AP02009 – Understanding the Flesh browning Disorder in Pink Lady™ Apples

J. Jobling, D. Tanner, I. Wilkinson, G. Brown, S. Tustin, B. Mitcham and A. Zanella

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J. Jobling, D. Tanner, I. Wilkinson, G. Brown, S. Tustin, B. Mitcham and A. Zanella

August 2004

This report provides a summary of interim results and project activities that meet the fifth milestone of the HAL project 'AP02009 - Understanding the flesh browning disorder in Pink Lady™ apples.' The report summarises further findings of research carried out by the six collaborating research agencies. The objective of this globally-coordinated project is to understand the causes and to develop strategies for the control of flesh browning disorders during storage of Pink Lady™ apples.

- The southern hemisphere storage trials are presently underway and the northern hemisphere trials that were recently completed, are currently being analysed.
- A project review meeting of all collaborators was held in Verona, Italy. This meeting was held prior to the 5th International Postharvest Symposium and included researchers from Italy and France as nominated by Pink Lady™ Europe (minutes presented in this report).
- Recent results continue to indicate that flesh browning is the result of a combination of factors, but the relative contribution of each factor has not yet been definitively identified. However some important disorder risk management methods have been established.
- Managing storage disorder risk requires commercial attention to the following factors. Pink Lady™ apples are sensitive to high CO_2 levels in storage. Step-wise cooling reduces the incidence of the disorder. Late harvested fruit are more susceptible to flesh browning disorder. Fruit from trees with a low crop load are more susceptible to flesh browning. If growers manage all these risk factors then the incidence of flesh browning will be significantly reduced.
- There is continuing effort on developing a climatic model that will use seasonal weather data to predict higher risk years. Initial data indicates that cool seasons may predispose the fruit to some forms of Pink Lady™ flesh browning disorder.

An important outcome from the project has been that the major Australian retailers have reduced blush colour specifications from 60% to 45 – 50% fruit surface coverage. This reduced blush requirement has been implemented as a result of research that showed that high blush standards caused growers to delay harvest and pick late, therefore increasing the risk of flesh browning occurring in storage. The supermarkets were encouraged to reduce the blush colour specification in an attempt to minimize the risk of flesh browning later in the year.

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PROJECT OVERVIEW

A summary of presentations made to the Verona meeting is presented here. Each group gave a brief overview of their research progress.

Pink Lady™ Europe
Currently Europe produces 80,000 tonnes of Pink Lady™. They are aiming to extend the supply season to April/May, which means extending the storage capability out to seven months. They therefore need to develop storage conditions that are risk free in terms of flesh browning and so Pink Lady™ Europe are enthusiastically supporting this research project.

David Tanner (Food Science Australia)
There are two hypothesise for the interaction between climate, fruit structure and seasonal incidence of flesh browning:

- Cooler seasons tend to produce denser fruit which could be more susceptible to damage and
- In seasons where the diurnal variation in temperature is low, colour development will be delayed and so too will harvest.

The project has shown that late harvested fruit are more susceptible to browning. We have a lot of data for many seasons for some districts and two full sets of data for all the participating regions. The data has highlighted some major seasonal differences between years and between districts but to date none of the environmental factors tested show a clear correlation with incidence of browning. The project has not coincided with seasons with a high incidence of flesh browning and so this makes it difficult to draw conclusions and correlations.

Stuart Tustin (HortResearch NZ)
HortResearch have found a strong relationship between cortical air space and fruit density. The correlation is strongest for cortical tissue sections compared with whole fruit, perhaps because the core volume can cause error in fruit density. So far within the project there has been a wide variation in fruit air content and fruit density among the districts and seasons measured. Italy has the densest fruit despite having one of the warmest seasons in 2003. There appears to be a more complex relationship involving environment, crop load and carbon balance that affects fruit structure or metabolism. Again there has not been a high incidence year during the life of the project and so no correlations between fruit structure and severity of flesh browning can be drawn. This work is continuing.

HortResearch also conducted a 'green life' study to evaluate the maturity characteristics of fruit from Hawkes Bay and Nelson. Interestingly the green life (number of days at 20°C from harvest to onset of the ethylene climacteric) of the fruit from Nelson was longer than from Hawkes Bay. This result is similar to the results from Australia where the coolest district of Tasmania had a longer green life than the warmer districts. This was an unexpected result and further research is planned to understand the implications for starch index and physiological maturity in relation to incidence of flesh browning.

Gordon Brown (Scientific Horticulture)
Gordon summarised the incidence of browning that he has seen in his trials since 1999 in Tasmania. He found that 2000 and 2002 were high incidence years. He found a significant effect of root disease in flesh browning risk. Reflective cloth dramatically increased colour and reduced the incidence of browning, presumably by enabling harvest at earlier maturity.

Beth Mitcham (UC Davis)
There has been a low incidence and low severity of browning (in 2002 incidence was also low for the same atmospheres but severity was high). The highest incidence and severity occurred with the use of 3% CO_2 in controlled atmospheres (CA) in 2003. In 2003 in California, maturity data for starch levels, respiration and ethylene production rates were measured. 1-MCP (Smartfresh^{TM}) had no effect on internal browning, but Diphenl Amine (DPA) prevented browning.

There were also differences in mineral content this season. There were lower NO_3 nitrogen levels, and higher NH_3 nitrogen, P, Ca, Zn, B and Mg.

Ian Wilkinson (Department of Primary Industries and Energy, Victoria)
At the 6 month storage assessment, fruit from Batlow region had a higher incidence of flesh browning than Goulburn Valley, but severity was low. Insufficient browning was observed to draw conclusions from the stepwise cooling experiment for 2003. The experiment will be repeated in 2004. The crop load trials are set up in storage for 2004 harvest season.

Angelo Zanella (Centre for Agriculture and Forestry, Laimburg, Italy)
In 2002 the incidence of flesh browning was high. In 2003 the incidence was very low in commercial stores. It was speculated that there is better nutrition (lower nitrogen levels) and also most CA operators have converted to using stepwise cooling. It is also important to note that the storage temperature used in Sud Tirol has become 2.5^{o}C and not 0.5^{o}C typically used in the southern hemisphere.

The current temperature and CA atmosphere recommendations are for 1.8% O_2 and 1.3% CO_2 at 2.5^{o}C, using stepwise cooling (1 week 5^{o}C while the room is being filled, 1 week 4^{o}C, 1 week 3^{o}C, then 2.5^{o}C and then the CA atmosphere is established).

The diffuse browning symptoms seen in Italy could be induced with high CO_2 but the results were inconsistent so there is more complexity to the disorder. Cold shock (rapid pull down to 0^{o}C) also increased the incidence of browning. Zanella *et al.* have carried out additional research into using Ultra Low Oxygen CA storage, coupled with the Satlantic Fluorescent sensors, but low O_2 compared with CO_2 concentration is a risk especially for late harvested Pink Lady^{TM} fruit.

Green life studies have also been completed on fruit from Sud Tirol, Italy. The results for the districts in California, Italy and Batlow, Australia were similar while Tasmania and Nelson (cooler regions) had longer green life periods relative to starch score.

Jenny Jobling (Sydney Postharvest Laboratory)
The data from the 2003 storage trial has been analysed and was presented. The risk of browning was highest for fruit stored in 2% O_2 and 1% CO_2 after 9 months in storage. The storage trial for 2004 has been set up and a ReTain® treatment has been included in the trial using fruit from Batlow. The fruit were treated 7 days before harvest and were harvest at a Ctifl score of 6. The aim was to determine if treatment with ReTain® could delay maturity and therefore reduce the incidence of browning. This trial using ReTain® was funded by Valent BioSciences as preliminary work for the proposed extension.

The green life study for Batlow and Tasmania has been completed and there are some interesting block effects at Batlow and differences between Tasmania and Batlow. It will be interesting to see if these differences are correlated to the incidence of flesh browning induced by waxing the fruit.

General additional comments from the meeting
Other research groups within Europe found that late harvested fruit, fruit from trees with a low crop load and fruit from young trees were most susceptible to the disorder. They also found that treatment with Ethephon increased the incidence of flesh browning.

There is a dramatic increase in the incidence and severity of browning after 7 days at 20^{o}C. Perhaps there is a stress on the fruit coming out of CA as well as going into CA conditions. There is a recommendation to increase the CA storage temperature to 2^{o}C to prevent the diffuse browning disorder.

Comments arising from discussions at the Postharvest Symposium, Verona, Italy
There were discussions with other postharvest physiologists at the conference following the presentation of our paper that summarised results from the project. There is an hypothesis that fruit may acclimatise to certain temperature ranges during seasonal growth and development. This seasonal preconditioning may alter sensitivity to particular storage regimes. These physiological responses could be considered as generic stress-related responses. Some experience in eastern USA has suggested that seasons which are colder than the respective 30-year district average have historically increased the incidence of internal breakdown during storage. This hypothesis will be incorporated into the climatic modelling component of the project.

New Initiatives
A research proposal to extend the project using money from voluntary contributions until October 2007 has been submitted. The outcome of this proposal will determine how we proceed in terms of writing up our research results and final report.

PROJECT COMMUNICATION ACTIVTIES

- Regular conference calls have been carried out between the Pink Lady^{TM} collaborators and the Reference Group organised by Affco.
- Summary of results for the project presented at grower meetings in the Perth Hills, Manjimup and Donnybrook, March 2004.
- Articles were written summarising the results of the project for Tree Fruits, February Edition, Good Fruit and Vegetable March Edition and Southern Farmer May Edition.
- A paper was presented at the 5th International Postharvest Conference in Verona, Italy, June 2004. The conference paper will be published in Acta Horticulturae.

ACKNOWLEDGEMENTS

The research conducted in NZ for this was made possible by the efforts and collaboration by many HortResearch staff. The significant contributions of Shayna Ward, Paul Brookfield, Sarah Legg of Hawke's Bay Research Centre, Ian McIvor of Palmerston North Research Centre and Shona Seymour of Nelson Research Centre is acknowledged with thanks.

APPENDIX I

Pink Lady™ Flesh Browning Meeting Agenda

Date: Sunday 6th June 2004

Venue: Hotel Acacademia
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Time:
1.00pm Horticulture Australia Team meeting
1.30pm Work Meeting with Pink Lady™ Europe researchers

Agenda:

1.00 – 1.30pm	Horticulture Australia Team - Discuss research progress and discuss future plans and presentation of date/publications.
1.30 –3.00pm	Presentations from Australian and European researchers. and discussions - 7 people Jenny Jobling (SPL), Stuart Tustin (HortResearch), David Tanner (Food Science Australia), Beth Mitcham (UC Davis), Angelo Zanella (Laimburg), Gordon Brown (Scientific Hort), Ian Wilkinson (NRE Victoria)
3.00 – 3.30pm	Brief presentations from researches coming with APLE Please prepare a 5 minute summary of your main research results Vincent Mathieu Hurtiger (CTIFL), Marie-Eve Biargues (CEFEL), Lisa Cavicchi (Cisa Mario Neri), Dr Testoni (IVTPA, Milano)
3:30- 4:30pm	Discussion on Pink Lady™ Internal Flesh Browning. 1. Compare the different symptoms from around the world 2. Are there patterns of climate/maturity/crop management for each symptom type? 3. Future research avenues
Facilities:	A laptop and data projector will be available courtesy of Pink Lady™ Europe. Please bring presentations as powerpoint on a CD.

Meeting Organisers:

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APPENDIX II

Copy of the draft manuscript of the paper presented to the 5th International Postharvest Symposium,Verona, Italy, June 2004.

FLESH BROWNING OF PINK LADY™ APPLES: WHY DO SYMPTOMS OCCUR? RESULTS FROM AN INTERNATIONAL COLLABORATIVE STUDY.

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KEYWORDS: Pink Lady™ apple, flesh browning, maturity, density, crop load, CO_2

ABSTRACT

Pink Lady™ apples are an important new variety with unique market advantages as a result of its quality and market identity. This image is at risk of being damaged as a result of the flesh browning disorder that has been a problem for both domestic and exported fruit over recent years. The disorder is sporadic in nature and occurs in both air and CA storage. This disorder seems to be the result of a combination of factors that have been implicated in other storage disorders of apples. An international team of researchers from 4 countries from both the Northern and Southern hemispheres contribute to this project. Our preliminary results show that Pink Lady™ apples are sensitive to high CO_2 levels in storage and that late harvested fruit are more susceptible to the disorder. We also have data showing that differences in temperatures during early fruit development among regions are correlated with differences in fruit density and air content of the fruit cortex measured at maturity. We therefore hypothesize that climatic and production practices, such as crop load can influence either the structure of fruit and/or its ripening metabolism and in turn predispose fruit to the disorder. We propose that the structure of the fruit influences fruit sensitivity to CO_2. It is also likely that seasonal conditions influence maturity.

INTRODUCTION

The internal browning disorder of Pink Lady™ apples is the manifestation of some undefined physiological condition of fruit at harvest, which predispose them to damage during storage under certain conditions. Two types of browning disorder are currently known. One type of internal browning disorder can be correlated with certain seasonal conditions and reduced fruit O_2 and CO_2 gas permeability and is expressed in both air and CA storage (example: Braeburn Browning Disorder). A second form of internal browning occurs only in CA storage and is not correlated with fruit gas permeance, but is a CO_2 related injury (example: Fuji browning disorder (Volz et al. 1998). The cause of flesh browning in Pink Lady™ apples is still not known but research has begun investigating factors that have been a problem for other varieties.

The physiological condition of apple fruit at harvest is the integration of seasonal effects on fruit growth, crop load and tree effects on fruit development and the stage of fruit ripening. The most obvious example of this is that fruit growth is more "typey" in cooler growing districts (e.g. Pink Lady™ in Tasmania, Braeburn in Otago, NZ). Seasonal 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). Therefore it is likely that seasonal conditions predispose Pink Lady™ apples to develop flesh browning during storage.

Flesh browning of Pink Lady™ apples may also result from some of the same factors as the Braeburn browning disorder (BBD). The BBD injury is the result of high CO_2 levels in the flesh of the fruit causing damage. Braeburn is a very dense fruit. Rajapakse (1990) reported that the skin resistance of Braebum to O_2 diffusion is two and four times higher than that of Granny Smith and Cox's Orange Pippin respectively. Pink Lady™ is also a dense apple and may have relatively high internal CO_2 levels and this could be a factor in the development of flesh browning symptoms. Johnson et al. (1998) also found that there was a correlation between increased internal CO_2 injury with high resistance to gas diffusion in some varieites. When high resistance apple cultivars are placed in low O_2 or elevated CO_2 regimes, the internal atmospheres may be modified to a point at which BBD is readily induced. Yearsley et al (1997) also reported that high internal CO_2 contributes to the development of CO_2 related physiological disorders. It is likely that CO_2 also plays a role in flesh browning in Pink Lady™ apples and this will be investigated by the research team.

Lau (1998) found that the incidence of BBD markedly increased after cool growing seasons (<1300 Degree Days >10^{o}C) especially in fruit picked at a more advanced maturity. They also found that shellac waxing increased occurrence of BBD as it increased the skin's resistance to gas diffusion, and that storage in elevated CO_2 and or low O_2 atmospheres also increased the incidence of the disorder (Lau, 1998). The permeability of apples was found to be lower in fruit from cooler regions of New Zealand (Elgar et al., 1999). It was reported that the skin permeance was lower in fruit from Central Otago than from Hawke's Bay. These results correlate to the anecdotal reports of the incidence of flesh browning in Pink Lady™ apples. It seems that more advanced fruit from cooler districts such as, Tasmania and Central Otago, are most susceptible to this disorder. Lau (1998) proposed that cool growing conditions may alter cellular metabolism, reduce skin and tissue diffusivity and/or increase fruit susceptibility to elevated CO_2 and low O_2. A similar hypothesis is proposed for flesh browning of Pink Lady™ apples.

It is important to note that another part of this project is to develop storage and marketing strategies to minimise the incidence of this disorder in high risk seasons. Some management factors to consider are outlined by Little and Peggie (1987) who reported results from a comprehensive 8 year storage trial. They found that ULO storage was best for most varieties in terms of maintaining firmness and reducing the incidence of scald. They also reported that stepwise cooling reduced the incidence of low temperature breakdown in Jonathan apples and that it was important to establish the CA atmosphere quickly. Removal of ethylene also increased the storability of the fruit. These management strategies will be investigated in an effort to prevent susceptible Pink Lady™ apples developing flesh browning in storage.

The complex of seasonal and orchard factors affects the biophysical properties of fruit such as cellular structure, nutrition, tissue density, gas permeability and wax cuticle properties of the fruit. It is therefore likely that the flesh browning disorder of Pink Lady™ apples is the result of a combination of these factors. This research project draws together an experienced international team to address these factors over several districts and seasons in order to better understand the flesh browning disorder of Pink Lady™ apples.

MATERIALS AND METHODS

Crop Load Experiment

This trial was carried out in New Zealand over two six-year old orchard blocks of Pink Lady™ apple grown on M.26 rootstock, one in the Hawke's Bay and one in the Nelson region of New Zealand in the 2002-03 season. On each site, a high crop and a low crop treatment were set using hand thinning in early December. Growers thinned trees to a commercial crop load, which was used as the high crop treatment. Trees for the low crop treatment were further thinned to half the commercial crop load by removing every second fruit over the whole tree.

Fruit were harvested at an average starch score of 3.5 and at 8.5 (CTIFL 10 point starch pattern index scale). From both crop load treatments, 250 fruit per replicate were picked, selecting the most highly coloured fruit at each maturity. Five, 100-fruit replicates for both Air and CA storage treatments were assembled using 20 fruit chosen at random from every field replicate. Fruit were immediately static cooled to 1°C and within 7 days placed into CA atmosphere (2% O_2: 2% CO_2) or air stored conditions at 0.5-1.0°C. The late harvest maturity treatment was set up in the same way. In the late harvested treatment, fruit was stored in air but not under a CA atmosphere.

After 24 weeks, treatments were removed from storage and held at 20°C for 7 days. Fruit were then cut and scored for internal browning disorders. Disorders were rated according to incidence, type of browning and severity of browning. All data were analysed using general linear models with means compared by paired T-tests of least squares means using SAS statistical procedures (SAS Corporation).

CA Storage Matrix

Fruit was sourced from five apple growing regions of Australia, Batlow (New South Wales), Goulburn Valley (Victoria), Yarra Valley (Victoria), Houn Valley (Tasmania), Manjimup (Western Australia). The fruit was harvested at two maturities, based on 3.5 and 8.5 CTIFL average starch scores. Fruit were stored in CA tents at 1°C. The atmosphere was controlled using a Bishop Oxystat analyser. Atmospheres were within 0.5% of the set points. Seven storage atmospheres were used in the matrix; Air, 3% O_2: 2% CO_2, 3% O_2: 1% CO_2, 3% O_2: 0.5% CO_2, 1.5% O_2: 2% CO_2, 1.5% O_2: 1% CO_2 and 1.5% O_2: 0.5% CO_2. There were 3 storage periods 2, 4 and 6 months after harvest.

There were 5 replicates per atmosphere. Assessments were for incidence (percentage of fruit with any flesh browning, irrespective of the severity) and severity based on 1 to 5 scale ratings where 1 = none, 2 = trace, 3 = slight, 4 = moderate and 5 = severe. Seven hundred fruit were assessed for each maturity at the end of each storage period per region. The fruit was held at 20°C for 6 days before assessing the fruit for internal flesh browning.

Seasonal Temperature Analysis and Fruit Growth and Structural Analysis

Hourly temperature data was collected from weather stations on or near the properties included in the storage trials. At 50 days after full bloom (DAFB) 100 fruit per site were harvested and the fruit diameter, fresh weight and dry weight were measured. At harvest samples of fruit from each district around the world were sent to New Zealand for analysis of cell size, air space and density. This information from 50DAFB and at harvest have been correlated with seasonal temperature accumulation models.

Induction of Flesh Browning

Two harvests of fruit from Batlow NSW, Australia were used in this experiment; one had a starch score of 3.5 and the other 8.5 using the CTIFL starch chart. Fruit were either double

waxed with a commercial apple wax using a commercial packing line or were left un-waxed before being placed in storage. Two replicates of 100 fruit per maturity were stored in the following atmospheres at 0°C; 21% O_2: 0.03% CO_2; 21% O_2: 1% CO_2, 2% O_2: 0.03% CO_2 and 2% O_2: 1% CO_2. After 4 and 9 months the fruit were removed from storage and held at 20°C for 7 days. Fruit were then cut and scored for internal browning. All data were analysed using general linear models with means comparisons by KLsd (5% level of significance) using SimStat (Provalis ver, 2.5).

Green Life and maturity Study

Ethylene production of apples picked at different maturities was analysed at constant 20°C in order to record the time-course of climacteric rise in ethylene production. This assessment was done with fruit from California, USA; South Tyrol, Italy; Houn Valley, Tasmania and Batlow, New South Wales Australia during the 2003 and 2004 harvest seasons.

Apples were harvested up to 4 times during the harvest season (8 to 10 fruit for each level). The starch levels were scored using the CTIFL 10 point scale and ranged from a score of 3 to 8 in the three countries. Analyses were carried out at regular intervals at 20°C. Measurements were done on individual fruit sealed for a known time in individual glass jars with a septum fitted lids. After a known period of time a 1mL headspace sample was taken and analysed by gas chromatograph.

RESULTS AND DISCUSSION

Our initial results have shown that Pink Lady™ apples are sensitive to CO_2 and that late harvested fruit are more susceptible to browning than earlier harvested fruit. Fruit from trees with a low crop load are also more susceptible to the disorder. Our results also illustrate how seasonal conditions influence fruit structure. There are three different types of symptoms of flesh browning in Pink Lady™ apples. We have named them radial (browning radiates out from the core), diffuse browning (has a scalloped pattern under the skin) and pits and cavities (typical CO_2 injury symptom). Generally Europe, New Zealand and Tasmania in Australia see the diffuse browning and the other districts see the radial browning symptoms. To date we cannot say whether the symptoms are the result of the same or different sets of factors.

Each collaborator has contributed to specific aspects of the research and the counter season of the USA and Italy has meant that we have collected a lot of important information in a relatively short time. Table 1 summarises some of the main effects from the New Zealand trial. The effect of maturity has also been reported in the USA and Australian storage trials (data not shown).

There were significant effects of site and crop load on incidence of flesh browning in fruit harvested early at SPI 3-3.5, but overall early harvested fruit had the lowest incidence of flesh browning. A higher incidence of flesh browning occurred in fruit from the second harvest, picked at SPI 6.6-7.1, increasing from 3 to 10% from the Hawke's Bay site and from 6 to 13% from the Nelson site. The differences in incidence of flesh browning between sites or crop loads were not evident at these higher expression levels from the later harvest. There was no difference in incidence of flesh browning with fruit stored in air or CA conditions, suggesting that storage atmosphere may not be a primary cause of flesh browning disorder. There were no significant interactions between sites, crop load or storage regime for either harvest date.

Despite an increase in the proportion of fruit with internal browning from the later harvest date, the relative proportion of fruits in severity grades was not greatly different between the two harvests.

Table 1. Summary of main effects of factors evaluated for their association with development of flesh browning disorder of Pink Lady™ apple during storage.

Main Effect	Treatment	Maturity	% Incidence Flesh Browning	Probability
Site	Hawke's Bay	Harv. 1	3.0	<0.0001
	Nelson	Harv. 1	6.0	
Site	Hawke's Bay	Harv. 2	10.6	ns
	Nelson	Harv. 2	13.6	
Crop Load	High	Harv. 1	3.7	0.014
	Low	Harv. 1	5.3	
Crop Load	High	Harv. 2	14.3	ns
	Low	Harv. 2	9.9	
Storage method	Air	Harv. 1	4.9	ns
	CA	Harv. 1	4.1	

Overall the main effect from the later harvest date (higher maturity) was for an increase in the number of fruits expressing flesh browning but not an increase in severity of symptoms being expressed by individual fruits. This data highlights the fact that district, crop load and maturity all have a role to play in the induction of flesh browning symptoms. The results from the Australian storage trial also indicate a district effect (Figure 1).

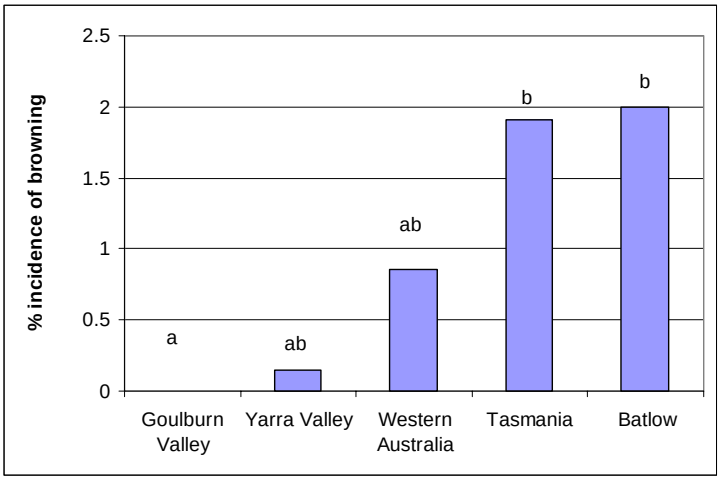

Figure 1. Incidence of flesh browning in different districts in the Australian storage matrix trial in 2003 after 4 months in storage. Regions with a different letter are significantly different at the 5% level of significance.

The district effect may be the result of significant differences in fruit density and percent airspace which were evident among the sites. These regional data were used to plot the change in fruit cortical air space in response to fruit or cortical tissue density and show close relationships between density and air content of the tissue (Figure 2).

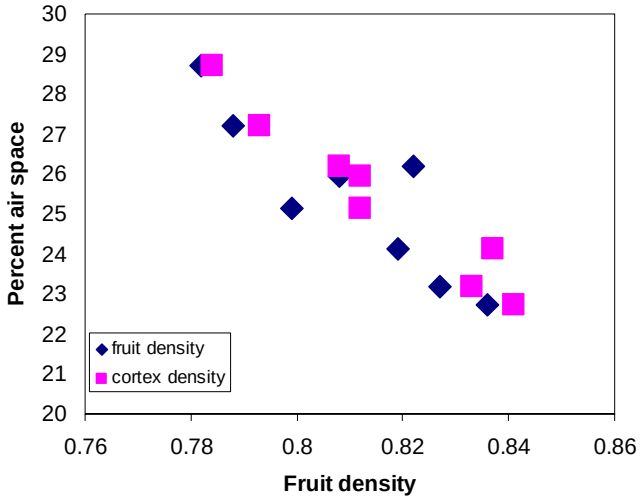

Figure 2. The relationship between the change in fruit or cortical tissue density and the air content of Pink Lady™ apples derived using fruit from eight distinct geographical sources (R^2 = 0.73 for fruit density and R^2=0.93 for cortical tissue density).

As well as a district effect on fruit density there was also a seasonal effect on fruit density. However the effect of climate on fruit structure is not always this simple across all districts and all seasons. The development of an accurate predictive model may require more complex analysis over several seasons.

The storage trials in Sydney, Australia and California, USA indicate that CO_2 is also a factor in the development of symptoms (Figure 3). Flesh browning could be induced by adding CO_2 to the storage atmosphere as well as by double waxing which significantly increased the internal CO_2 level in the fruit. There was an interaction between CO_2 level and O_2 level in the atmosphere with low O_2 and high CO_2 inducing the highest incidence of symptoms.

Double waxing fruit significantly increased the incidence of flesh browning symptoms. The average internal CO_2 level for waxed fruit was 3.1% compared to 1.9% for un-waxed fruit and waxed fruit stored in atmospheres with added CO_2 showed the highest incidence of flesh browning symptoms (data not shown). The induction trials also showed that after 4 months in storage the most significant factor was maturity at harvest with late harvested fruit having a higher incidence of browning than earlier harvested fruit (data not shown). After 9 months storage maturity was still the main factor but the incidence of flesh browning increased for later harvested fruit stored in an atmosphere of 2% O_2 and 1% CO_2 (Figure 3). Growers may be able to reduce the risk of radial flesh browning symptoms by ensuring the fruit are put into storage in a preclimacteric state and that the level of CO_2 in the atmosphere is kept to a minimum.

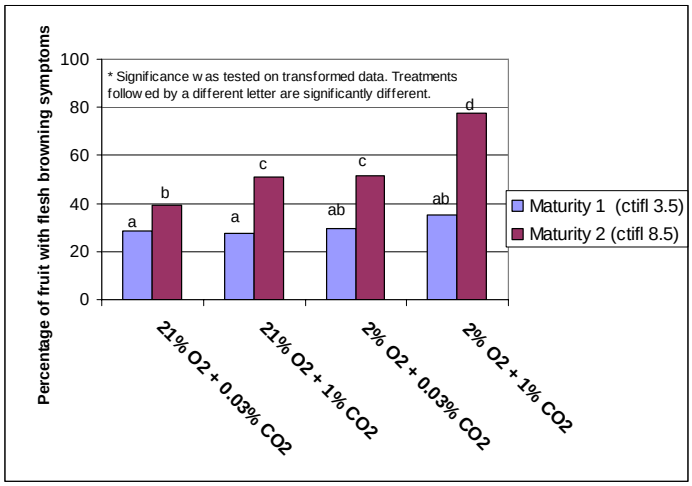

Figure 3. The effect of high CO_2 on the incidence of flesh browning in Pink Lady™ apples from Batlow, Australia after 9 months in storage in the 2003 season.

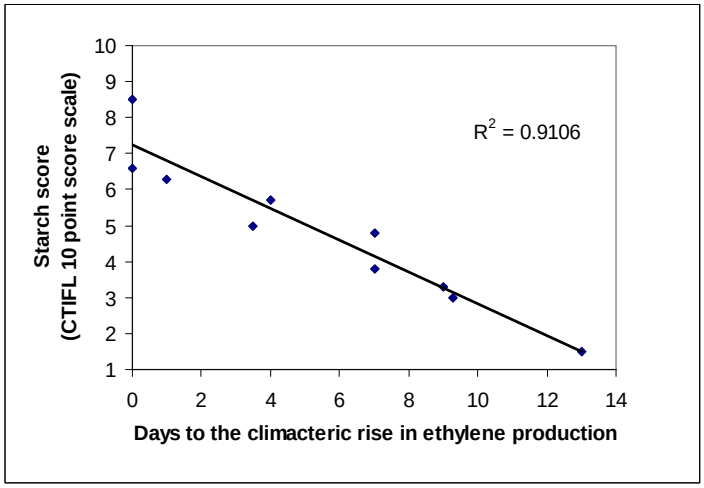

Figure 4. The relationship between starch score at harvest (CTIFL 10 point scale) and the number of days after harvest when the climacteric rise in ethylene production begins (fruit from USA, Italian and Australian experiments combined).

One of the difficulties for growers is the prediction of harvest maturity. The starch score is the simplest method of harvest prediction but its accuracy from season to season and between districts is limited. A detailed maturity study has been carried out for Pink Lady™ apples particularly now that we know that harvest maturity is a critical factor in predisposing the fruit to the flesh browning disorder. The project aims to correlate starch score to the days to the climacteric rise in ethylene production in an effort to determine how this changes between season and between districts (Figure 4). It may be possible to modify the interpretation of the starch charts to make them a more accurate tool that takes into account the seasonal and district climatic variations.

Our preliminary results show that Pink Lady™ apples are more likely to develop flesh browning symptoms if they are harvested late and are exposed to atmospheres containing high CO_2. There is also a temperature response with step wise cooling significantly reducing the incidence of browning. Fruit from trees with a low crop load are also at risk of developing flesh browning and it is likely that seasonal weather conditions and their effect on fruit structure and physiology also play a role in the disorder. However more seasonal weather data is required before a robust predictive climatic model can be established.

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