples after 45 days in CA storage. The level of PPO activity was similar for apples kept in either air or CA storage. The results showed that the protective mechanism of the oxidant-antioxidant mechanisms was more closely associated with the level of browning than the activity of specific browning enzymes like PPO. This result shows that further investigation of the protective effect of ascorbic acid is warranted.

Keywords: ascorbic acid, calcium, cell viability, CO₂ injury, electrolyte leakage, hydrogen peroxide, *Malus x domestica* Borkh, mineral composition, polyphenol oxidase.

Introduction

It is well known that the addition of controlled atmospheres (CA) during low temperature storage can preserve the quality of apple fruit for extended periods. However, CA can also cause physiological disorders, such as CO₂-induced internal flesh browning (FB) in susceptible apple varieties (Volz et al., 1998; Lau, 1998). Susceptibility to the CO₂-induced internal FB disorder of Pink Lady™ apples may result from the interaction of yet unknown preharvest and postharvest factors, as well as the physiological state of the fruit at harvest. Flesh browning in Pink Lady™ apples generally occurs intermittently and in unpredictable patterns which makes it a difficult disorder for growers to manage.

CO₂ induced FB disorders are thought to be the consequence of oxidative damage caused by high concentrations of CO₂ that can be aggravated by low concentrations of O₂ (Lau, 1998). Accumulation of CO₂ inside the cells could cause an oxidative stress which damages cell membranes. Research has shown that hydrogen peroxide has been related with stress conditions

(Rea et al., 2004) and in pears it has been related with CO₂ stress (Lauguedierre et al, 2001). Stress and high peroxide level can result in membrane dysfunction (Sears and Eisember, 1961; Frenkel and Patterson, 1973). Leakage of membranes could potentially lead to an accumulation in the cytosol of plastid enzymes like polyphenol oxidase (PPO) and its vacuole-stored substrates, the phenolic compounds. The oxidative reaction between enzyme and substrate would result in the formation of quinone compounds, leading to the brown discolouration of the flesh. Finally, leakage of membranes can lead to cell death.

Membrane integrity depends on several interacting factors. For example the availability and concentration of certain nutrients, such as calcium and boron have been shown to have an influence on membrane integrity. Boron is an important stabilizer of cell wall structure (O'Neill and York, 2003) and also has a role in the plasma membrane (Parr and Loughman, 1983). Calcium has also been shown to play an important role in cell membrane structure to stabilize phospholipids (Marinos, 1962). Picchioni et al (1995) concluded that Ca⁺² infiltrated into fruit after harvest improved membrane organization and function during postharvest life. Additionally, De Castro et al (2006??) found a relationship between high Ca⁺² and reduced susceptibility of Pink LadyTM apples to FB.

Another parameter related to the development of flesh browning may be the level of antioxidants in the tissue. Antioxidants protect the stability of the cell membrane. The antioxidants, ascorbic acid and glutathione, which are found in high concentrations in chloroplasts and other cellular compartments, are crucial for plant defense against oxidative stress (Noctor and Foyer, 1998). Ascorbic acid functions as the main defence against oxidative stress damage and reactive oxygen species (ROS). Ascorbic acid and calcium interact to preserve the stability of the membranes. In CaCl₂-treated apples, ascorbic acid content decreased significantly more than in untreated apples during storage (Drake and Spayd, 1983). Veltman et al (1999a) related decreasing ascorbic acid concentrations in high-CO₂-stored pears with the appearance of brown core. This may also be the case for the expression of flesh browning in Pink LadyTM apples.

In relation to CO₂ injury, Maguire and Mackay (2003) proposed the mechanism by which high concentrations of CO₂ inside the apple may develop stress-originated free radicals, which may lead to membrane degradation that would cause decompartmentalisation and this decompartmentalisation would lead to internal browning development. The goal of this research was to identify the biochemical processes that contribute to the development of FB in individual apples and account for populations of damaged and undamaged apples in representative sub samples. Data will be presented on the viability of affected cells; the protective role of ascorbic acid; PPO solubility and activity enhancement by CO₂; hydrogen peroxide; membrane leakage, and the relationship of calcium concentration with FB susceptibility.

Materials and methods

Fruit material

On 21 September, 5 and 22 October 2004, and 28 September and 27 October 2005, Pink LadyTM apples were harvested in the early morning from five 40-tree plots in a single orchard near Stockton, California, USA. Approximately 50 apples were harvested per tree. On the day of harvest, 30 fruit were selected randomly among the plots to determine starch content. The apples were cut in half equatorially, dipped for 1 minute in iodine-potassium iodide (3%) and rinsed in

fresh water. The starch levels were scored using a CTIFL (Centre technique interprofessionnel des fruits et legumes, Paris, France) 10 point scale.

Storage and internal browning evaluation

In 2004, all fruit were stored in air or 1.5% O₂ in a factorial with 1.0, 3.0 and 5.0% CO₂ at 0.5°C for 2, 4 or 6 months. Fruit were sorted and cooled at 0.5°C for 24 h before being placed into CA storage. In 2005, all fruit were stored in air or 1.5% O₂ and 5.0% CO₂, 1.5% O₂ + <0.5% CO₂ and 19% O₂ + 5% CO₂ at 0.5°C for 2 or 4 months. At harvest two in 2004 and one in 2005, one subset of fruit was immersed for 5 min in 2200 ppm diphenylamine (Pace International, Seattle, WA) in water, air dried at 20°C, pre-cooled overnight at 0.5°C and then placed into air or CA storage at 0.5°C. A second subset of fruit was placed into CA storage for 2 or 4 months after a 4 week delay in air at 0.5°C.

For FB evaluation after storage, 125 fruit per treatment were cut equatorially in three equally spaced locations resulting in ~2-cm thick slices, and the percentage area of flesh area browning per equatorial slice was visually estimated.

Cell viability

The viability of cells in the brown tissue was determined using intracellular enzymatic hydrolysis of fluorescein diacetate (Heslop-Harrison and Heslop-Harrison, 1970). Fluorescein diacetate (Sigma chemical, St Louis, MO) was dissolved in acetone (Fisher scientific, Fairlawn, NJ) (2mg/ml) and diluted 1:500 with a 0.4 M sucrose solution. Observations of the fluorescein chromatic reaction were made with a Leica MZ 12 fluorescence microscope (Wetzlar, Germany) using mercury arc source (HBO 100w, Osram lamp, Chicago, IL) exciting and T-2 barrier filters. The fluorescent light emission of individual cells was observed with an IP 28 photomultiplier tube and recorded with a Leica DC 300F digital camera system (Wetzlar, Germany). Viable cells dissociate fluorescein diacetate and can be easily seen with the fluorescence microscope. When cells are non-viable, they are not able to dissociate fluorescein diacetate and a dark area is observed.

Polyphenol oxidase

a. Subcellular fractionation

A plastid fraction was separated from a soluble fraction using a modified method of Mayer et al (1964). Fresh apple flesh (6g) was homogenized with 6 mL of cold extraction buffer (0.1M NaK phosphate buffer (pH 7.2) containing 0.4 M sucrose and 0.001M ascorbic acid). The homogenate was filtered with 4 sheets of gauze. The filtrate was kept on ice and designated as the crude fraction. Two replications of 1 ml each were centrifuged (4000 x g) for 5 minutes. The precipitate formed was washed and resuspended in 1 ml of cold buffer (0.1M NaK phosphate buffer containing 0.4M sucrose) and designated as the 'plastid' (including 'cell wall') fraction. The supernatant was kept on ice and designated as the 'soluble' (soluble and mitochondrial) fraction.

b. Enzyme activity

Activity was measured spectrophotometrically as described by Espin et al (1995). Assays were performed at 20°C in 1.0 mL of medium containing dihydroxycinnamic acid, 3-methyl-2-benzothiazolidone hydrozone (MBTH) and acetic acid buffer. An aliquot (10 µL) of the fraction (crude, plastid or soluble) was added and the increase in absorbance at 500nm was monitored for

1 minute. The rate of absorbance increase per minute was calculated on the interval between the tenth and the twentieth second. One unit of enzyme activity was defined as the quantity of enzyme responsible for an increment in absorbance of 0.001/min.

Ascorbic Acid

Ascorbic acid was determined in apple fruit at harvest and after each period of storage in each atmosphere. The apples were divided after storage into two categories; undamaged, those without FB symptoms, and damaged, those with some flesh browning. Four apples of each category were selected following storage in 5% CO₂. For air and 3% CO₂-stored fruit, only 4 undamaged apples were selected for analysis. Skinless tissue samples (10g) were collected from the stem-end and the blossom-end halves. A whole transversal section (2 cm thick) that was located 3 cm from the stem or blossom end respectively, was obtained from each fruit and 10g of only healthy tissue was collected for damaged or undamaged apples and retired for analysis. Injured brown tissue in the case of the damaged apples was collected from everywhere inside the apple, wherever it appeared.

All samples were evaluated for reduced L-ascorbate and dehydroascorbate content based on the method of Zapata and Dufour (1992). Samples were frozen with liquid N₂, crushed with a mortar and pestle and then homogenized with 10 ml of an extraction solution (0.1 M citric acid, 0.05% EDTA, 4mM sodium fluoride, 5% methanol) for 1 min at high speed in a blender. The homogenate was filtered through cheesecloth and then centrifuged for 5 min at 12,000 x g at 2°C in a Sorvall RC-SB centrifuge (Sorvall Dupont Instruments, Wilmington, DE) using a SS-34 rotor. After adjusting the pH of the supernatant to 2.35-2.40, the sample was passed through a Sep-Pak C18 cartridge (Waters Assoc. Milford, MA) which had been preconditioned with 10 ml HPLC-grade methanol and 10 ml ultra pure water. The first 5 ml of eluent was discarded and the next 3 ml retained for analysis. As specified by Zapata and Dufour (1992), 37 min before injection onto the HPLC system, 1 ml of 1, 2- phenylenediamine (3.33 mg/ml) in methanol/water (5:95, v/v) was added. The mixture was immediately passed through a 0.45-mm filter (Acrodisc. Gelman Sciences, Ann Arbor, MI) into an amber sample vial and sealed.

The HPLC system consisted of a Hewlett Packard Series 1050 auto sampler, Series 1050 pump, and a Series 1040M diode array detector, operated by HP ChemStation software (Hewlett Packard, Waldbronn, Germany). A Waters pBondapak C18 reversed-phase column (Waters Assoc. Milford, MA), 30 cm x 3.9 mm i.d., was used for separation with a Bio-Rad Bio-sil Micro-Guard column (ODS-5S 4.6 mm x 3 cm i.d) (Beckman Coulter Inc, Fullerton, CA). The eluent was methanol/water (5:95, v/v) containing 5 mM hexadecyltrimethyl-ammonium bromide and 50 mM potassium dihydrogen phosphate, with the pH adjusted to 4.59. The flow rate was 1 ml/min. Detection was at 261 nm for reduced L-ascorbate and at 348 nm for dehydroascorbate. Retention times were 4.3 and 6.3 min for dehydroascorbate and reduced L-ascorbate, respectively. L-ascorbate and dehydroascorbate standards were supplied by Sigma-Aldrich, Inc (St. Louis, MO).

Electrolyte leakage

Prior to electrolyte leakage studies, we first determined the isotonic concentration based on the method of Saltveit (2002). Samples of apples randomly selected at harvest were monitored to study the weight loss or gain in different mannitol solutions (0.0-0.5 N for 210 minutes). The samples consisted of 20 discs of 1 cm diameter and 0.5 cm thickness. A 0.5 cm slice was cut along the equator of the apple, 0.25 cm each side of the equator. With a stainless

steel 1 cm-diameter cork borer, discs were cut from flesh halfway between the skin and the core. The discs were washed three times for about 1 min each in distilled H₂O, blotted dry and weighed (9 to 12 g total). Each sample was placed in a petri dish with 20 ml of the corresponding mannitol solution and gently shaken on a rotary shaker at a speed of 60 cycle/min. The solution was vacuum aspirated after 35, 75, 150 and 210 minutes and at each time the discs were weighed and fresh solution added.

Electrolyte leakage was determined in different locations within the apples at harvest and after 2 and 4 months of storage. After storage, apples were divided into undamaged and damaged apples. Eight apples of each category from each storage atmosphere were selected. Samples of approximately 3 g were collected, consisting of 5 discs of 1 cm diameter and 0.5 cm thick collected as previously described. Each sample of discs was put into a 50-ml centrifuge tube with 20 ml 0.35 M mannitol, and the conductivity recorded at 0, 15, 30, 45, 60, 80, 140, 200 and 250 min. The tubes with the discs were frozen and thawed twice before the final conductivity was measured as total conductivity. The pre-frozen conductivity readings were divided by the total conductivity for that sample and the product multiplied by 100 for percent conductivity. The slope of the line defined by the percentage of electrolytes measured at each time (80, 140 and 200 min) was calculated to define the slow rate of leakage through the membrane (Saltveit, 2002).

Determination of hydrogen peroxide

Five apples per treatment were selected and samples of 6 grams were collected from the stem-end and the blossom-end halves of each fruit as described for the ascorbic acid analysis. Then samples were frozen with liquid N₂, crushed with a mortar and pestle and homogenized with 5 ml of 5% trichloroacetic acid for 1 min at high speed in a blender. The homogenate was filtered through 4 layers of cheesecloth and centrifuged for 20 min at 20,000 x g at 2°C in a Sorvall RC-SB centrifuge (Sorvall Dupont Instruments, Wilmington, DE) using a SS-34 rotor. Hydrogen peroxide concentration was determined using the Bioxytech H₂O₂-560 colorimetric assay (OXIS International Inc., Portland, OR) based on the oxidation of ferrous ions (Fe⁺²) to ferric ions (Fe⁺³) by hydrogen peroxide under acidic conditions. The ferric ions bind with the indicator dye, xylenol orange, to form a stable, colored complex which can be measured at 560 nm. The standards were prepared by diluting 30% H₂O₂ (Fisher Sci., Fair Lawn, NJ) in water and were measured at the same time as the samples.

Mineral analysis

Individual apple mineral composition was measured from the stem-end and the blossom-end. Tissue from undamaged apples and healthy tissue from FB apples was collected after 2, 4 and 6 months of CA storage. A total of 60 apples were analysed per year, 30 undamaged and 30 damaged. A whole transversal section (2 cm thick) that was located 3 cm from the stem or blossom end respectively, was obtained from each fruit. Skinless tissue samples (5g) were collected. Samples were freeze-dried. All samples for mineral analysis were sent to a laboratory for analysis (UC-DANR Analytical Laboratory, Davis, Calif.) and analysed for calcium and boron by inductively coupled plasma atomic emission spectrometry (ICP-AES) as described by Meyer and Keliher (1992).

Statistical Analysis

The experiment had a factorial design with 2 storage times (2 and 4 months), two storage atmospheres (air and CA), two maturities and two locations within the fruit, stem and blossom-end. Fruit stored in 5% CO₂ were further divided into undamaged and damaged fruit. Data were analysed by storage atmosphere, location within the fruit and harvest maturity. There were 4 apples per treatment for ascorbic acid in 2004, 10 apples per treatment for the first harvest and 5 apples per treatment for the later harvest in 2005. There were 8, 5, 5 and 7 apples per treatment for electrolyte leakage, mineral analysis, hydrogen peroxide and PPO activity, respectively. Analysis of variance was computed by SAS Version 8.02 (SAS Institute Inc., Cary, NC). Multiple mean comparisons were performed using LSD and Tukey-Kramer adjustment ($\alpha=0.05$).

Results

Flesh browning

The incidence of CO₂-induced FB after 2 months storage was found to be higher in apples stored in 5% CO₂ + 1.5% O₂ than in 1 and 3% CO₂ + 1.5% O₂ in 2004 (Table 1). Air stored apples did not show FB. In 2005, the incidence of FB in apples was only 5% in 5% CO₂ + 1.5% O₂ compared to 15% in 2004. The percentage of FB in each atmosphere did not change between 2, 4 and 6 months of storage in any season. DPA inhibited FB completely and the delayed CA storage treatment significantly reduced FB incidence but did not eliminated the disorder (Table 1). Apples stored in 1.5% O₂ + <0.5%CO₂ never developed FB symptoms. Storage in 5% CO₂ with 19% O₂ reduced FB to 1% compared to 1.5% O₂.

The percentage of fruit with damage at the stem-end was higher (66%) than fruit that only had symptoms at the blossom-end (13%) (Table 2). There were 21% of fruit showing FB symptoms that were equally distributed in the entire apple.

Cell Viability

After two and four months of storage, the analysis of cell viability with fluorescein diacetate revealed that the brown tissue was a group of dead cells surrounded by healthy, viable cells (Fig. 1). Apples that did not show brown tissue did not have any non-viable cells after 2 or 4 months of CA or air storage.

Polyphenol oxidase activity

Total PPO activity increased during the first 15 days of storage in air or CA (1.5% O₂ + 5% CO₂) storage in 2005 (Fig. 2), and increases occurred in both the plastid and soluble fractions. The PPO activity remained constant and similar in air and CA until day 30. At 45 days after harvest, flesh browning damaged apples showed higher PPO activity in the tissue that was not brown. This increase in activity was mostly due to an increase in soluble PPO activity. After 60 days, PPO activity decreased slightly in both fractions. Undamaged apples in the same storage atmosphere did not show an increase in PPO activity by day 45, but activity increased slightly by day 60, in both fractions. In air storage, total PPO activity from day 30 to 60 decreased slightly, while there was a shift from plastid to soluble activity.

In 2004, PPO activity was determined after 6 months of storage. There were no differences in total, plastid or soluble PPO activity between apples stored in air and apples stored in 1.5% O₂ + 5% CO₂, and no differences in PPO activity between damaged and undamaged apples (data not shown). In all cases, approximately 90% of the total PPO activity was, by then, in the soluble fraction.

Ascorbic acid

Ascorbic acid levels decreased significantly with time in all storage conditions in late maturity fruit (starch index at harvest for 2004 was 8.5 and for 2005 was 8.0) (Fig. 3A). Apples stored in air had the lowest levels of ascorbic acid. After 4 months of storage in 2004, the initial value decreased by 70% (Fig. 3A). Apples stored in 3 or 5% CO₂ that were not damaged by FB only lost 30% of the initial amount of ascorbic acid after 4 months.

Although 5% CO₂ conserved ascorbic acid, once the apple was damaged by FB, the amount of ascorbic acid decreased to very low levels in the healthy tissue and in the brown tissue of the damaged apples (Fig. 3A and Fig. 4A). After two months, ascorbic acid concentration in the healthy tissue surrounding the affected tissue decreased to 35% of the initial amount and after 4 months to trace levels. Ascorbic acid concentration in the brown tissue was very low after 2 months of storage and after this time, the concentrations were very close to 0 mg/100g of fresh weight. In 2004, the loss of ascorbic acid in CA happened sometime between 2 and 4 months, however, in 2005, the loss occurred within the first two months of storage and after that it did not decrease significantly.

In the early maturity fruit (starch index at harvest 3.5) in 2005, ascorbic acid decreased significantly during the first two months in all storage conditions (Fig. 4A). Ascorbic acid concentration after two months was similar for undamaged apples stored in CA with 5% CO₂ + 1.5% O₂, 1.5% O₂ + <0.5% CO₂, DPA-treated apples stored in CA with 5% CO₂ + 1.5% O₂ or air, and untreated air stored apples. Damaged apples and apples stored in 19% O₂ with 5% CO₂ showed a significant decrease in ascorbic acid after 2 months. After 4 months, the DPA-treated apples stored in CA or air and apples subjected to delayed CA showed higher conservation of ascorbic acid.

Dehydroascorbic acid (DHA) concentrations followed a different pattern, it increased during the first two months in storage, and except for undamaged apples in CA it decreased after that (Fig. 3B). The surrounding non-affected tissue of damaged fruit stored in 5% CO₂ showed the highest DHA concentrations after 4 months (Fig. 3B). DHA levels were similar among the storage conditions after 2 months except for the brown tissue, where it was higher, and then decreased to very low levels in all samples except in damaged apples at 4 months, where DHA increased or decreased slightly in healthy and brown tissue respectively. In 2005, the dehydroascorbic acid concentrations were very low in apples from all storage conditions after 0, 2 and 4 months in storage (Fig. 4B).

The total ascorbic acid concentration in fruit at harvest was significantly higher in 2005 than in 2004 (3.04 vs. 2.25 mg/100 gfw) and the fruit had lower CO₂-induced flesh browning incidence in 2005. The concentration of ascorbic acid in the blossom-end of the apple was slightly higher in air and CA-stored apples not affected by FB (Table 2). Once the apple showed FB, ascorbic acid concentration was lower than in undamaged apples and similar in the stem and blossom-ends of the apple (data not shown).

Electrolyte leakage

There was an increase in electrolyte leakage rate with time in storage in all storage conditions (data not shown). The highest leakage rate values were found in the brown tissue of damaged apples stored at 1.5% O₂ + 5% CO₂, and there were no differences in electrolyte leakage rates of apples stored in air or undamaged apples and healthy tissue of damaged apples stored in 1.5% O₂ + 5% CO₂ was similar.

Healthy tissue samples from the stem-end of the fruit had a higher leakage rate than the blossom-end of the fruit in FB-damaged apples in 5% CO₂ and FB incidence was also higher in the stem end of these apples (Table 2). Samples from undamaged apples in 5% CO₂ or from other storage conditions showed similar electrolyte leakage rates in the stem and blossom-ends.

Hydrogen peroxide concentration

The concentration of hydrogen peroxide in apple flesh increased significantly during 2 months of storage in all storage conditions and treatments (Table 3). However, apples stored in 5% CO₂ showed statistically higher concentrations than apples stored in air. DPA treatment significantly reduced the accumulation of hydrogen peroxide but levels remained significantly higher than in air stored fruit. Healthy tissue from apples with FB (damaged apples) had similar H₂O₂ levels as undamaged apples kept under the same CA. Hydrogen peroxide levels in stem and blossom ends of the apples were similar (data not shown).

Mineral analysis

Calcium concentrations in the stem-end of the apple were significantly higher than the blossom-end (Table 2), however; the concentration of boron was significantly higher in the blossom-end than in the stem-end of the fruit.

Discussion

Changes with storage conditions, effect of FB on apple

It has been hypothesized that brown core in pear fruit is a consequence of decompartmentation of cellular compartments caused by membrane disintegration (Veltmann et al., 1999 b). After this decompartmentation, the PPO enzyme solubilizes from the plastid to the cytoplasm at the same time as phenolic compounds leak from the vacuole leading to quinone formation and polymerization to brown pigments.

Internal browning develops between 30 and 45 days in storage and the incidence of CO₂-induced FB in Pink Lady™ apples does not increase with time in storage. Polyphenol oxidase activity was not elevated in 5% CO₂ storage during the first 45 days in comparison to air stored fruit. We expected to see higher PPO activity in CA than in air storage, in relation with internal browning development. At 45 days, PPO activity in damaged apples was slightly higher than in undamaged apples in both plastid and soluble fractions. However, we were unable to measure PPO activity in damaged apples before 45 days, because at that time we did not see any brown symptoms, and therefore did not know at what point it became elevated. The high standard deviations of the means in every atmosphere condition was due to one or two apples with very high PPO activity, usually higher in the soluble fraction than in the plastid fraction (data not shown). Those apples could have been developing early symptoms of FB, perhaps there was membrane damage resulting in PPO leaking from the plastid to the soluble fraction.

Previous attempts to correlate browning directly to PPO enzyme activity and phenolic compounds in different apple cultivars have been contradictory (Nicolas et al, 1994). Barrett et al. (1991) found in 'Delicious' apples that after 28 weeks in CA, PPO activity was higher in the soluble fraction and decreased in the plastid and mitochondrial fraction with higher CO₂ concentration (8-12% in comparison to 3%). Our results did not show such an increase in PPO activity with high CO₂ but our CO₂ concentration (5%) was much lower than that reported by Barrett et al. (1991). Murata et al. (1997) demonstrated in 'Fuji' apple that the higher PPO concentration is in the plastid and that PPO enzyme is solubilised and proteolyzed during storage

and ripening. That may explain the movement of the enzyme from the plastid to the soluble fraction and a consequently higher PPO activity in the soluble fraction after 6 months in our trials.

There was a large difference in the ascorbic acid (AA) content between apples stored in CA which had developed FB and healthy, undamaged apples stored under the same conditions. Storage in CA, especially with low oxygen, can help to conserve AA in the fruit, unless the apple develops FB. Apples stored in CA with 19% O₂ and 5% CO₂ lost AA as fast as damaged apples in CA with 1.5% O₂ + 5% CO₂, although less than 1% of fruit showed symptoms of FB. It appears that the low O₂ component of the CA preserves the AA concentration while the high CO₂ component of the mixture creates oxidative stress in susceptible apples and generates a loss of AA and causes FB symptoms to be expressed. Ascorbic acid oxidation is strongly related to tissue browning. Browning generally, does not occur until all ascorbic acid is oxidized, as ascorbic acid is one of the PPO enzyme's preferred substrates (Ponting and Joslyn, 1948). This would explain why in this work there was a low ascorbic acid concentration in the brown tissue. The results showed an interesting decrease in the concentration of AA in the healthy areas not affected by FB in damaged apples. Ascorbic acid decreases to trace levels in the brown tissue, in relation with cell death, but AA also decreases in distant tissue, not dead or brown, within the same apple. Several studies propose a role for H₂O₂ as a signal for the activation of stress-response and defense pathways. H₂O₂ produced during stress response is thought to diffuse into cells (Mittler, 2002; Love et al, 2005) together with other plant signals and activate many of the plant defenses, including programmed cell death. Ascorbic acid may decrease under the systemic response of the whole fruit.

Frenkel and Patterson (1974) found that pears in CA containing high concentrations of CO₂ showed alterations in different organelles including plastids and other membrane systems such as the tonoplast and plasma membrane. The electrolyte leakage analysis of Pink Lady™ apples in this work did not show that the rate of leakage due to membrane degradation was the main difference between apples stored in air or CA or was the main difference between damaged and undamaged apples stored in high CO₂ concentrations. Electrolyte leakage in brown tissues was high because the cells were dead. We could not detect any other differences in membrane leakage between apples stored under air or CA or even between healthy tissue of damaged apples. The effect of CO₂ may be greater on plastid membranes than on the plasma membrane and our method may be only measuring the latter. Secondly, the CO₂ effect may be very specific to tissue that becomes brown; for some reason that tissue may be very susceptible and we do not have the means to target that tissue for study before it dies. Finally, the electrolyte leakage analysis may not be sensitive enough to pick up very small differences in levels of electrolyte leakage between the treatments.

The non-viability of cells affected by FB explains the high rate of electrolyte leakage and the low concentration of ascorbic acid in these tissues. We were first able to detect FB after 45 days in storage and cells affected by FB were already dead at that time. It is hypothesised that the ascorbic acid was oxidized to DHA and the non-viable cells were unable to reduce the latter back to the former because of a lack of energy and resources.

Apples held in CA accumulated more H₂O₂ than apples stored in air, indicating stress from the high CO₂ concentrations in storage. Apples stored in high CO₂ atmospheres also produced more ethylene than apples kept in low CO₂ atmospheres (De Castro et al, 2006), this could also be an indicator of apples stored under high CO₂ levels developing stress. The fact that H₂O₂ was not higher in the healthy tissue of damaged apples may indicate that the greater stress

leading to browning in some tissues may be due to other factors specific to that tissue. It is interesting to note that while DPA did not affect the loss of AA during the first two months of storage, it did reduce the accumulation of H₂O₂. However, after 4 months of either air or CA storage, apples treated with DPA had higher levels of AA. Apples that were held in air storage for 4 weeks before CA storage also conserved high AA concentration. Because delayed CA also partially reduced the incidence of FB in some seasons, this supports a relationship between ascorbic acid levels and the development of FB.

Comparison of stem and blossom end

The results showed that the stem-end of the fruit showed a higher incidence of FB than the blossom-end. The difference in FB susceptibility between stem and blossom end tissues of the fruit was related to higher concentrations of ascorbic acid in the stem-end tissue, except in apples affected by FB. In these apples with FB, the difference was slight, perhaps because the ascorbic acid was used to prevent oxidation and degradation caused by the high CO₂. The role of calcium in cellular membranes has been broadly researched. There is strong evidence of a role for calcium in maintaining the stability of membranes (Marinos, 1962). However, we have seen a higher FB severity in the stem-end of the fruit where the concentration of calcium was statistically higher. However, the boron concentration was lower in the stem-end, and it also may help to maintain membrane stability and cell wall (O'Neill and York, 2003; Parr and Loughman, 1983). The rate of electrolyte leakage was also significantly higher in the stem-end of the fruit. Factors like higher ascorbic acid concentration, higher boron concentration and lower electrolyte leakage rate at the blossom-end could explain the fact that the blossom-end of the fruit had a 5 fold lower incidence of FB compared to the stem-end of the fruit.

CO₂-induced flesh browning

It is clear that CO₂-induced flesh browning cannot be related to a single biochemical parameter at harvest or during storage. High CO₂ in storage is a condition leading to development of FB and can be further aggravated by low oxygen concentrations (De Castro et al, 2006). Ascorbic acid concentration appears to be the most highly correlated factor associated with tissue susceptibility to the disorder but there is some uncertainty about the mechanisms of its involvement. Ascorbic acid may be preventing the oxidative effect of high CO₂ stress on membranes, reducing lipid peroxidation as Shalata and Neumann (2001) concluded. We still do not know if it is the concentration of AA at harvest which determines the susceptibility of apples or if there is another biochemical factor that defines the susceptibility. We have seen some increase in PPO activity in damaged apples in comparison to activity in undamaged apples in CA or air storage by day 45 in storage. But 2 weeks later, PPO activity in undamaged CA stored apples also increased. We can not conclude that PPO leaks from plastids to the cytoplasm due to the degradation of membranes and that PPO has a high activity in the soluble fraction in CA stored apples. In summary, it appears that CO₂ stress results in elevated oxidative level in cells, probably more in areas of susceptible tissues. When AA is consumed in this oxidative environment, membranes are damaged, enzymes and substrates mix to form brown pigments, and eventually the cells die. DPA, with its antioxidant properties, reduces the peroxidation and the disruption of the membranes and delays the expression of symptoms.

The results showed that high CO₂ concentration in CA storage does not damage all apples and because the undamaged apples do not lose much of their ascorbic acid content, we propose that there is another factor making the apples susceptible to FB beyond the high CO₂

concentration. It seems that only a certain percentage of the apples are susceptible to FB and the percentage varies between years. Even further, only a portion of the apple is affected by FB, the brown area does not extend to the whole flesh, even though the healthy tissue in the same apple has very low ascorbic acid concentration and high DHA concentration. There is must be another characteristic of the apple tissue that makes it susceptible to FB when exposed to high CO₂ concentrations in storage. Among the biochemical factors, it appears that ascorbic acid and H₂O₂ concentrations are the most highly correlated to the expression of FB of the factors investigated in this work, and further work is required to better understand the biochemical mechanism of CO₂-induced flesh browning in Pink Lady™ apples.

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Table 1. Incidence (%) of flesh browning in 2004 and 2005 after 2 months storage in air or controlled atmosphere (CO₂ with 1.5% O₂) at 0.5°C.

Year		% CO ₂		
		1	3	5
2004	Harvest 1 ^z	13 ^w	20	31
	Harvest 2	2	10	16
	Harvest 2, DPA ^y	--	--	0
	Harvest 3	1	5	15
	Harvest 3, 4-week delay ^x	0	4	3
2005	Harvest 1	--	--	5 ^v
	Harvest 1, DPA	--	--	0
	Harvest 3	--	--	5

^z Early, intermediate and late harvests correspond to starch indices of 3.5, 6.5 and 8.5 respectively.

^y 2200 ppm diphenylamine

^x Four week delay at 0.5 °C before CA storage.

^w Minimum Significant Difference, within 2004, according to Tukey's test 16% ($\alpha=0.05$)

^v No significant differences in 2005 ($\alpha=0.05$).

Table 2. Flesh browning incidence in relationship with ascorbic acid, calcium and boron concentrations and electrolyte leakage rate for stem and blossom end apple tissue.

Location	Flesh Browning Incidence (%)	Ascorbic Acid (mg/100 gfw)	Flesh Calcium ($\mu\text{g/gfw}$)	Flesh Boron ($\mu\text{g/gfw}$)	Electrolyte leakage rate (%/h)
Stem	66	1.3a ^z	33.6a	5.2a	1.9 ^y
Blossom	13 ^x	1.5b	31.1b	5.8b	1.4 ^y

^z Different letters indicate significant differences ($p < 0.05$) according to Tukey's test.

^y Corresponding to a difference: $\text{Pr} > |t| = 0.06$.

^x Percentage of damaged fruit showing more severity on the stem-end or on the blossom-end. There were 21% of fruit showing FB equally in the entire apple. Data were pooled across treatments, either from tissue samples collected from undamaged apples (air and undamaged CA for ascorbic acid) or pooled from one single storage condition (1.5% O₂ plus 5%CO₂) from healthy tissue of undamaged and damaged apples (calcium, boron and electrolyte leakage rate).

Table 3. Hydrogen peroxide concentration in apples at harvest and after 2 months storage in air or in controlled atmosphere (CA) in 2005. From the apples stored in CA, we differentiate between healthy tissue from apples affected (damaged) or not affected by flesh browning (undamaged).

Evaluation time	Storage Atmosphere	[Hydrogen peroxide] $\mu\text{mol/Kg fw}$
Harvest	--	8.61 a ^x
2 months	Air	15.32 b
2 months	CA ^z undamaged	20.81 d
2 months	CA damaged	19.88 cd
2 months	CA plus DPA ^y	17.56 c

^z CA conditions: 1.5% O₂ + 5% CO₂.

^y 2200 ppm diphenylamine at harvest.

^x Letters correspond to LSD mean separation ($\alpha = 0.05$), n=5 apples.

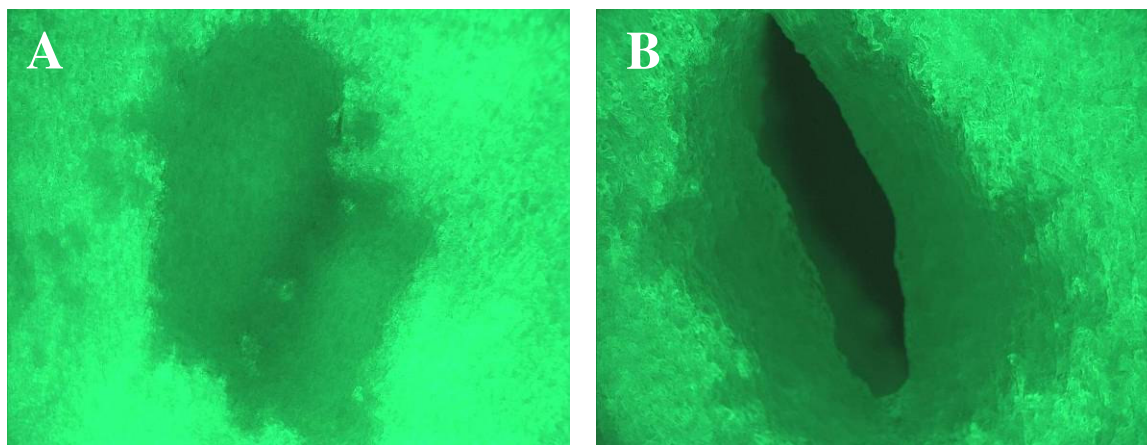


Figure 1. Cell viability by induced fluorescence analysis in apples stored in 1.5% O₂ + 5% CO₂ for four months at 0.5°C and showing flesh browning injury. Cells without fluorescence were dead and cells with fluorescence were viable. The pattern matched perfectly with the pattern of flesh browning, all brown cells were non-viable, surrounding non browned cells were viable. A) Flesh of a damaged apple affected by flesh browning. B) Flesh of a damaged apple affected by flesh browning with CO₂- induced cavity.

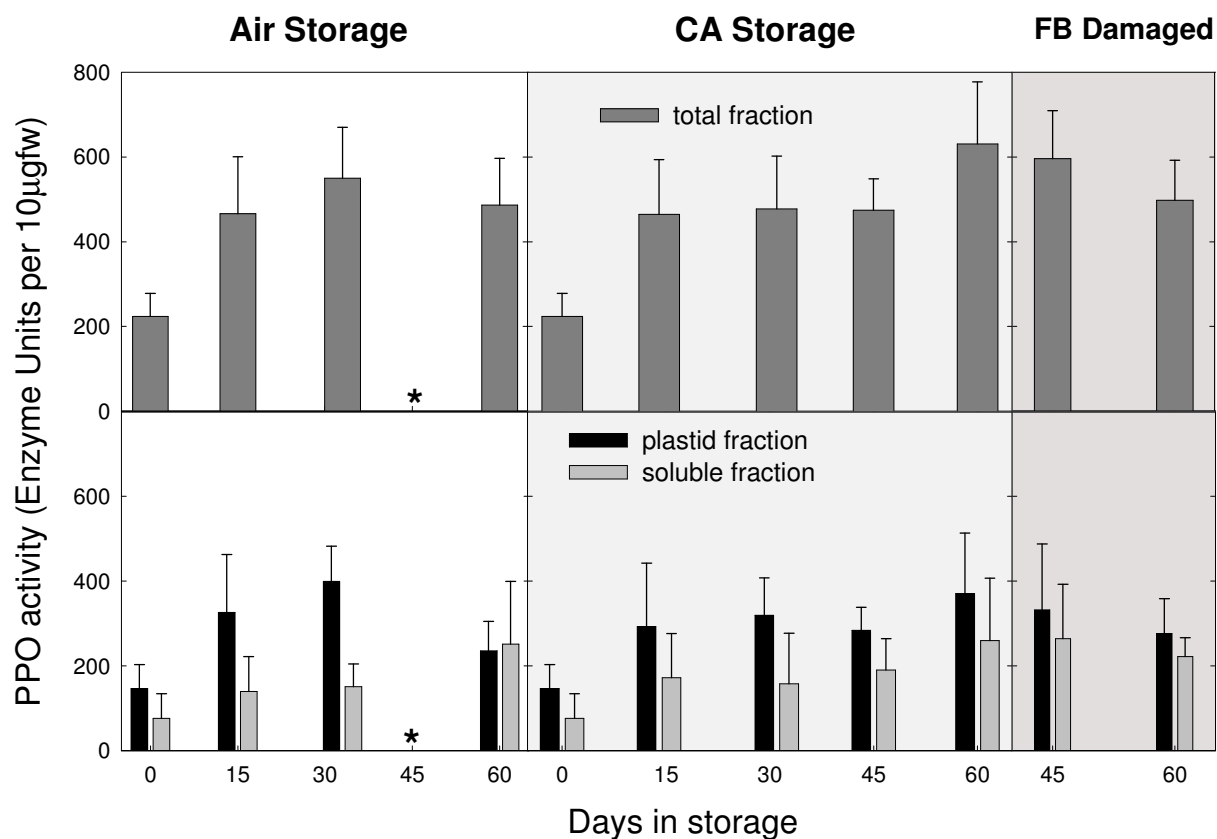


Figure 2. Polyphenol oxidase activity (enzyme units per 10µg of flesh fresh weight) from plastid and soluble fractions or total (plastid plus soluble fractions) in apples stored for 2 months at 0.5°C in air or CA (1.5% O₂ + 5% CO₂). Damaged apples showed flesh browning and flesh browning was not detected before 45 days. * No analysis done.

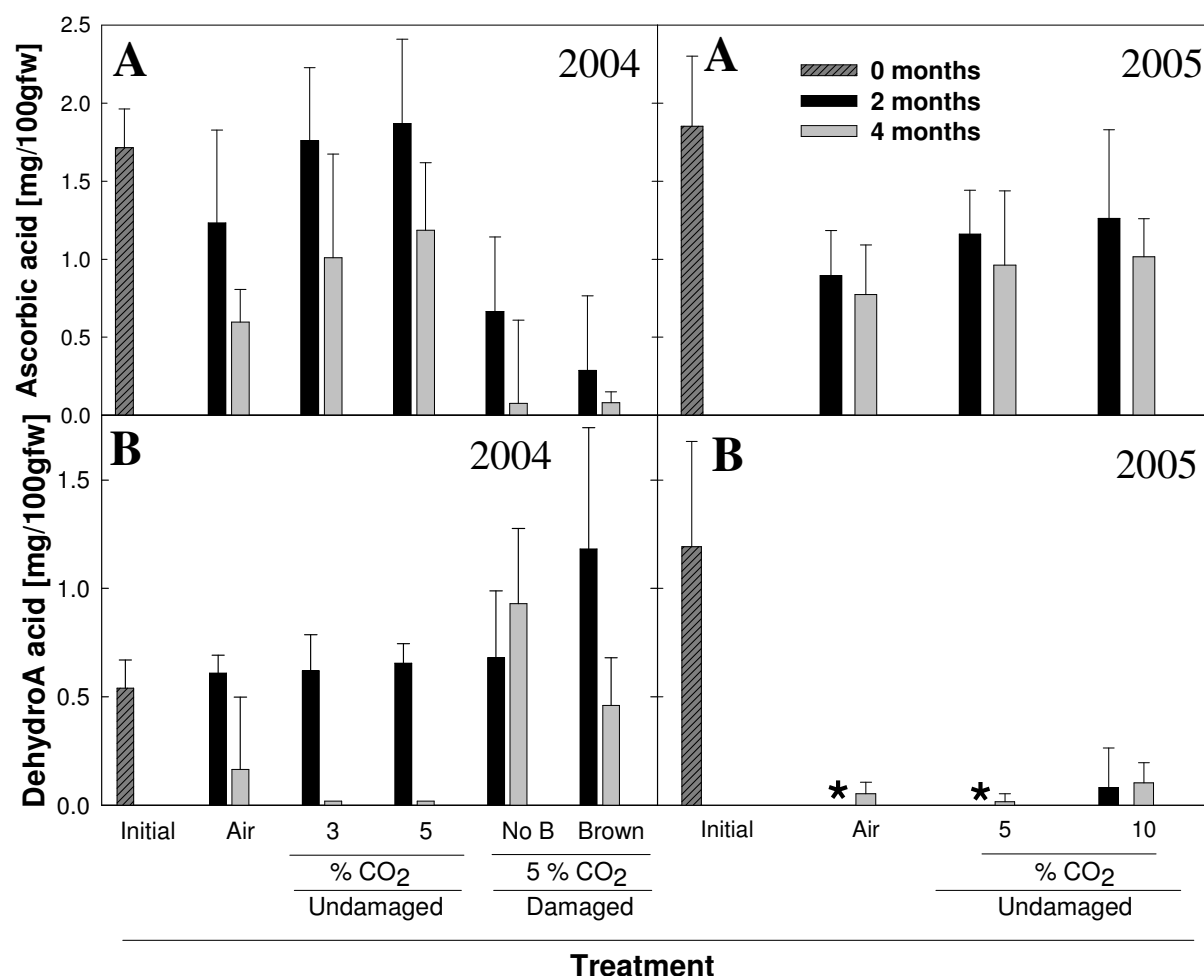


Figure 3. Ascorbic acid (A) and dehydroascorbic (dehydroA) acid (B) (mg/100g fresh weight) concentrations in apples at harvest and after storage at 0.5°C in air or 1.5% O₂ with 3% or 5% CO₂ in 2004, and in air or 1.5% O₂ with 5% or 10% CO₂ in 2005 for 2 and 4 months. Apples stored in 5% CO₂ were divided into undamaged and damaged fruit. Damaged fruit were further divided into brown (brown tissue from damaged apples) and not brown tissue (no B, healthy tissue from damaged apples). Bars correspond to the average of 4 apples and their standard deviation in 2004 and 5 apples in 2005. Starch score at harvest = 8.5 in 2004 and 8.0 in 2005. * DHA not detected.

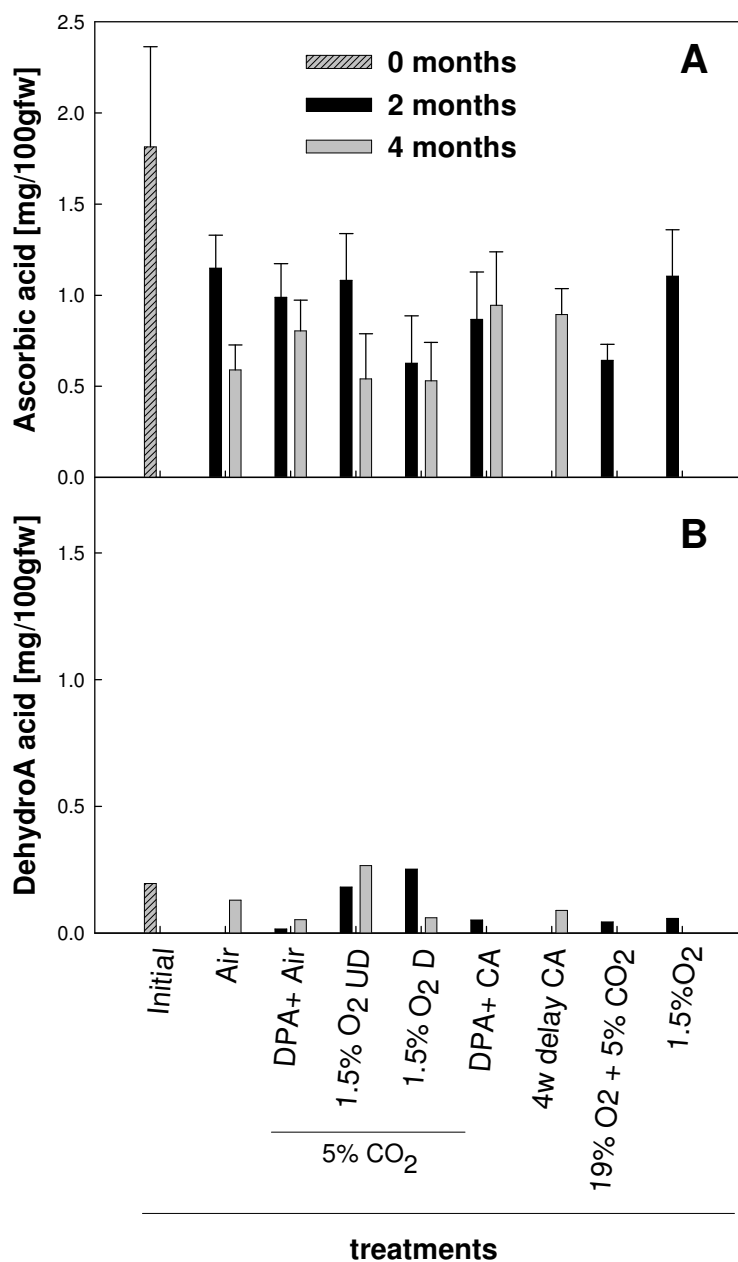


Figure 4. Ascorbic acid (A) and dehydroascorbic (dehydroA) acid (B) (mg/100g fresh weight) concentrations in apples at harvest and after 2 and 4 months storage in 2005 at 0.5°C in air, CA (1.5% O₂ + 5% CO₂), 19% O₂ + 5% CO₂ or 1.5% O₂ (with <0.5% CO₂). Additional fruit treated with 2200 ppm diphenylamine (DPA) were stored in air or CA. One set of fruit were held 4 weeks at 0.5°C in air before CA. Apples stored in 1.5% O₂ + 5% CO₂ were divided into undamaged (UD) and damaged (D) fruit. Bars correspond to standard deviation. Starch index at harvest 3.5.

8. Carbon Dioxide-Induced Flesh Browning in Pink Lady Apples

Elena de Castro, Bill Biasi, Stuart Tustin, David Tanner, Jennifer Jobling and Elizabeth Mitcham
Department of Plant Sciences, University of California, Davis, CA 95616

Abstract

To investigate a flesh browning disorder in Pink Lady™ Apple (*Malus domestica* Borkh, Pink Lady*) fruit were harvested from the same orchard each year from 2002 to 2005, at two or three maturity stages each year. Fruit were stored after 24h cooling in air or controlled atmospheres (CA) at 0.5°C. Additional subsets of fruit were exposed to 1 ppm 1-methylcyclopropane (1-MCP) for 24 h, dipped in 2200 ppm diphenylamine (DPA) for 5 minutes or held in air at 0.5°C for 2 or 4 weeks before CA storage. Incidence of CO₂-induced flesh browning (FB) was determined after storage. FB was not seen in air-stored fruit, but appeared in CA-stored fruit as soon as 2 months after harvest, and the incidence did not increase after longer storage times. Additionally, FB increased with increasing CO₂ concentration and decreasing O₂ concentration in storage. 1-MCP did not significantly affect the incidence of FB while DPA inhibited it completely. Delaying CA by 2 or 4 weeks reduced the incidence of FB, but did not inhibit it completely. When comparing similar storage atmospheres for the four seasons, FB incidence was severe, low, moderate and low-moderate in 2002, 2003, 2004 and 2005, respectively. The concentrations of B, Ca, and Mg in apple flesh were significantly higher in 2003 corresponding with the lowest incidence of FB and apples damaged by flesh browning had lower Ca concentration in their flesh. Additionally, the 2003 season which had the lowest incidence of browning had the warmest field temperatures during the growing and harvest periods, and accumulated the highest number of growing degree days >10°C during the season.

Keywords: CO₂ injury, Cripps Pink, delayed CA, diphenylamine, DPA, *Malus x domestica*, 1-methylcyclopropane, mineral composition, temperature.

*Cripps Pink apples of an appropriate quality may be sold using the trademarked brand name Pink Lady™.

Introduction

The Pink Lady™ apple was developed in Australia in the late 1960's from a cross between Golden Delicious and Lady Williams. Since then, it has been sought after as a result of its sweet-tart flavor and crunchy texture. However, in recent years Pink Lady™ apples have shown a susceptibility to developing internal browning in storage.

Controlled atmosphere (CA) storage extends the life and preserves the quality of Pink Lady™ apples (Cripps et al., 1993). However, CA can also cause physiological disorders in susceptible apples. For example internal flesh browning in 'Fuji' apples (Volz et al., 1998) and Braeburn Browning Disorder (BBD) (Lau, 1998). Internal flesh browning (FB) disorder of Pink Lady™ apples may result from the interaction of yet to be identified pre and postharvest factors, as well as the physiological state of the fruit at harvest. The occurrence of FB in Pink Lady™ apples generally occurs intermittently and in unpredictable patterns. There are at least three different manifestations of this physiological disorder. One is diffuse flesh browning, which appears to be related to chilling injury (James et al. 2006, Bramlage and Meir, 1990); the second is radial browning which is related to senescent breakdown (Wilkinson and Fidler, 1973.) and the

third is CO₂ injury (brown spots that may develop into cavities), associated with CA storage (Lau, 1998).

There are several factors that predispose apple fruit to postharvest storage disorders. Variations in mineral composition are widely recognized to affect fruit quality after harvest (Bramlage et al., 1980; Sharples, 1980). Mineral composition greatly influences postharvest quality retention, and calcium is dominant in this respect. Trees high in nitrogen are usually vigorous trees with low crop loads and large fruit, which are high in nitrogen and low in Ca. Boron has a role in the structure of the plasma membrane (Parr and Loughman, 1983) and calcium is important in all cell membranes to stabilize phospholipids (Marinos, 1962).

The postharvest quality of fruit is superior when the number of cells in the fruit is maximized (Little and Holmes, 2000). This is mostly influenced by crop load and the environmental conditions during the first four weeks of development after full bloom. Cell division occurs during the first 50 days after full bloom (dafb) and the climatic conditions during this time are critical for the structure of the fruit (Austin et al., 1999). Light crop loads result in fruit with higher density than from trees with more commercial size crop loads (Bussakorn et al., 2001). In light cropping seasons, marginal winter chilling may limit cell division, resulting in bigger apples with lower numbers of cells, which are, then, more susceptible to postharvest disorders, like Braeburn Browning Disorder (BBD) (Elgar et al., 1999).

Biennial bearing or alternate year cropping occurs when apples have 'on' years, with abundant crops, and 'off' years with little crop (Singh, 1948). Fruit spurs of many apple cultivars tend to be biennial, including Pink Lady™. Heavy flowering during the 'on' year results in small, poorly colored, low quality fruit. The following year, flowering is light and generates fruit that are too large, susceptible to physiological disorders and generally of poor quality (Monselise and Goldschmidt, 1982; Ferguson and Watkins, 1992). Some apples, 'Laxton's Superb' and 'Beauty of Bath' for example, are naturally biennial bearing, while others are tipped into this mode by a frosty spring for example, when no blossom is pollinated. This tendency for biennial bearing spurs may be a factor in the sporadic occurrence of the FB disorder in Pink Lady™ apples.

Not only after full bloom, but also during harvest, climate has a great effect on postharvest development of physiological disorders (de Villiers, 1961). Lau (1998) assessed the relationship between the incidence of Braeburn Browning Disorder (BBD) and cumulative growing degree days higher than 10°C from 1-May to harvest. Lau concluded that a cool growing season could increase the susceptibility of the apple to CO₂-injury. Other weather effects are more related with harvest time. Diurnal fluctuations in temperature, light intensity and other developmental and environmental factors can significantly affect the concentration of antioxidants, anthocyanins, carotenoids, sugars and ascorbic acid, as well as fruit size (Kondo, 1992; Johnson and Andrews, 1997; Johnson et al., 2001).

Fruit maturity has been demonstrated to be a factor in susceptibility to internal browning in some apple varieties (Voltz et al., 1998 and Lau, 1998). Diphenylamine is an antioxidant used commercially to inhibit storage scald in apples (Lau, 1990). Its antioxidant properties markedly reduced both external and internal CO₂ injuries in some apple varieties (Watkins et al., 1997; Fernandez-Trujillo et al., 2001). Preliminary work with Fuji apples has shown that the susceptibility of apples to CO₂ injury is highest during the first few weeks of storage after harvest. Delaying the establishment of CA reduced internal browning while fruit quality was retained (Argenta et al., 2000). The application of DPA or delaying the start of CA storage may help to reduce the incidence of FB in Pink Lady™ apples.

Our objectives were to determine the most important pre-harvest and postharvest factors that affect the susceptibility of Pink Lady™ apples to develop CO₂-induced internal flesh browning and investigate possible solutions.

Materials and methods

Fruit Material

On 21 September and 20 October 2002, 6 and 20 October and 6 November 2003, 21 September and 5 and 22 October 2004 and 29 September and 15 October 2005, Pink Lady™ apples were harvested in the early morning, from five 40-tree plots in the same orchard near Stockton, California, USA. Approximately 50 apples were harvested randomly per tree, and each tree was harvested once.

Quality and Internal Browning Evaluation

On the day of harvest, 30 fruit were selected randomly among the plots to determine firmness and starch content. Firmness was measured as resistance to penetration with an 11-mm probe on opposite sides of the fruit after removing a small area of peel using a Fruit Texture Analyser (Güss, South Africa). The apples were then cut in half, dipped for two minutes in iodine-potassium-iodide (3%) and rinsed in fresh water. The starch levels were scored using a CTIFL (Centre technique interprofessionnel des fruits et légumes, Paris, France) 10 point scale. For FB evaluation after storage, 80, 105, 125 and 125 fruit per treatment in 2002, 2003, 2004 and 2005, respectively, were cut equatorially in three equally spaced locations resulting in ~2-cm thick slices, and the percentage of flesh area showing browning was visually estimated.

Treatments

The day following each harvest, after holding overnight at 10°C, fruit were sorted to obtain undamaged fruit of uniform size and color and immediately cooled to 0.5°C in air for 24 hours before starting the CA treatment. Following a determined storage period plus 5 days at 20°C, fruit were assessed for FB firmness and other quality attributes as previously described.

Harvest maturities each year were around the same starch index, harvest 1, ~ 3.5, harvest 2, ~ 6.0, and harvest 3, ~8.5 (CTIFL). The CA storage conditions varied between years. In 2002 apples were stored in air, or 1.5, 3.0 or 21% O₂ in a factorial design with 1.0, 3.0 and 5.0% CO₂. In 2003, fruit were stored in air or 2% O₂ in a factorial with 1% and 3% CO₂. In 2004, fruit were stored in air or 1.5% O₂ in a factorial with 1.0, 3.0 and 5.0% CO₂ and in 2005, fruit were stored in air or 1.5% O₂ + 5.0% CO₂. The storage temperature was always 0.5°C and the storage time varied between 2 and 6 months.

Additional apples from the second harvest in 2003 were pre-cooled at 0.5°C and placed into 50 L steel tanks for 1-MCP treatment. A small electric fan was placed inside each tank to ensure even distribution of 1-MCP gas around the fruit. The tank lids were constructed so as to fit into water filled troughs, forming a hermetic seal. Smart Fresh® tablets were used to generate 1 ppm of 1-MCP gas. Apples were treated with 1-MCP for 24 hours at 0.5°C and then transferred to air or CA at 0.5°C. A second set of fruit from the same harvest in 2003, and additional fruit from the second and first harvest in 2004 and 2005, respectively, were treated with a 2200 ppm DPA in water by immersion for 5 min., air dried at 20°C and pre-cooled overnight at 0.5°C before storage in air or CA at 0.5°C. Additional fruit from the same (2005) or another harvest (2003 and 2004), were placed into CA storage after 2 or 4 weeks delay in air at 0.5°C. All fruit with these additional treatments were stored for 4 months.

Density

Density of apples was calculated every season from 2002 to 2004 using fruit of intermediate maturity (starch score ~ 6.0, CTILF) after 3 months of storage in air at 0.5°C. Five fruit were selected, one from each block and weight and buoyancy were recorded. Buoyancy was measured by immersing the apple under water and recording the force that the apple pushed to go up to the water surface. The apple was submerged under a hemispheric sensor that was connected to a balance and recording the force (in grams). To calculate the density from the weight and the buoyancy, we use the formula:

$$\delta (\text{density}) = (\text{Weight} - \text{Buoyancy}) / \text{Weight}.$$

Mineral Analysis

Tissue samples consisted of two unpeeled wedges from each of five fruit per block. The wedges were chopped and a sample of approximately 1 g was collected including some skin, and mixed with the other apple samples so around 5g from each block were collected for analysis. The samples were dried at 90°C for 48h, heated at 500°C for 4h, and dissolved in 1N nitric acid. All samples were sent to a laboratory for analysis (UC-DANR Analytical Laboratory, Davis, California, USA.). Samples were analysed for Ca, B and Mg by inductively coupled plasma atomic emission spectrometry (ICP-AES) as described by Meyer and Keliher (1992) and by atomic emission spectrometry (AES) for K.

Individual apple mineral composition was also measured in healthy tissue of fruit with and without FB after storage in 1.5% O₂ + 5% CO₂ for 2, 4 and 6 months in 2004 and 2 and 4 months in 2005. A total of 30 apples were analysed each year. After each storage time, apples were selected, one half with FB and one half without. Samples were freeze-dried and sent to a laboratory for analysis as previously described.

Weather Data

The weather data were collected from a weather station, very near the orchard, in Farmington, California (California Weather Data, University of California, Statewide Integrated Pest Management Program). The average daily air temperature was recorded for the 50 days after full bloom, beginning on March 4, 7, 21 and 17 for 2002, 2003, 2004 and 2005, respectively.

To calculate the growing degree days (GDD) ≥ 10 during one period, the base of the calculation (10) was subtracted from the daily average temperature for every day of that period. The positive remainders were added to calculate the cumulative growing degree days (CGDD) ≥ 10 . To calculate the CGDD $< 10^\circ\text{C}$ during one period, the base of the calculation (10) was subtracted from the daily temperature average for every day of that period, and the negative remainders were added to obtain the total CGDD $< 10^\circ\text{C}$.

Statistical Analysis

The experimental design consisted of two or three harvest maturities, storage time (2 and/or 4 and/or 6 months) and treatment (atmosphere, DPA, 1-MCP and delayed CA). Each year was analyzed separately by harvest date and time in storage and a factorial design was employed with 5 repetitions of 16, 21, 25 and 25 individual fruit each consecutive year. Analysis of variance was computed by SAS Version 8.02 (SAS Institute Inc., Cary, NC). Least square means were employed due to missing values in some treatments. Multiple mean comparisons were

performed using Tukey-Kramer adjustment which was necessary due to the large number of mean comparisons required, and the need to maintain a low experimental error rate ($\alpha=0.05$).

Results

High CO₂ and low O₂ related injury

The incidence of fruit with FB increased with greater CO₂ and with lower O₂ concentrations during storage, even when the O₂ concentration was 21% (Fig. 1). The incidence of FB increased about 27% at 5% CO₂ and 58% at 3% CO₂ when the O₂ content decreased from 3 to 1.5%. The incidence of FB injury was somewhat greater in fruit from the late harvest in 2002, but was not statistically significant, and FB incidence was similar among harvests in 2003 and 2005. However, in 2004, the FB incidence of the first harvest (21.3%) was significantly higher than for the following harvests (9.6 and 6.6%, data not shown). No change in FB incidence was observed between 2, 4 and 6 months of storage (data not shown), and fruit stored in air never exhibited FB at any time, a pattern that was consistent among years.

Effect of 1-MCP, DPA and delayed CA

Treatment of fruit with 1-MCP before storage did not reduce the incidence of FB (data not shown). DPA inhibited FB completely in three consecutive seasons, an example is shown in Table 1. Delays of 2 and 4 weeks in cold storage at 0.5°C before placing the apples in CA storage decreased the incidence of FB after 4 months of storage (Table 2). However, only the four week delay before 5% CO₂ storage significantly reduced FB, with a 75% decrease compared to no delay in CA storage. Fruit from the 4-week delay were significantly softer (4 N or 7% decrease in 3% CO₂ and 5 N or 9% decrease in 5% CO₂) than non-delayed fruit, while the 2-week delayed fruit had similar firmness as the non-delayed controls after 4 months storage (data not shown).

Mineral content

We observed the relationship between FB incidences each year with fruit mineral content (Fig.2). In 2003, fruit had a lower incidence of FB and had significantly higher Mg, B, and Ca content and significantly lower content of K than in 2002, 2004 and 2005. However, the 2005 season with low-moderate FB incidence, had the lowest Ca concentration of all seasons and lower Mg, B and higher K than in 2003. There was no relationship between NO₃⁻, NH₄⁺, Zn, Fe, Cu, P or S concentration and FB (data not shown). When individual apples were studied, Ca concentration in the flesh was significantly lower in apples stored in CA and damaged by FB than in undamaged apples kept under the same CA conditions (28.1 vs. 36.6 µg/gfw Ca).

Seasonal weather

The incidence of flesh browning in Pink Lady™ apple is variable season to season. For the intermediate maturity harvest in 2002, the percentage of flesh browning (FB) was as high as 14% and in 2003, the percentage of apples affected was as low as 1.3% (Table 3). In 2003 and 2005 when the percentage of FB was low, the fruit was large (183-200 g). In 2002 and 2004, when the percentage of FB was higher than in 2003 and 2005, the crop load was slightly higher, corresponding with a smaller fruit size (173-176 g).

The daily average air temperature and CGDD $\geq 10^{\circ}\text{C}$ during the 50 dafb did not relate to the pattern of FB susceptibility among seasons (Table 4). However, the CGDD $< 10^{\circ}\text{C}$ was highest in 2002, the season with the highest incidence of FB. However, the relationship was not

strong in other seasons. The density of the fruit at harvest may relate to incidence of FB as well and to the weather during the 50 dafb. The season with the highest density at harvest had the highest incidence of FB (2002).

We also determined the CGDD $\geq 10^{\circ}\text{C}$ from 1-May to harvest. Season 2003 presented the highest accumulation for every harvest maturity, corresponding with the lowest incidence of FB (Table 5). The CGDD $\geq 10^{\circ}\text{C}$ measured from May 1 to October 31, were also higher in 2003 (2,002) than the 7 year average from 1998 to 2005 (1,923), while season 2002 presented the lowest CGDD $\geq 10^{\circ}\text{C}$ (1,838) corresponding with the highest incidence of FB.

Discussion

Our results show that CO₂-induced FB can be strongly associated with the CO₂ concentration in storage. A low concentration of O₂ increases the incidence of the disorder but 21% O₂ does not prevent it when CO₂ is elevated. These results agree with Watkins et al (1997), Lau (1998) and Voltz et al (1998) who proposed that the incidence of flesh browning and external browning were greater with higher CO₂ concentration. However, other factors appear to modulate fruit susceptibility, as we can conclude from the differences in incidence of FB among seasons.

In 2002, there was a trend towards more FB in the late harvested than in the early harvested fruit; however, the effect of harvest date was not statistically significant. On the contrary, in 2004 the effect of harvest date was significant and the earliest harvest was more affected by FB. This result is contradictory to previously reported trends by Volz et al. (1998) and Lau (1998). Both groups found that delaying harvest generated a higher incidence of FB and internal cavities (IC) in 'Fuji' and 'Braeburn' apples, respectively. Lau (1998) attributed this to an increase in respiration rate, skin resistance and sensitivity to low O₂ and elevated CO₂ atmospheres in the late harvested fruit compared with the early harvested fruit. However, Elgar et al. (1999) found that early harvested 'Braeburn' apples showed a higher incidence of internal cavities than the delayed harvest fruit. Additionally, Smock and Blanpied (1963) and Meheriuk (1977) associated higher levels of external CO₂ injury with earlier harvests.

DPA completely inhibited the appearance of CO₂ injury. Others have also shown that this antioxidant can inhibit CO₂-induced external and internal browning as well as storage scald in stored apples (Watkins et al., 1997; Lau, 1990; Fernandez-Trujillo et al., 2001). Delaying the introduction of CA in cold storage has also been shown to reduce CO₂ injury, both externally (Watkins et al., 1997) and internally (Argenta et al., 2000). The reduction in damage was not seen until the delay was longer than 5 or 6 weeks for external or internal injury, respectively for Watkins (1997) and Argenta (2000), although in Fuji a 4 week delay has been shown to eliminate CO₂-induced FB (unpublished data). For Pink LadyTM, we observed a significant reduction in CO₂ injury after a 4 week delay in CA with 5% CO₂. Perhaps this cold period before CA storage allows for a reduction in respiration rates, stabilization of membranes and production of ascorbic acid after the stress of harvest in which the fruit is separated from the tree, its main source of nutrients. Firmness of Pink LadyTM apples that were held in cold storage for four weeks before CA storage was statistically lower than that of apples placed directly in CA with 1.5% O₂ plus 3 or 5% CO₂. After 5 months in CA, Watkins et al. (1997) also found a difference in firmness of $\sim 4.5\text{N}$ between 'Empire' apples stored in CA following a 10-day delay in cold storage and the fruit placed directly in CA. Argenta et al. (2000) found significant firmness loss after 8 months of storage between 'Fuji' apples placed directly in CA and apples that were delayed from 2 to 12 weeks. We did not observe any significant effect on FB incidence in the

apples that were treated with 1-MCP following CA storage. The FB disorder does not appear to be related with ethylene action.

During four seasons of apple storage, a slight correlation between high levels of Ca, Mg and B and low FB incidence was observed. Lau and Looney (1978) investigated differences in mineral content of 'Golden Delicious' apples and found a greater incidence of CO₂ injury associated with higher fruit N, Mn and Zn and lower K and Mg, but no association with Ca concentrations. Ferguson and Watkins (1992) concluded that low Ca concentrations could increase the susceptibility of apple fruit to physiological disorders. Postharvest application of CaCl₂ reduces susceptibility of apples to many long-term cold storage disorders (Ramdane and Nisnaken, 1999). However, 2005 presented the lowest Ca and B concentrations of all four seasons but the incidence of FB was only low-moderate. This result may indicate that high Ca can reduce the susceptibility to FB, but lack of Ca does not necessarily lead to high susceptibility to the disorder. When we investigated the mineral content of individual apples, those apples with FB after storage were the apples with lower Ca concentration. Did the low Ca concentration make those apples more susceptible to FB than apples with high Ca concentration due to membrane structure and stability? Perring and Plochanski (1975) and Hopfinger and Poovariah (1978) discovered that Ca concentrations were higher in the pit area in bitter pit affected apples. Mechanical wounding and corking disorders also induce minerals to move into the affected part of the fruit (Faust et al., 1968). It is possible that Ca migrated to the areas affected with FB within the apples and therefore, the Ca concentration decreased in the healthy tissues within the apple where we collected the tissue for mineral analysis. In addition, when we tested the mineral content of the brown tissue it contained high amounts of Ca (data not shown). The relationship of Ca with CO₂-induced FB remains uncertain. High concentrations of Ca appear to reduce the susceptibility of the apple to the disorder; however, Ca alone does not explain all the variability we have observed among seasons.

Our results in 2002 fit the supposition that cool temperatures and decreased cell division during the 50 dafb can lead to an increase in fruit density later in the season. High fruit density is suspected to be a precursor to development of high internal CO₂ levels during CA storage and hence, internal browning. Consequently, we could infer, that a high number of CGDD <10°C, during the 50 days after full bloom, would correlate to a greater incidence of CO₂-induced FB. In 2002, we observed the highest CGDD <10°C, the highest density at harvest and the highest incidence of flesh browning. The incidence of CO₂-induced FB seems to relate with density at harvest. Weather during the growing season and during the harvest season might have a further effect on fruit density as well.

The climate during the growing season seems to influence FB susceptibility. During the harvest season, the concentration of antioxidants is increasing and a warmer temperature with full sunlight can help to accumulate a greater amount of ascorbic acid (Johnson and Andrews, 1997) that will prevent later oxidation of the tissue in storage. During the four years of this study, there was a slight correlation between weather during the growing and harvest periods and CO₂-induced FB susceptibility. Our results agree with previous studies that found a relationship between cool growing seasons (May to harvest) and increased incidence of Braeburn Browning Disorder (Lau, 1998). Lau concluded that a total number of CGDD $\geq 10^{\circ}\text{C}$ less than 1300, resulted in an increased incidence of internal browning. For our trial with Pink LadyTM, it is difficult to propose a specific threshold number; however, it is clear that 2002, with the lowest number of CGDD $\geq 10^{\circ}\text{C}$ had the highest incidence of FB and 2003, the year with largest number of CGDD $\geq 10^{\circ}\text{C}$, had the lowest incidence of FB.

CO₂-induced FB may be related with biennial bearing and, consequently, with Ca concentration. In the “off” years, 2003 and 2005, when the crop load was low and fruit size was slightly larger; the incidence of FB was low. However, low crop load has been related to low cell number, large cell size, low Ca concentration and susceptibility to physiological disorders (Ferguson and Watkins, 1989), and higher incidence of BBD (Elgar et al., 1999). While in the “on” years (2002 and 2004), in which the apples were smaller because of heavy crop load and which has been related to higher concentration of Ca and low incidence of physiological disorders, apples had a higher incidence of FB. Monselise and Goldschmidt (1982) stated that heavy flowering during the “on” year results in small, poorly colored, low quality fruit. In addition, one of the “off” seasons, 2003, with a low crop load and large fruit, had the highest Ca concentration and the lowest FB. And the next “off” season, 2005, had the lowest Ca concentration and low FB as well. Biennial bearing may have a large influence on FB susceptibility, while the weather, calcium concentration and other secondary factors attenuate this biennial pattern.

It is clear that fruit susceptibility to FB cannot be related to a single condition before harvest or during storage. High CO₂ concentration is the main factor causing FB; however, its effect is modulated by a list of known and unknown interacting factors. Among these factors, harvest date, mineral nutrition and seasonal weather conditions have shown a relationship with apple fruit susceptibility to high CO₂-induced FB and warrant further study.

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Table 1. Incidence (%) of flesh browning in Pink Lady™ apples harvested at starch score 6.0 in 2004 after 4 months storage in air or controlled atmosphere (CO₂ with 1.5% O₂) at 0.5°C.

% CO₂	Untreated	DPA^y
(0.03) Air	0 a ^z	0 a
1	2 b	0 a
3	8 bc	0 a
5	18 c	0 a

^zDifferent letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

^y2200 ppm diphenylamine

Table 2. Incidence (%) of flesh browning in Pink Lady™ apples harvested at starch score 8.5 in 2004 after 4 months storage in air or controlled atmosphere (CO₂ with 1.5% O₂) at 0.5°C preceded by a 0, 2 or 4 week delay in cold storage.

% CO₂	Delay in CA storage (weeks)		
	0	2	4
1	1 cd ^z	0 d	0 d
3	5 bc	3 cd	4 bcd
5	13 a	8 b	3 cd

^zDifferent letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

Table 3. Average percentage of apples of intermediate maturity (starch index ~ 6.5) presenting flesh browning after storage in 3% CO₂ with either 1.5% or 2% O₂ pooled across storage time and crop load and average fruit size in 2002 to 2005.

Season	Atmosphere		Relative flesh browning	Crop Load (Kg) ^z	Average Fruit Size (g)
	1.5% O ₂ + 3% CO ₂	2% O ₂ + 3% CO ₂			
2002	14	--	High	273,806	173
2003	--	1.3	Low	221,353	183
2004	8	5.6	Moderate	334,569	176
2005	<6		Low-Moderate	271,248	200

^zTotal production in orchard

Table 4. Flesh browning incidence and cumulative growing degree days (CGDD) $\geq 10^\circ\text{C}$, CGDD $< 10^\circ\text{C}$ and density of the fruit at harvest and mean daily average air temperature for the 50 days after full bloom (dafb) in 2002 to 2005.

Season	CO ₂ -induced FB	CGDD $\geq 10^\circ\text{C}$	CGDD $< 10^\circ\text{C}$	Density at harvest (Kg/L)
2002	high	178	17.3	0.822 a ^z
2003	low	156	2.4	0.797 b
2004	moderate	322	0.0	0.802 b
2005	low-moderate	178	1.6	-- ^y

^zDifferent letters indicate significant differences ($p \leq 0.05$) according to Tukey's test.

^yNo data collected

Table 5. Flesh browning incidence and cumulative growing degree days, CGDD $\geq 10^\circ\text{C}$, for the period 1-May to harvest for each of the harvests and for the period 1-May until 31-Oct (average time for last harvest) in 2002 through 2005 and the average value for 1998-2005.

Year	FB Incidence	05/01-harvest, CGDD $\geq 10^\circ\text{C}$			05/01-10/31, CGDD $\geq 10^\circ\text{C}$
		H1	H2	H3	H3
2002	high	1571	1609	..	1838
2003	low	1820	1940	2004	2004
2004	moderate	1605	1732	1831	1846
2005	low-moderate	1634	1738	1828	1842
7 year Ave^z					1923

^z Average value for 1998-2005.

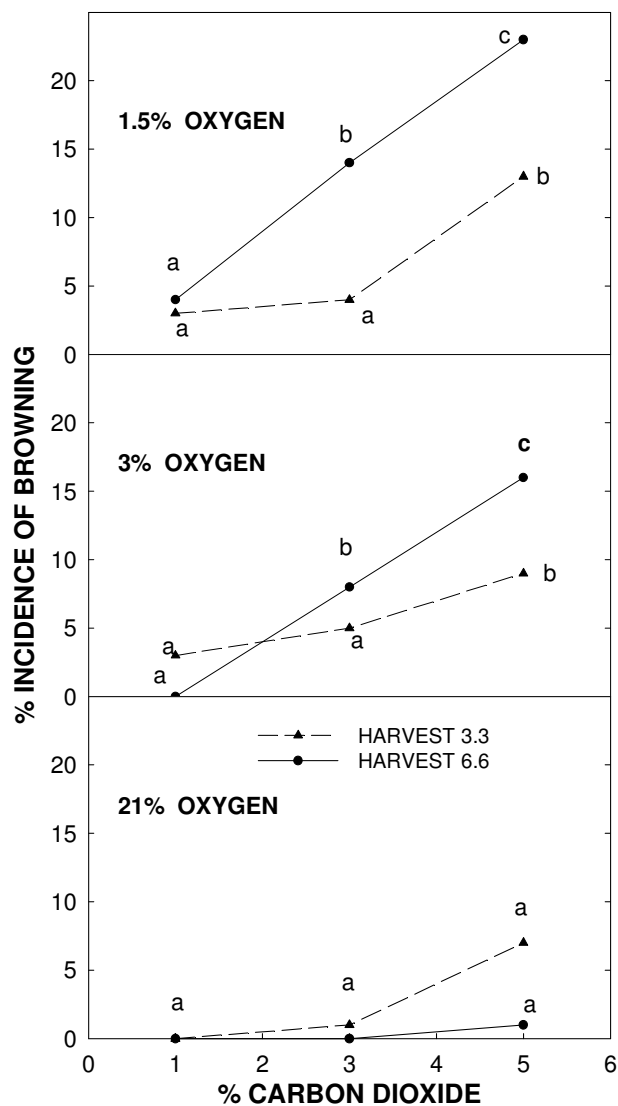


Figure 1. Relationship between the incidence of flesh browning after 2 months storage at 0.5°C in apples harvested at two maturities (starch indices 3.5 and 6.5) in 2002, and the concentrations of CO₂ and O₂ in storage. Different letters within an O₂ concentration indicate significant differences (p ≤ 0.05) according to Tukey's test.

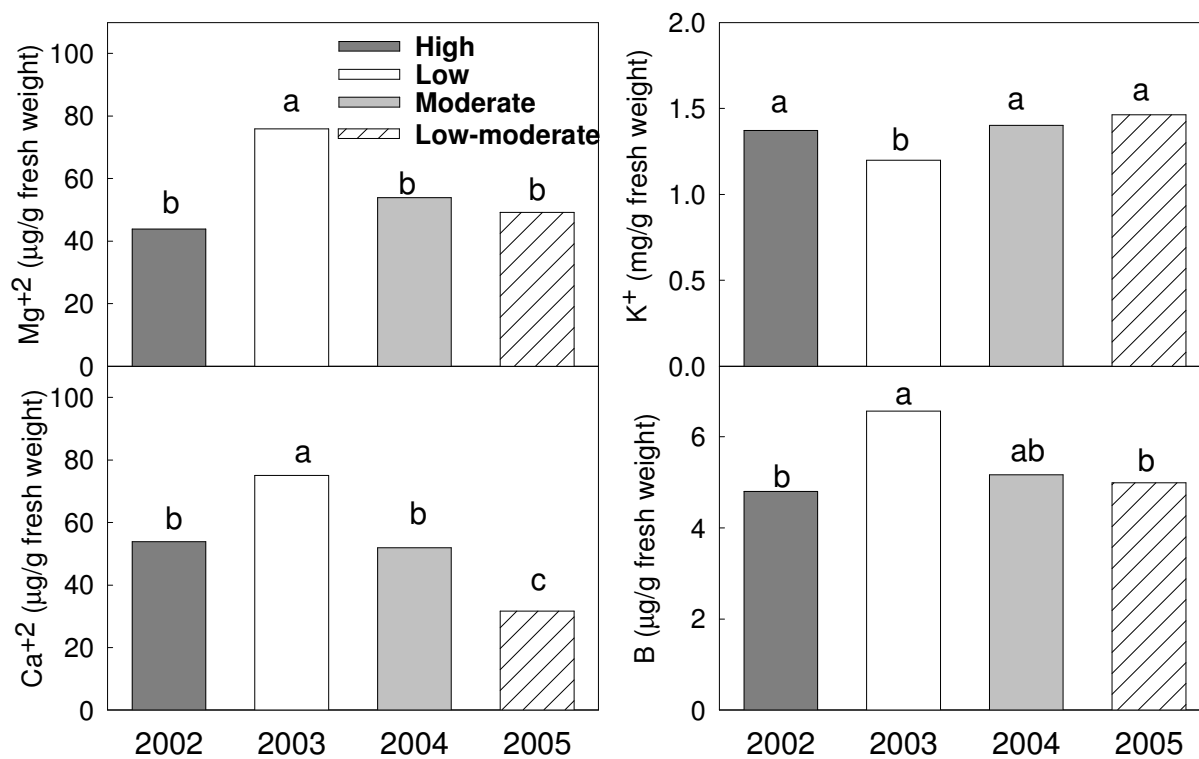


Figure 2. Calcium (Ca), potassium (K), magnesium (Mg) and boron (B) content per gram fresh weight in apples at harvest in 2002 to 2005. Different letters indicate significant differences within a mineral ($p \leq 0.05$) according to Tukey's test. High, moderate, low-moderate and low correspond to the severity of flesh browning incidence for that year.

9. Influence of harvest maturity and storage on the incidence of flesh browning in ‘Cripps Pink’ apples in New Zealand

D.S. Tustin¹, G.A. Dayatilake¹, S.M. Seymour².

1 HortResearch, Hawke’s Bay Research Centre, PB 1401, Havelock North

2 HortResearch, Nelson Research Centre, PO Box 220, Motueka

INTRODUCTION

In all of our trials since 2004, results have shown low and insignificant levels of flesh browning in stored fruit. Therefore we confined the 2006-07 study to a trial to ascertain if incidence of flesh browning is affected by the relationship between harvest maturity and storage duration and to explore if the diffuse flesh browning disorder occasionally experienced in New Zealand grown ‘Cripp’s Pink’ apple may be a time-course related senescence disorder.

MATERIAL AND METHODS

Fruit were selectively harvested to export red colour standards from ten trees in a block of ‘Cripp’s Pink’/M.26 apples at Hoddy’s Vailima Orchard, Nelson. This block of trees has been used for all our studies in the flesh browning project. An initial batch of four hundred fruit was harvested, coincided with the grower’s first selective commercial harvest date (SPI=5.4, Early harvest) and a second batch was harvested between two and three weeks later once fruit had reached a more advanced stage of maturity (SPI=7.9, late harvest). Fruit were received at Hawke’s Bay Research Centre within 24 hours of harvest. Fruit were randomly assigned to four replicates of 100 fruit for each of two storage duration treatments of 16 weeks or 26 weeks in air at 0.5°C. Ten fruit were randomly taken from each replicate for immediate maturity and quality assessment within 1 day of harvest. Table 1 presents details of treatments used to investigate the effects of harvest maturity and storage period on flesh browning of ‘Cripp’s Pink’ apple grown in New Zealand (Table 1).

Table 1. Table of maturity and storage treatments.

Treatment	Maturity stage	Storage period
1	Early	16 weeks
2	Early	26 weeks
3	Late	16 weeks
4	Late	26 weeks

Storage treatments used 80 fruit per replicate for each harvest date x storage duration treatment. Fruit maturity and quality traits were determined using the following attributes:

- Fruit fresh weight (g)
- Fruit firmness (Kgf)
- Skin background colour (CTIFL swatch)
- Percent fruit surface with red blush coverage

- Soluble solids concentration (%)
- Starch pattern index (CTIFL swatch)
-

Fruit were removed from storage and equilibrated at 20°C for 24 hours and then assessed for incidence and severity of internal flesh browning by making three transverse cuts across the longitudinal axis halfway between the stem end and the equator, across the equator and halfway between the equator and the calyx end and were assessed using the following ratings for flesh browning expression.

1. Severity of browning
The intensity of the brown colour in affected tissues was categorized into four levels using the project browning intensity scale.
2. Area of browning
Area of browning was expressed as a percent of the cut surface of the fruit using four categories defined as <10%, 11 – 20%, 21 – 40% and 41%<.
3. Type of browning
The nature of expression of tissue browning was categorized as ‘diffuse’ or ‘radial’ according to definitions used in the project.
4. Location of browning
The relative location of tissue browning identified as ‘stem end’, ‘middle region’ or the calyx end of the fruit.

The percentage of affected fruit was calculated from the total sample assessed in each replicate and data were subjected to angular transformation before statistical analysis. Statistical analysis of results was conducted using ANOVA procedure in GenStat 9.1 (Lawes Agricultural Trust, Rothamstead Experimental Station, 2006).

RESULTS AND DISCUSSION

1. Fruit quality at harvest

Fruit firmness, percent blush coverage and the soluble solids concentration of ‘Cripps Pink’ fruit showed significant increases when harvested at higher maturity (Table 2). Further increase in fruit size was also evident from the delay in harvest. The mean starch pattern index (SPI) was well separated between ‘Early’ and ‘Late’ harvests.

All of the observed trends were consistent with expectations of the influence of harvest date on fruit quality and maturity traits.

Table 2. Effect of maturity on fruit quality at harvest

Harvest period	Fresh wt (g)	Firmness (kgf)	Background colour	Red blush coverage (%)	Soluble solids (%)	SPI
Early	197.9	7.372	5.50	66.6	13.80	5.425
Late	210.0	6.890	5.65	79.2	14.23	7.925
F prob.	NS	0.001	NS	0.001	0.013	0.001
LSD 5%		0.2632		6.16	0.169	0.331

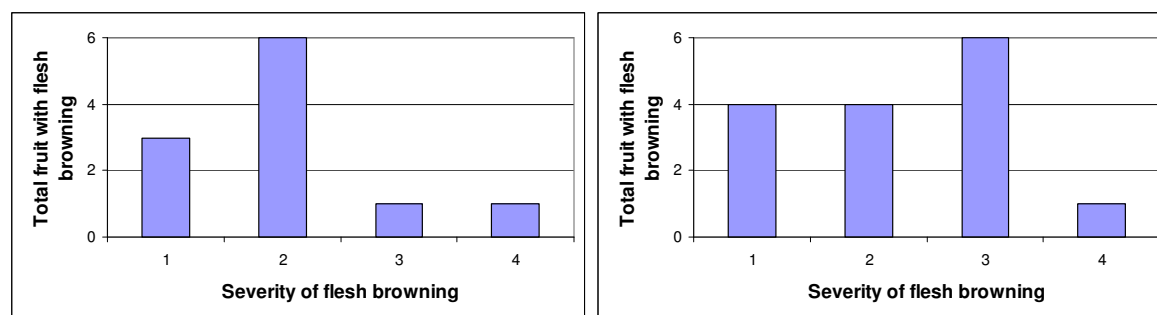
2. The incidence and severity of flesh browning in stored fruit

Fruit expressing flesh browning occurred at a low mean frequency and was not different between early and late harvested fruit and did not differ between storage durations of 16 and 26 weeks (Table 3).

Table 3. The effect of harvest maturity and storage period on percent incidence of flesh browning in ‘Cripp’s Pink’ apple (back transformed means).

Harvest maturity	Storage period	Percent incidence of FB
Early	16 weeks	3.1
Early	26 weeks	1.2
Late	16 weeks	3.6
Late	26 weeks	3.1
F prob.		NS (0.819)
LSD (5%)		

In the very low proportion of fruit that expressed flesh browning, the severity of flesh browning showed a general trend to be higher in fruit that were harvested at higher maturity (Figure 1). Numbers of fruits affected were so low that statistical comparisons were not possible. High maturity of fruit at harvest could not be related to increases in incidence of flesh browning, just a trend for slightly greater severity of expression.



1a. 'Early' harvested fruit

1b. 'Late' harvested fruit

Figure 1. The effect of harvest maturity and storage period on severity of flesh browning in 'Cripps's Pink' apple.

The proportion of total cut surface area with discoloured flesh tissue was less than 10% in most of the affected fruit, irrespective of the harvest maturity and the duration of storage. The type of flesh browning observed was almost always of the 'diffuse' category with only rare observation of 'radial' browning and in no discernable patterns attributable to harvest maturity or storage period (data not presented). Where flesh browning was observed it most commonly occurred in the stem end and the middle regions of the fruit.

CONCLUSIONS

The results from the 2006 maturity and storage trial continue a trend in recent years for a very low incidence of flesh browning in 'Cripps's Pink' apple, in which maximum incidence recorded was only 3.6%. Delaying harvest to give fruit with advanced maturity did not cause any increase in incidence of flesh browning which contrasts with our results from 2003 when markedly higher levels of browning were experienced. Extending the storage interval by 10 weeks to 26 weeks duration had no added effect on flesh browning incidence than with fruit stored for 16 weeks. Neither fruit maturity nor storage duration could be related to the development of flesh browning in the 2006 trials. The orchard block from which all trial fruit has been sourced since 2003 has a history of regular cropping at crop levels of 70-80 tonnes per hectare from 2004 onwards, which may be an orchard attribute that has reduced and maintained the extremely low occurrence of flesh browning during storage.

Flesh browning and fruit shape

Throughout the successive years of this project we have recorded one constantly recurring feature in fruit that develop flesh browning. Fruit that express flesh browning in New Zealand 'Cripps's Pink' are almost always mildly misshapen. The tissues that express flesh browning are usually associated with the region of the fruit in the misshapen zone. Misshapeness is defined as fruit with an irregular transverse outline diverging from generally round (normal) to an oval or asymmetric transverse outline (Figures 2, 3, 4).



Figure 2: Characteristics of fruit shape associated with susceptibility of New Zealand- grown 'Cripp's Pink' apple to flesh browning disorder during storage. In the left hand image the central apple is a normally-shaped fruit surrounded by four examples of misshapen fruit in each corner. The right hand image is a typical example of the expression of flesh browning associated with the misshapen region of the fruit cortex.

In many cases the irregular fruit shape is associated with zones of fruit cortex that appear to have expanded to a greater extent than the rest of the fruit tissue giving a segment or area of raised skin surface, sometimes ridged not unlike sectoral chimera mutations often seen with apple skin colour. Sometimes this expanded volume of fruit tissue is as much as one third of the surface area so that the fruit does not appear especially abnormal (Figures 3, 4). Once cut transversely through the equator, the irregular shape of fruit is more easily recognised –with the presence of flesh browning after storage. In most cases the misshapen fruit are still sufficiently uniform that they would not be rejected on a packing line.



Figure 3. The flesh browning associated with mildly irregular fruit shape. Tissues in the overgrown (ridged) segments showing expression of flesh browning.



Figure 4. Further examples of mildly irregular fruit shape and associated flesh browning expressed in the zones of increased fruit cortex growth.

10. Effects of fruit maturity at harvest and ReTain™ treatment on incidence and severity of flesh browning of ‘Cripps Pink’ apple during storage.

The objective of this experiment is to evaluate the effects of ReTain™ (AVG) in altering maturation time of fruit in relation to the seasonal red colour development and to measure effects on fruit maturity and ReTain™ on storage quality and incidence of flesh browning during storage. The study will also determine the effects of ReTain™ on the development of red blush in ‘Cripps Pink’ in relation to stage of maturity.

Fruit from the Nelson region in New Zealand were used for these experiments because of the greater problem with delayed red colour development relative to fruit maturity at harvest of ‘Cripps Pink’ apple grown in Nelson compared with the Hawke’s Bay region.

The trial site was located at Hoddy’s Vailima Orchards, using the same block of trees on which all our previous studies in the project have been conducted.

Replicated blocks of trees were used for ReTain™ treatments applied to multi- tree plots.

1. Untreated control
2. Trees sprayed with ReTain™ according to commercial recommendations 28 days before expected ‘go-date’ at rate of 830g/Ha in 1000 L water with 50ml/100L with Freeway organosilicone wetter.

Fruit were harvested at several different stages of maturity to make up the maturity and associated storage and flesh browning studies. The study of flesh browning in storage was set up using four replicates of 100 fruit per replicate for each treatment. Twenty fruit were assessed immediately and 80 fruit were placed into air storage at 0.5⁰ C for 16 weeks.

The first pair of treatments were fruit harvested from untreated control and ReTain™ treated plots when fruit sampled from the control plots averaged an SPI of 3.5 (CTIFL chart).

The second pair of treatments sampled the same plots of untreated and ReTain™ treated trees, when the maturity of fruit from untreated trees indicated an SPI of 8.5.

An additional plot of trees, treated with ReTain™ was sampled on each of the four dates that the grower made commercial picks of the block. These treatments arose as a variation from the original plan, when an anticipated delay in onset of fruit maturation did not eventuate in response to ReTain™ treatment.

Twenty fruit were held at room temperature for 24 hours after harvest and assessed for fruit attributes indicative of stage of maturity: Blush intensity – CTIFL colour swatch, Background colour - CTIFL colour swatch, Starch pattern index – CTIFL 10-point starch score chart. Means of fruit characteristics for each treatment measured at harvest, immediately prior to storage, are presented in Table 1.

Table 1. Maturity characteristics at harvest of fruit from untreated and ReTain™-treated trees of ‘Cripps Pink’ apple grown in the Nelson region of New Zealand.

Treatment	Blush intensity	Background colour	Starch index
Untreated control, SPI 3.5	3.80b	4.05a	3.15a
ReTain™, SPI 3.5	3.12a	3.92a	2.90a
Untreated control, SPI 8.5	6.93g	4.90c	7.03d
ReTain™, SPI 8.5	6.27f	4.97c	6.22c
Commercial harvests			
ReTain™, first pick	4.66c	3.95a	4.12b
ReTain™, second pick	5.50d	4.50b	6.25c
ReTain™, third pick	5.67e	4.82c	7.20d
ReTain™, fourth pick	5.30d	5.02c	8.22e
P value	<0.001	<0.001	<0.001
LSD (5%)	0.37	0.31	0.50

Incidence of flesh browning in fruit at harvest was not detected

ReTain™ treatment did not significantly delay onset of fruit maturation but did reduce red colour intensity slightly of fruit picked on the first harvest date. With fruit harvested at high maturity, ReTain™ appeared to have slowed the rate of fruit maturation between the early and late harvest and red blush intensity was also slightly less. Actual SPI achieved at the late harvest was somewhat less than originally intended. Despite a detectable reduction in red blush intensity caused by ReTain™ treatment, the sequential harvests timed with the commercial harvesting of the block, show that ReTain™ treatment did not adversely affect overall fruit red blush development. But ReTain™ treatment did not enhance fruit red blush intensity by delaying the time of onset of fruit maturation.

Fruit assessment after 16 weeks air storage.

Fruit were removed from cool store after 16 weeks of air storage at 0.5⁰C and held at 20⁰C for 24 hours, then assessed. Incidence of flesh browning was assessed by cutting fruit in three transverse slices: i) halfway between the stem end and the equator, ii) the equator and iii) halfway between the equator and the calyx end and then examining cut surfaces for flesh browning.

If flesh browning was detected, the symptoms were rated using under three categories:

1) *Severity* - according to the intensity of brown colour in the discoloured area, five categories of increasing severity were defined as internal browning with level 1 being no browning and

severity levels 2, 3, 4 and 5 in increasing intensity of colouration.

2) *Area* (% of affect area of the total cut surface. Four levels were used as ratings <10%, 11% – 20%, 21% – 40%, >40%.

3) *Pattern* of the flesh browning expression, whether diffuse or radial symptoms.

Very low incidence of flesh browning of between one and three percent was encountered across treatments and among the range of fruit maturities evaluated (Table 2). The low incidence of flesh browning did not enable any statistical separation of treatments and no indicative trends were evident either.

Table 2. Incidence of flesh browning in fruit of ‘Cripps Pink’ apple after 16 weeks storage at 0.5⁰C in air, from untreated and ReTainTM-treated plots within a Nelson, New Zealand orchard, harvested over a range of fruit maturity. (means are back-transformations of angular-transformed data used in analysis)

Treatment	Mean percent flesh browning
UTC: Untreated control, picked at 3.5 SPI	1.82
ReTain®, picked at 3.5 SPI	2.66
Untreated control, picked at 8.5 SPI	1.15
ReTain® treated, picked at 8.5 SPI	1.10
ReTain®, picked at the time of 1 st commercial pick	1.25
ReTain®, picked at the time of 2 nd commercial pick	1.52
ReTain®, picked at the time of 3 rd commercial pick	2.28
ReTain®, picked at the time of 4 th commercial pick	1.41
F pr.	NS

In discussion with the project team in the December telephone hook-up, it was considered that a minimum storage interval of 6 months may be an important requirement to achieve expression of flesh browning at levels that enable the evaluation of remedial treatments. Our use of 16 weeks which is relevant to the New Zealand ‘Cripps Pink’ growers may be an interval that is too short for other than the most highly susceptible lines of fruit.

11. Seasonal fruit development of ‘Cripps Pink’ apples in New Zealand.

Stuart Tustin, Shayna Ward, Shona Seymour, Daya Dayatilake, Ken Breen, Robert Diack.

During our final review in the original project, it was identified that we did not have good data on the seasonal growth and development of Cripps Pink apple, in particular detailed understanding of the early fruit development. In the 2004-05 seasonal cycle we have undertaken the measurement of fruit growth and the changes in fruit volume, fruit density and fruit dry matter content during fruit development. (Figures1-4).

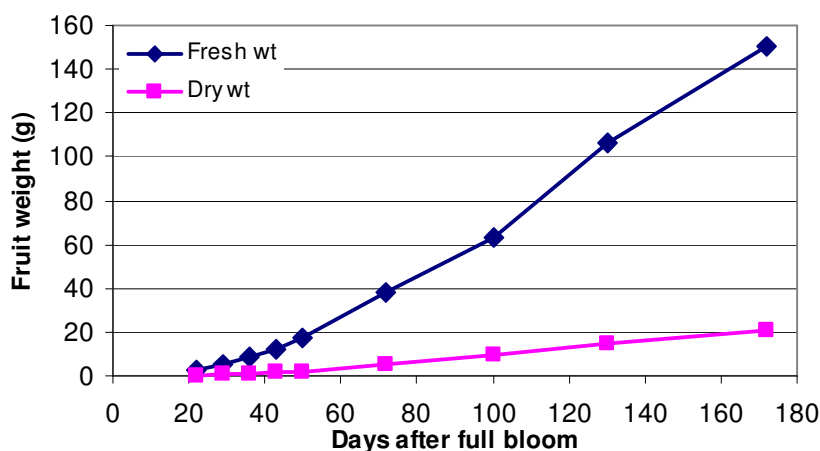


Figure 1. Seasonal fruit growth (fresh weight and dry weight) of ‘Cripps Pink’ apple, grown in the Hawke’s Bay region of New Zealand.

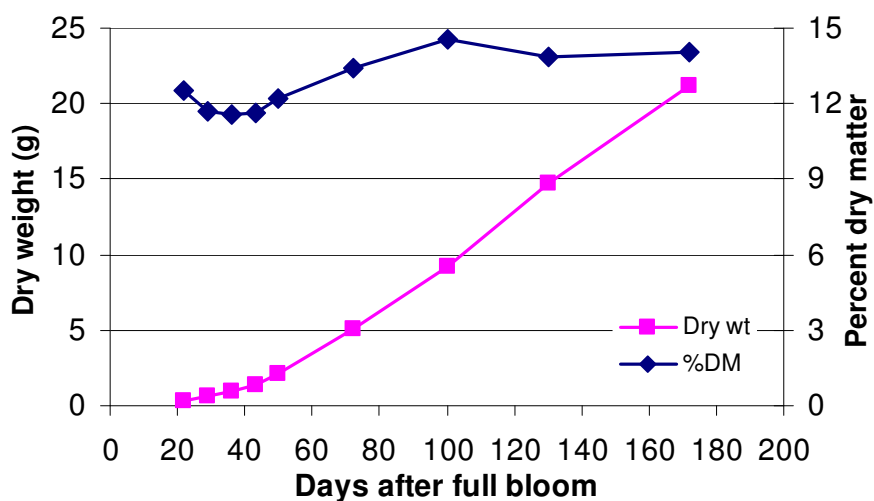


Figure 2. Seasonal changes in fruit dry weight and fruit dry matter content of ‘Cripps Pink’ apple grown in the Hawke’s Bay region of New Zealand.

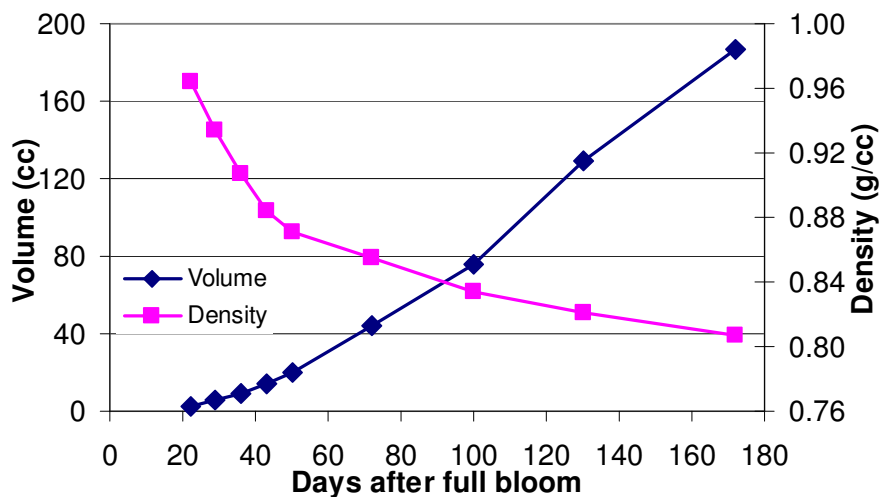


Figure 3. Seasonal changes in fruit volume and fruit density of 'Cripps Pink' apple grown in the Hawke's Bay region of New Zealand.

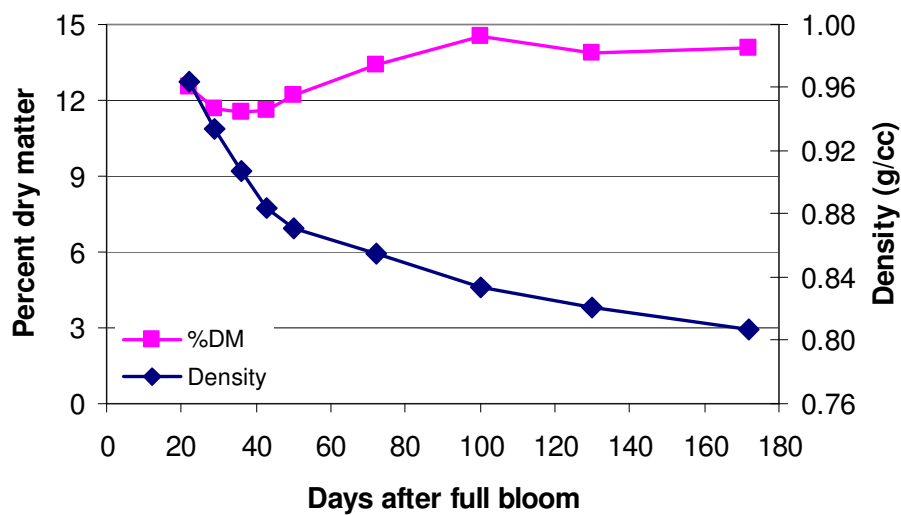


Figure 4. Seasonal changes in fruit dry matter content and fruit density of 'Cripps Pink' apple grown in the Hawke's Bay region of New Zealand.

12. Summary Climatic Data from Food Science Australia

Analysis of seasonal climatic factors has identified several key times throughout the growth and development of Pink Lady™ apples that are hypothesised to relate to the development of the Flesh Browning (FB) disorder.

It has previously been shown that districts or seasons can be classified as being at risk of developing either Radial or Diffuse types of FB depending on the accumulation of Growing Degree Days (GDD) above 10°C. This is an important classification to make as this will then influence the other risk factors identified and will also determine the storage recommendation for the fruit.

Analysis of major apple growing districts (including Hawkes Bay and Nelson in New Zealand, Goulburn Valley, Yarra Valley, Huon Valley, Batlow and Manjimup in Australia) have indicated that a seasonal GDD (from full bloom to harvest) below 1300 results in a risk of developing Diffuse FB (DFB). A seasonal GDD between 1300 and 1700 results in the development of Radial FB (RFB) during storage and when the seasonal GDD exceeds 1700 it appears that there is no risk of developing either DFB or RFB, warm districts such as this are only at risk of developing CO₂ injury during storage.

Once the type of FB that the season or district is prone to developing has been established, further climatic risk factors can then be identified. For RFB, another climatic risk factor is the GDD accumulation during the first 50 days after full bloom (DAFB). This period of time is linked to the density of the fruit with cool seasons resulting in an increased period of cell division. Fruit with an increased cell density are more prone to the development of CO₂ related RFB and to CO₂ injury. This should be given increased consideration when the fruit are going to be stored in Controlled Atmosphere (CA) storage due to the increased CO₂ in the storage atmosphere in CA storage.

Both RFB and DFB are also influenced by late season climatic factors such as the GDD and the diurnal temperature difference. The GDD during the last 60 days prior to harvest have been linked to the development of chilling injuries in other apple cultivars. The 2004/05 seasons storage results indicate the significant role that storage temperature had in the development of both DFB and RFB indicating the role that chilling injury has in the development of this disorder. Diurnal temperature differences leading up to harvest are primarily related to the development of red blush colour on the skin of the fruit. For optimal blush colour development, a high cumulative diurnal temperature difference (CDTD) with day temperatures above 20°C and nights below 10°C is required. As blush colour is one of the primary quality characteristics of the Cripps Pink (CP) apple, especially for exported fruit, there is a risk that blush colour development overrides the harvesting of the fruit at the correct physiological maturity for long-term storage. It has been found that fruit are left on the tree beyond the ethylene climacteric in order to meet the requirements for red blush. This results in the fruit being harvested in an over mature state, which has been shown to increase the risk of developing both DFB and RFB during storage.

The following is a summary of the climatic conditions observed in the 2005/06 season for several of the growing districts which have participated in the FB project.

New Zealand

- ***Hawkes Bay***

This district is one of the cooler districts in the trial. The GDD for the entire 2005/06 season was 1157 (Food Science Report Table 1) which classifies this district as having a risk of developing DFB during storage. The entire season GDD was higher than in previous seasons, this indicates that the incidence of DFB for the 2005/06 season would likely be lower than in previous seasons.

In the 2005/06 season, the CDTD was lower than previous seasons, indicating a risk of harvest of over mature fruit. The CDTD for the last 60 days before harvest in this district is in the region of 600 – 750 (Food Science report Figure 24), which indicates that even a warm season in this district will still be at risk of delayed harvest.

The accumulation of GDD for the last 60 days prior to harvest showed a relatively warm late season (Food Science Report Table 4) reducing the risk of chilling injury and DFB during storage under normal storage conditions.

- ***Nelson***

This district has also been classified as having a risk of developing DFB during storage. The GDD for the entire 2005/06 season was 1106 (Food Science Report Table 1). Similarly to Hawkes Bay, this was a warmer season than the previous five seasons, indicating a reduced risk of developing DFB during storage.

The CDTD for the 2005/06 season was consistent to that found in the previous five seasons indicating no increased risk for the harvest of over mature fruit. Similarly to Hawkes Bay, the CDTD for the last 60 days prior to harvest is between 600 – 700 (Food Science Report Figure 25) depending on the season. This indicates that even a warm season in this district will have a disadvantage for developing optimal red blush.

Similarly to Hawkes Bay, Nelson also showed a relatively warm late season (Food Science Report Table 4) reducing the risk of chilling injury and DFB during storage under normal storage conditions.

Australia

- ***Batlow***

The GDD for the entire 2005/06 season was 1543 (Food Science Report Table 1), this classifies the district as having a risk of developing RFB during storage. The entire season GDD was higher than the previous season, indicating that the incidence of RFB would be lower than the previous season.

In the 2005/06 growing season, the accumulation of GDD during the 50 DAFB was lower than the previous seasons (Food Science Report Figure 7) indicating the potential for an increased risk of RFB and CO₂ injury during storage.

The CDTD for the 2005/06 season was not dissimilar to that of previous seasons (Food Science Report Figure 29) indicating no increased risk of delayed harvest. The accumulation of GDD in the late season reached 431 (Food Science Report Table 4) indicating no increased risk of chilling injury during storage under normal storage conditions.

- ***Goulburn Valley***

The Goulburn Valley is the warmest district assessed in Australia. This district has been classified as being at risk of developing RFB. The GDD for the entire 2005/06 season was 1706 (Food Science Report Table 1). This value indicates that that this season, the Goulburn Valley has little or no risk of developing RFB during storage.

In the 2005/06 growing season, the accumulation of GDD during the 50 DAFB reached over 300 (Food Science Report Figure 4) and did not indicate an increased risk of RFB or CO₂ injury during storage. In comparison to previous seasons, this was a relatively average accumulation of GDD for this climatic risk time.

The CDTD during the late season indicated no risk of delayed harvest for the Goulburn Valley. The CDTD this season was higher than that of previous seasons indicating a higher potential for optimal blush colour. The seasonal CDTD for this region is in excess of 1100 (Food Science Report Figure 26) indicating optimal conditions for the development of red blush. The Goulburn Valley accumulated 562 GDD during the last 60 days before harvest (Food Science Report Table 4) indicating no risk of developing chilling injury during storage under normal storage conditions.

- ***Yarra Valley***

This district is on the climatic borderline between DFB and RFB and in some seasons, both types of FB have been observed. The GDD for the entire 2005/06 season was 1234 (Food Science Report Table 1). This indicates that the 2005/06 season in the Yarra Valley would be borderline between DFB and RFB in terms of the expression of symptoms.

The accumulation of GDD during the 50 DAFB was higher than previous seasons (Food Science Report Figure 5) and did not indicate an increased risk of RFB or CO₂ injury during storage.

The CDTD was higher than previous seasons (Food Science Report Figure 27) indicating a reduced risk of late harvest than has been observed in previous seasons.

The accumulation of GDD in the late season reached 391 (Food Science Report Table 4) indicating a low risk of chilling injury during storage under normal storage conditions.

- ***Manjimup***

Manjimup is also on the climatic borderline between DFB and RFB. The 2005/06 season was cooler than previously recorded seasons, with a GDD of 1079 (Food Science Report Table 1). This indicates that this season would be at a significant risk of developing DFB during storage.

Despite the cooler than average season, the CDTD observed in Manjimup was higher than average (Food Science Report Figure 30). This indicates optimal blush development conditions and a reduced risk of the harvest of over mature fruit.

Manjimup had a cooler late season than in previous years (Food Science Report Table 4) indicating an increased risk of developing chilling injury during storage under normal storage conditions.

A Review of the final milestone report for Project “AP02009” relating to Pink Lady apples –March 2005.

By Colin R. Little

The project has completed its second season now and many aspects associated with the post harvest handling of the apple variety ‘Pink Lady’ have been investigated. Of prime importance are the adverse effects of over maturity status, high carbon dioxide and or low oxygen and low storage temperature on the sensitivity of Pink Lady to Flesh Browning (FB).

Findings which relate to storage regimes and fruit storeability.

While maturity has been identified as a major factor associated with the onset of FB during storage and of the incidence, severity and type of FB manifested, no specific levels of maturity, based on the EUROFRUIT (‘ctifl’ starch scoring standard) or other approved starch standards, have been given to say when the fruit are suitable for short term CA (STCA) medium term CA (MTCA) or long term CA (LTCA). Neither has there been information given as to the likely duration of storage for the different storage categories with fruit sourced from the various regional locations. However, having said this I realise that to satisfy this requirement a lot more detailed work would need to be done.

The positive findings made so far in relation to storage regimes are that it is desirable to hold carbon dioxide below 1.0%, particularly during the early stage of CA storage, and to hold oxygen at levels not lower than 1.5% and between 1.5% and 1.8%. With regard to the effect of storage temperature, ‘step-wise’ cooling, the avoidance of rapid initial cooling and the use of a higher long-term storage temperature (to 3.0°C) have each given some reduction in FB. However, there is still some confusion over how to manage step-wise cooling. As I see it, the first 14 days of storage is at 4°C. In this period the first 7 days of storage is during room filling under non – CA conditions. Room sealing and oxygen reduction occurs on or about day - 9, and while in the final stages of oxygen reduction the temperature is reduced to 2°C on day 14, and remains at this level until day 21 when it is reduced to 1°C and remains at this level for the duration of the CA storage period.

Findings, from the work conducted at Laimburg in the Adige Alpine Valley of Northern Italy, concur with the Knoxfield work in showing the advantage of step-wise cooling as a means of reducing initial low temperature stress and minimising both categories of FB. The Italian work goes further in two regards. Firstly, the use of ‘chill shock’ (which was rapid initial cooling to 0°C) had the opposite effect to step-wise cooling and tended to increase the incidence of FB. Secondly there was data showing a reduction in FB by using a continuous storage temperature of 4°C in conjunction with low oxygen (1.8%) CA, but in this study there was no measurement of chlorophyll degradation (yellowing) which may be associated with such a high temperature regime during storage.

Yellowing is a condition that the Australian Supermarket buyers are asked to use as a means of down grading the buying price.

Research initiated to predict, in advance, the potential for FB in a current season.

The idea here was to see if a simple post harvest treatment could be used which would give pre-warning as to whether Pink Lady apples from the current harvest had a low, medium or high propensity to subsequent FB which may occur later in storage.

A double waxing applied prior to storage was chosen as a treatment that would be likely to reduce skin permeability by blocking transpiration through the lenticels, and in turn would decrease gas exhalation and increase the inter-cellular carbon dioxide status.

The treatment worked well in one season but not so well in the second season. Loss of effectiveness in the second season seems to have been due to the high overall incidence of FB that occurred in fruit from the test blocks in that season. It seems that where predisposition to FB is high, the disorder occurs at a high level without the predictive treatment. Progression in the incidence of the disorder is then only minimal with the addition of the predictive treatment.

There is still value in the idea of using a predictive indicator but, to be useful to the industry, assessments would need to be done in each regional location in each season. Then assessments would be needed after only short-term storage so that pre-warning of the predicted incidence of FB could be issued in time for pack-house operators to modify the storage duration of the portion of fruit they had allocated to (LTCA).

Regional location and district variability.

There were differences in levels of FB found at various regions and locations within a region. The incidence of FB within a specific region, and between regions, varied within the same season.

For instance, there were differences in FB incidence between three Northern Italian locations (Branzolo, Leifers and Laimburg) within the same region and in the same season. In the same context, differences between orchards in the Goulburn Valley district were appreciable where the climatic conditions, within the season, were similar and very nearly identical. Possible causes for the differences encountered were:- soil type, irrigation regime, tree vigour, severity of endemic phytophthora infection, bud line and selection of grafting scions, pollination, and incidence and severity of sun burn. Pink Lady under net were less affected by solarisation and samples selected for maturity testing were more uniform.

It is likely that temperature profiles would have been most uniform across the Goulburn Valley district which is in open plain country with a dry interior climate where water evaporation exceeds precipitation over eight months of the year. On the other hand, the Batlow district is located in mountainous country where the western flanks of some valleys come under shadow in mid afternoon during April when Pink Lady are in the ripening phase. In addition, orchards in the lower regions are at an altitude of 300 metres and those in the higher regions are at 1100metres. There are valleys, ridges, and high plateaus within the apple growing area of the Batlow district. It is possible in an average season to see a dusting of snow on surrounding hills after the passage of a cold front in April. However, if *high pressure* weather systems enter a

cycle where the easterly movement is slow across South Eastern Australia and there is a tendency for ridging up the east coast, hot continental air from the interior dominates the temperature regime at Batlow, regardless of the effect of altitude. These late autumn weather patterns are not encountered often

The Huon Valley district of southern Tasmania was shown to have a cool climate and within this region, there was a higher probability for the development on FB in Pink Lady. However, there was a weaker correlation between 'greenlife' maturity assessment and 'starch' maturity assessment in the case of Huon Valley fruit compared to Goulburn Valley fruit. This may indicate that Huon Valley Pink Lady scheduled for long term CA storage may need to be harvested at 'ctifl' Plate 2, rather than at Plate 4 as is the case for most other districts.

Gala, Red Delicious and Jonagold grown in the Houn Valley are regarded highly for maximum red colour expression, firmness and long-term storage and shelf life. If Pink Lady colour early in this region then maybe they should be picked early.

Is the FB problem in Tasmania confined to the Huon Valley district only or does it also show in the Tamar and at Legana (North Eastern Tasmania) ??.

The effect of temperature variability between seasons on the incidence of FB in Pink Lady.

Where temperature profiles were assessed in the first 50 days of fruit growth, over the full growing season, and in the 60 day period leading in to harvest it was possible to classify districts as being warm, medium warm or cool. Within the two year test period the between season variability in temperature profiles measured was minimal.

The between season variability in the incidence of FB was more pronounced.

It is possible therefore, that short term high intensity aberrations in weather events may have more impact on the physiology of Pink Lady than long- term profiles.

A cold snap in early April followed by mild temperature is ideal for red colour development and for a consistent rate in the progression of the ripening phase. An opposite effect is evident if a prolonged warm weather event occurs during the ripening phase.

With regard to weather conditions that occur in the 50 days following on from full bloom, it is possible that wind velocity, hours of sunshine and canopy wetness may alter the 'chill factor component' existing within a temperature profile. Wind velocity, cloud cover and precipitation can seriously affect a chemical thinning program. These parameters are more difficult to measure on an hour by hour basis.

If early thinning fails there are more fruit retained in the inner lower canopy. These fruit are always a problem at harvest time. If late thinning fails there are more fruit in the upper portion of the tree and these fruit are off weak blossom and are more prone to wind and sun burn during the growth and maturation phase.

Physiological factors that may influence the incidence of FB.

Fruit density and air content within the cortical tissue has been used to see if these physiological characteristics relate in any way to the development of FB during storage.

It was found that as the apple tissue density increased air content (%) within the tissue decreased. There was also some indication that high density or low air content led to some increase in FB but this was not entirely consistent for each district in each season. Pink Lady apples from the Huon (Tasmania) and the Yarra Valley (Southern Victoria) had tissue of high density and low air content but the apples from one location Tasmania had a high incidence of FB and the Yarra Valley had a low incidence of FB.

Early fruit growth was faster and dry matter content was higher in fruitlets sourced from warm locations rather than cooler locations. In addition there was a trend for lower FB in fruit sourced from warm rather than cool areas. An inconsistency occurred with Batlow apples which showed high FB in one season and not in the other. This may have been due to a change in block location between seasons.

Maturity assessment

The use of starch Plates, Plate 1 (ctifl) to Plate 10 (ctifl) is a method that could be adopted by the industry and used by individual growers. Change in the rate of change in starch indication can be influenced by an unusually warm weather event (slows the rate of change and dilutes red colour) or a vigorous cold snap (accelerates the rate of change and enhances red colour area and intensity). The starch test is subjective and relies on a colour chart and the accuracy of the assessor in matching actual apple staining with the colour plates given on an approved chart such as the (ctifl) chart. When using the starch test there is a need to standardise sample selection by avoiding inner shaded, outer sun bleached, damaged, scuffed, and insect affected apples.

Generally a 10 fruit sample taken twice weekly from a selected block is sufficient. Not only should the mean Plate value be given but there should be a statement as to the variability. (Example, mean 'ctifl' is 4.0. Range is 3.0 to 5.0). This would signify the existence of a suitable maturity status for long-term CA storage in Pink Lady sourced from most district locations. A 'ctifl' 4.0 with one apple showing 6.0 would show that although the mean score is acceptable and the apples are suitable for long-term storage there is 10% of the sample population that could develop FB if allocated to long term CA.

Greenlife assessments measure the time span in days for an apple sample taken at a given starch score to show a significant change in the rate of change of ethylene produced by the apple sample when it is held at a constant temperature.

Apples harvested at a 'ctifl' starch score of 4.0, indicating immaturity may take 13 days (Batlow) and 22 days (Huon Tasmania) to show an accelerated rate of ethylene production. Using the 'greenlife' method for assessing maturity status it was found that Pink Lady apples from the Huon in Tasmania were different to Pink Lady from most of the other locations in the trial.

The 'greenlife' method of maturity assessment is recognised as a more accurate means of assessing maturity than the starch test, and in districts where there is a wide variation between the two assessment methods, the benchmark parameters set for the starch test may need to be reviewed.

Immediate post harvest treatments used to counter the effect internal ethylene status as a stimulus to accelerated ripening.

Smartfresh (MCP-1) applied in an appropriate manner at a prescribed concentration after harvest and immediately prior to CA storage has significantly improved the degree of firmness retention in Pink Lady apples stored for short, medium and long term periods.

While the retention of firmness gained from MCP-1 was greater following standard CA storage than low oxygen CA there was still some advantage in using MCP-1 even where low oxygen CA was used. So far as firmness was concerned, MCP-1 followed by regular CA (2.0% carbon dioxide: 3.5% oxygen) was marginally better than using low oxygen CA (1.0% carbon dioxide : 1.8% oxygen)with no MCP-1 (Agricultural Research Centre, Laimburg Italy).

The data from Laimburg showed that MCP-1 was equally effective in retaining the firmness of Pink Lady apples during 6 months of storage with fruit picked on 7 October (ctifl. 5.0) or on 21 October (ctifl 7.0). There is evidence that Pink Lady picked at (ctifl. 8.0 – 9.0) or when there is a presence of greasiness that MCP-1 is less effective. It is possible that at such an advanced stage of maturity the internal ethylene status in the apple is at a level where its effect on senescence is irreversible.

MCP-1 applied after harvest gave higher titratable acidity values after 6 months of storage than was the case with non-MCPA-1 treated apples. MCPA-1 had no affect on sugar levels and there was an inconsistent response in relation to the reduction of FB.

There is no data available to show the effect of MCPA-1 on chlorophyll degradation (skin yellowing) or on red colour enhancement during storage.

MCP-1 did control scald on Pink Lady apples that got no DPA treatment. However, as Pink Lady has a low susceptibility to scald a more challenging test would be needed to see if MCP-1 controlled scald on Granny Smith or Red Delicious.

Temperature accumulation in the 50 day period after full bloom.

It has been suggested that a low level of early season temperature accumulation is likely to cause a higher incidence of FB following 4 to 6 months of CA storage. In the research findings presented the extent or degree of temperature accumulation has been quantified. Actual 'growing degree days (GDD) at 10°C and > 10°C were calculated. The various districts within Australia, New Zealand, Italy and in one location in California can be shown as having a low, medium or high potential for temperature accumulation in the period to 50 days after full bloom (50. dafb). In addition, the variability in temperature accumulation between two seasons is documented.

Fruitlet density from a number of test locations was measured but there was no consistent trend to show that fruitlets with higher density were always from cooler locations. In this context, Pink Lady responded with less consistency than Braeburn.

Temperature accumulation during the late maturation and ripening phase (60 days prior to harvest on to harvest).

In this exercise diurnal difference (the measured range between minimum and maximum temperature recordings) was taken as the prime criteria rather than temperature accumulation above or below a set benchmark. No emphasis was given to the actual temperature regime during the ripening phase (5 week period).

This part of the project was designed to see if a lower accumulated diurnal difference led to a higher incidence of FB. The thought here was that in regions where there was a low difference between the day-time maximum and night-time minimum (warm nights with warm days or cool nights with cool days) there would be a delay in red colour development. In such a situation there would be a reluctance to harvest at 'ctifl' 4.0 for long term CA due to poor red colour expression and a preference to harvest at 'ctifl' 7 when colour was beginning to show. This latter option is acceptable if the late picked fruit are marketed directly or scheduled for short term CA (STCA) only.

Temperature profile for the whole season.

There are seasons that produce firm fruit or soft fruit, highly coloured or marginally coloured fruit, sweet and not so sweet fruit, and fruit that are very prone or marginally prone to FB. What are the climatic factors that cause these differences?

Is it fruit set, blossom vigour, the effectiveness of chemical thinning, or rainfall distribution. It has been reported that the uptake of nitrate from organic matter on the orchard floor that was measured by regular sap analyses was significantly increased following a sustained rain event in early February 2005. This led to a surge in vegetative growth which was un-seasonal.

The comment by Professor Chris Watkins that we should look at a 2°C deviation within a 50 year temperature average is sound if we are convinced that seasonal temperature variation is the prime seasonal factor that is associated with the susceptibility of Pink Lady apples to FB.

There is anecdotal evidence that seasons with high FB incidence occur every 5 to 7 years. Comprehensive climatic data from a number of regions covering two of these events would require trials that continued over a period of 10 to 14 years.

Conclusions

In research covering only two seasons in the northern and southern hemispheres, a great deal has been accomplished. The research findings have re-confirmed the need for an accurate assessment of fruit maturity with criteria given for short, medium and long-term CA storage.

Results to date have again shown the need to minimise carbon dioxide levels in CA storage to as low as 0.5% and to maintain oxygen at above 1.5%.

The use of step-wise cooling or slow initial cooling during the first 3 weeks of CA storage has again been shown to reduce FB. In addition, the post-harvest application of MCP-1 has allowed the use of higher storage temperatures (to 2.0°C) without loss of firmness or increase in yellowing during extended CA storage.

The use of double waxing applied as a method of gaining early warning of the likelihood of a season of high FB potential has given reasonable results but may not be accurate enough in locations where susceptibility to FB is high.

The notion that high fruit density relates to high propensity to FB does not correlate with an acceptable degree of consistency as is the case with Braeburn in New Zealand. This is unfortunate as it complicates the means by which we can more fully understanding what causes FB in Pink Lady.

The results showing that 'greenlife' assessment did not always give a clear linkage to maturity assessment based on starch plates is of some concern. As 'greenlife' assessment in all districts in all seasons is not possible logistically and it is therefore urgent that work is done to fine tune starch procedures for those districts where there is no tight linkage between 'greenlife' and starch assessments.

There has been little further evidence showing a relationship between mineral levels below or above benchmark values or the effect of low crop on the susceptibility of Pink Lady to FB.

Detailed work to test the notion that the ambient temperature regime during early fruit development, the late maturation and ripening phase, or for all of the fruit growth period has not produced consistent evidence to link temperature profiles with fruitlet or mature fruit density. While there are instances where a lower level of temperature accumulation did give higher fruit density and a greater susceptibility to FB, there were other locations where the reverse applied. Nonetheless, the overall trend was for fruit out of the warmer locations to be less prone to FB than those from cooler locations.

This research, which wants to link seasonal and within district differences to climatic parameters is worthwhile as there is no doubt there is a linkage but the difficulty is in identifying the specific linkage. Such research requires time, possibly 10 to 14 years, and the data collected would require specialised analysis to yield a commercial solution. Identification of three brands of FB seems to have complicated the understanding of what is happening, in a physiological sense to Pink Lady during storage. Supermarket buyers want fruit that are totally free of any impediment.

One criticism I have in relation to the work done is that there has been no mention of red colour expression or intensity in the data presented. However, this omission can be excused, as the prime focus of the programme was to address the FB problem. So far as supermarket buyers are concerned, if there is not a required colour standard the fruit is not acceptable regardless of size, crop load, sugar level, district location, density or any other parameter. While they require colour

at an unattainable level they are also ever ready to reject fruit later in the season if there is any indication of even trace levels of FB.

It is sad to admit that in the future the only Pink Lady produced will be of the selected red strains Rosy Glow, Ruby Pink and other selected colour sports. We have no idea what the susceptibility of these over coloured strains is to FB. We do know that for export to Europe, the bi-coloured standard Pink Lady is preferred.

A vote of thanks.

I have attempted to go through the mass of data presented and to make my comments on what I would like if I were a grower. In doing this I may have been over critical on some of the rationale used in this multi disciplined project. This is not because I entirely disagree with it. I am probably a little disappointed that some of the detailed work did not give 'break through' results. Pink Lady did not respond in line with the way Braeburn did in relation to density. Nonetheless there were some instances where it did. Braeburn requires specific regional conditions for maximum performance. So does Cameo. Maybe we are lucky with Pink Lady in that it is less fastidious in relation to what specific growing conditions it needs. What has marginalised Pink Lady is the unreasonable requirement for more and more colour demanded by buyers that do not understand and have no interest in the physiology of one of the world's best apple cultivar.

Finally, congratulations to a team that was given a 'mission impossible' to solve all problems associated with the growing, storage, and marketing of Pink Lady grown in just about all of the apple growing apple districts in the world. A lot has been accomplished in only two seasons. Reporting has been on time and in detail in spite of the volume of data from around the world that had to be processed.