

# AP02009 – Understanding the Flesh Browning Disorder in Pink Lady apples

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# EXECUTIVE SUMMARY

## AP02009 - Understanding the Flesh Browning Disorder in Pink Lady apples

Milestone Report 3 to Pipfruit New Zealand

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

August 2003

During this milestone fruit were harvested in Australia and New Zealand and placed into several storage experiments. Work has also begun on the analysis of fruit nutrition, fruit cell structure and the climatic temperature models for the Southern Hemisphere regions. In California the final removal of fruit from CA storage has been completed. The experiments and results from each laboratory are outlined below.

### Summary of Project Results

- Type 3 symptoms of internal browning can be induced in CA atmospheres with high carbon dioxide and low oxygen. The incidence is higher in later harvested fruit but does not change with time in storage.
- The climatic models show that there are some interesting differences between districts for the periods 50 days after full bloom and the period around harvest. This will be investigated further and correlated with the incidence of flesh browning from the different districts in the trial.
- The modelled temperature data for the 2002/2003 season shows that the fruit with a higher accumulation of heat units 50 days after full bloom correlates with less dense fruit. It will be interesting to see if this pattern also matches the incidence of flesh browning with our hypothesis being that fruit from cooler districts/seasons are denser and therefore more susceptible to the flesh browning disorder (Type 2 symptoms).

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## SYDNEY POSTHARVEST LABORATORY/ FOOD SCIENCE AUSTRALIA

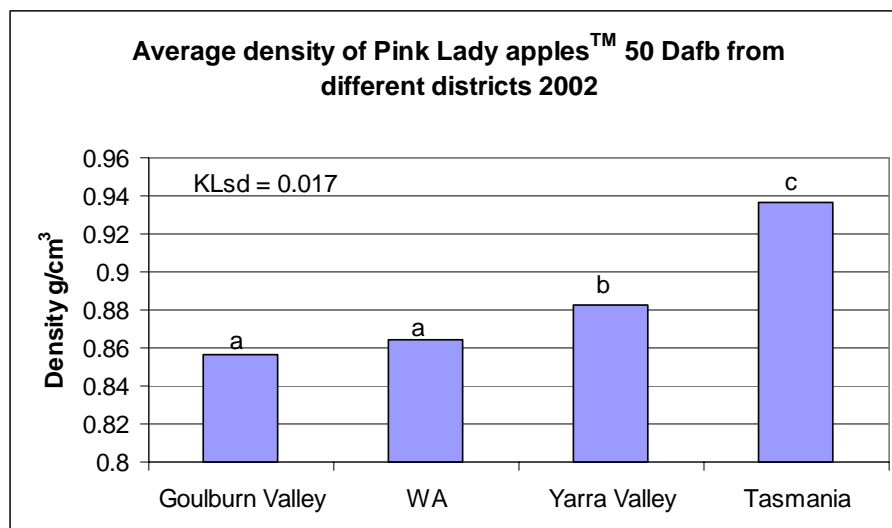
**Jenny Jobling and David Tanner**

The fruit used were from Batlow and the harvest dates were 23/5/03 (ctifl 3.5) and 1/5/03 (ctifl 8.5) and the CA matrix was air, 2% O<sub>2</sub> + 1% CO<sub>2</sub> and 21% O<sub>2</sub> and 1% CO<sub>2</sub> and 2% O<sub>2</sub> and <0.03% CO<sub>2</sub>. The Batlow fruit are grown on M9 rootstock in the European weeping habit. There were 2 treatments unwaxed and a double waxed treatment. The fruit were waxed on a commercial packing line. The aim is to reduce fruit permeability to induce flesh browning.

Samples have been freeze dried and extraction has been started for the nutrient analysis of samples from each district. Other fruit is at 0.5°C for permeability analysis and samples were sent to NZ to be used for the cell structure analysis.

The fruit density data for 50 days after full bloom has been analysed and shows an interesting result with fruit from Tasmania and the Yarra Valley being significantly denser than fruit from WA and the Goulburn Valley (Figure 1).

**Figure 1.** The average density of Pink Lady™ apples 50 Days after full bloom from different districts in Australia, samples taken October 2002.



It will be interesting to see if this difference is still apparent at harvest and if it correlates with the incidence of flesh browning during storage.

*Historic analysis of temperature data.*

David Tanner has written a summary paper on the modelling of the historical temperature data. He had difficulty getting hourly temperature data from weather stations near the apple growing districts.

Graphical presentations of monthly cumulative day-degrees below 4, 7, 10 and 17°C, and monthly diurnal temperature fluctuation were used to evaluate years and regions with low September/October temperatures and those districts that were likely to have problems with colour development in April. It appears that there are likely risk regions each year, but without strong data on internal browning from a range of growers within a region it is difficult to make clear conclusions.

There was some interesting data that illustrated there may be a link between cool temperatures during the 50 dafb and Radial (Type 2) browning and the low diurnal variation at harvest being related to Diffuse (Type 1) browning. However this needs to be explored in more detail with the current season's data. David Tanner and Stuart Tustin will co-ordinate the collection of data and the models to use for this seasons data.

# INSTITUTE OF HORTICULTURAL DEVELOPMENT, AUSTRALIA

## **Ian Wilkinson**

Pink Lady apple samples from Tatura, Yarra Valley, Batlow, Tasmania and WA went into CA storage over the period 8/4/2003 to the 30/4/2003. All districts other than WA had 2 maturities (Ctfl starch plate targets were 3.5 and 8.5). The maturity varied slightly from the targets but early and late maturity fruit was harvested to investigate the effect of maturity on flesh browning (FB). The CA combination matrix included 2%, 1% and 0.5% CO<sub>2</sub> combined with 3% or 1.5% O<sub>2</sub> plus there is an air control. There has been some difficulty at achieving the target CO<sub>2</sub> atmospheres and some overlap in the CO<sub>2</sub> concentrations is probable.

The CA storage facilities were set-up in April 2003. Due to the large quantity of fruit (volume and weight) used for the CA storage experiment specially built shelving and CA tents were purchased (see attached photograph).

The assessment of the samples after 2 months storage has just commenced. To date only the fruit from Tatura, Yarra Valley and Batlow first pick has been assessed and no FB has been observed. The Pink Lady apples will be assessed for flesh browning up to the end of October.

The industry will be kept informed as to the incidence and severity of FB.

At the AFFCO / APAL FB workshop December 2002 it was suggested that step wise cooling may assist in preventing FB. Therefore, in 2003 an extra step-wise cooling experiment was set-up. Fruit from the Tatura second harvest were used for the experiment. Fruit was either held at 1°C or 2.5°C continuously or step-wise cooled for 2 weeks at 4°C followed by 2 weeks at 2.5°C and thereafter 1°C. The CA storage atmosphere is 2.5% O<sub>2</sub> plus 2% CO<sub>2</sub>. The fruit will be assessed in September.

Seasonal conditions probably have a major impact on FB. Therefore, temperature data is being monitored at each trial site. The Tatura and Yarra Valley loggers are downloaded every three months and the data is forwarded to Stuart Tustin (Hort Research, NZ). In addition, the temperature data for 1996 - 2002 was obtained from the Tatura weather station and forwarded to David Tanner (Food Science Australia, North Ryde). Stuart and David will co-ordinate the collection of data and modelling. They will model the temperature data during the 50 days after full bloom (dafb) and at harvest to establish if there are links with the incidence of FB.

### *Next Steps:*

The data from the CA storage and the step-wise cooling experiments will be analysed and the effects of storage atmosphere and cooling on FB reported.

In the June telephone hook-up of the Pink Lady collaborators meeting, it was suggested that fruit with low seed counts have been shown to have lower calcium levels and are more prone to storage disorders. Therefore, the number of seeds in the fruit will be counted on at least one removal per region and this will be correlated with FB.

In the project contract the proposed CA matrix combinations were to be 1, 3, 5% CO<sub>2</sub> and 1.5%, 3% O<sub>2</sub> for the USA and Australian trials. The CO<sub>2</sub> levels were changed for the Australian work because it was felt by the Pink Lady collaborators that USA experiment would establish high CO<sub>2</sub> FB disorders and that additional information could be gained from the Australian trial by using atmospheres closer to commercial storage conditions.

# HORTRESEARCH, NEW ZEALAND

## Stuart Tustin

Two districts Nelson and Hawkes Bay with a light crop load and a normal commercial crop load. The difference in crop load was 50%. There were 2 maturities 3.5 and 8.5 (ctifl chart). The later maturity is just in air rather than CA. The CA storage is 2% O<sub>2</sub> and 2% CO<sub>2</sub>. They will remove fruit every 2 weeks to determine when symptoms begin to occur.

The method for the cell structure analysis is being developed for Pink Lady using the NZ fruit. The original method was for Braeburn.

### *1. Effects of crop load on development of flesh browning*

At one orchard in each of the Nelson and Hawke's Bay regions, export quality fruit were harvested from high crop (commercial crop load) and low crop (50% of commercial crop load) treatments, during April 2003. Harvest date was determined when fruit samples reached an average starch pattern index of 3.5 units. Storage treatments were set up where fruit from high and low crop treatments were stored at 0.5°C, in a 2% O<sub>2</sub>/2%CO<sub>2</sub> controlled atmosphere or in air. Additional fruit samples from the two crop load treatments were stored in air, for regular sampling to monitor for the development of flesh browning symptoms, as a guide to the timing for fruit evaluations after storage.

A second harvest of fruit from high and low crop load treatments from both Nelson and Hawke's Bay orchards was taken when the average starch pattern index reached 7-8 units. These fruit were stored in air only. Samples for monitoring the development of flesh browning symptoms were also placed into storage.

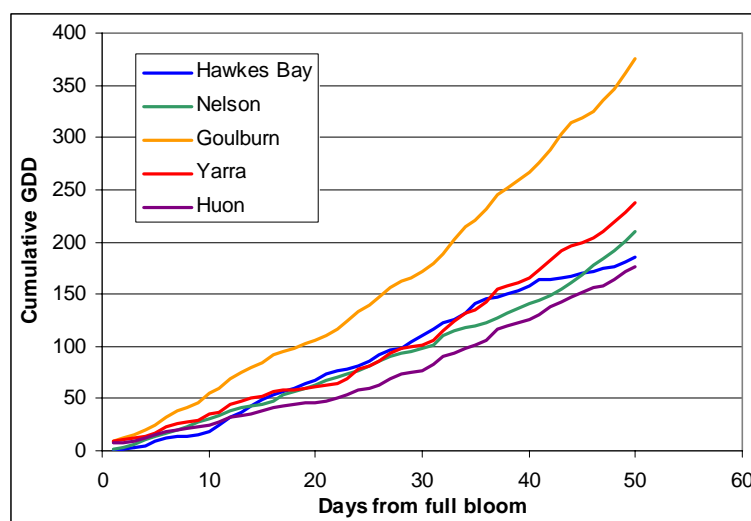
As of 25 June, 2003, monitored samples after 8 and 10 weeks storage from the first harvest date and 8 weeks storage from the second harvest date have been assessed. Incidence of flesh browning in stored fruit has been minimal thus far.

### *2. Fruit development phenology*

Mean daily temperatures and temperature accumulation for the total seasonal interval from bud break until mid May have been calculated for those sites for which we have complete records.

Preliminary comparisons of temperature accumulation (GDD, base 10°C) from full bloom until 50 days after full bloom (dafb), among regions within Australia and New Zealand have been conducted (Figure 2).

Differences in the seasonal trend of growing degree-day accumulation were evident among sites. The most marked difference was in the higher rate of temperature accumulation of the Goulburn Valley site compared with all other locations.



**Figure 2.** Patterns of temperature accumulation (degree-days, base10°C) in the spring of 2002 from three fruit growing regions in Southeast Australia and two regions of New Zealand.

The properties of young fruits measured at 50 dafb from the same five sites and Batlow are presented in the following table (Table 1).

Region	Fruit diameter (mm)	Fresh weight (g)	DM percentage ( $\pm 2x$ stdev.)
Goulburn, Vic.	29.8	12.83	15.3 $\pm$ 1.80
Yarra, Vic.	28.1	10.82	12.8 $\pm$ 1.20
Huon, Tas.	25.4	8.46	11.6 $\pm$ 1.08
Nelson, NZ.	30.3	13.78	12.6 $\pm$ 1.02
Batlow, NSW	31.1	15.63	10.8 $\pm$ 2.30
Hawke's Bay, NZ.	28.2	10.46	11.5 $\pm$ 0.72

**Table 1.** Properties of Pink Lady fruit sampled at 50 days after full bloom collected from sites in four fruit growing regions in Southeast Australia and two regions of New Zealand.

The pattern of accumulated degree-days follows a similar trend to the density data in Figure 1, with fruit from cooler districts being denser than those from warmer districts. It will be interesting to see how the cell structure of the fruit from the different districts compares.

## **SCIENTIFIC HORTICULTURE, TASMANIA, AUSTRALIA**

### **Gordon Brown**

Since the previous milestone the fruit have matured, been harvested and sent to various locations for CA storage and laboratory analysis. Unfortunately one of the boxes of fruit from Tasmania and destined for NSW for the nutrition and permeability studies was lost by the courier. This is particularly disappointing in light of the cell density figures for the 50 DAFB data where it was found that Tasmanian fruit had extremely high cell densities. All other samples are believed to have arrived at their destinations.

The last half of the 2002/3 season in Tasmania was extremely mild. The lowest recorded temperature on the data logger located at the experimental site up to harvest was 1.3°C and this occurred on December 26. There were only 5 days below 3°C from December till the completion of harvest and only 2 days above 35°C.

## UNIVERSITY OF CALIFORNIA, DAVIS, USA

### **Beth Mitcham**

We have completed our first year of this study. Pink Lady apples were harvested on September 20 and October 18, 2002. Fruit were stored in air, or 1.5, 3.0 or 21% O<sub>2</sub> in a factorial with 1, 3 and 5% CO<sub>2</sub> at 0.5C for 2 and 6 months. Following storage plus 5 days at 20C, fruit were assessed for internal browning.

The percentage of fruit affected with internal browning increased with greater CO<sub>2</sub> concentration and with lower O<sub>2</sub> concentration. The incidence of injury was very low when the oxygen concentration was near 20%, even with high CO<sub>2</sub>; however, the damage clearly increased with CO<sub>2</sub> concentration at each level of O<sub>2</sub>.

The incidence of injury was greater in fruit from the second harvest, but there was not a great difference in incidence between 2 and 6 months of storage. The severity of internal browning (percentage of the cut apple surface browned) was greater in apples held in low O<sub>2</sub> atmospheres, but there was not as much relationship with changing CO<sub>2</sub> concentration. The intensity of the brown colour did not vary much between the different treatments.

**Table 2.** Percent incidence, severity and intensity of brown discoloration in Pink Lady apples harvested on September 20 or October 18, 2003 and stored at 0.5C for 2 or 6 months.

<b>2 months</b>		Score for Intensity of Brown Color		% Incidence of Internal Browning		Severity (% of Cut Surface Affected)	
% O <sub>2</sub>	% CO <sub>2</sub>	H1	H2	H1	H2	H1	H2
Air	Air	1.0	1.0	0.0	0.0	0.0	0.0
1.5	1	4.2	4.0	3.0	4.0	3.2	1.7
	3	4.0	4.1	4.0	14.0	1.3	1.6
	5	4.5	4.1	13.0	23.0	3.1	1.9
3.0	1	3.7	1.0	3.0	0.0	2.0	0.0
	3	4.1	3.4	5.0	8.0	1.2	2.4
	5	4.2	4.1	9.0	16.0	3.2	1.8
21	1	1.0	1.0	0.0	0.0	0.0	0.0
	3	3.0	1.0	1.0	0.0	1.0	0.0
	5	3.5	4.0	7.0	1.0	1.0	1.0
<b>6 months</b>							
Air	Air	1.0	1.0	0.0	0.0	0.0	0.0
1.5	1	4.0	4.0	1.0	2.0	3.0	3.5
	3	3.9	3.8	9.0	14.0	2.7	2.2
	5	3.6	4.0	11.0	28.0	2.5	2.7
3.0	1	3.5	1.0	2.0	0.0	2.0	0.0
	3	3.4	3.7	10.0	3.0	1.6	2.3
	5	3.3	3.6	17.0	17.0	1.6	2.0
21	1	-	-	-	-	-	-
	3	2.0	4.0	1.0	2.0	1.0	2.0
	5	-	-	-	-	-	-

Intensity Score: 1=none; 5=severe.

Severity Score: 0=none; 1=1-10%; 2=11-20%; 3=21-40%; 4=>40%.

A mineral analysis was conducted on a composite sample of 5 apples per rep. Rep 5 had the lowest CA, highest Mg, and lower levels of Zn and Fe, as well as lower nitrogen, and had the lowest incidence and severity of internal browning. However, for the remaining apple samples, there was not a strong relationship between any of these elements and internal browning.

**Table 3.** Mineral analysis of Pink Lady apples per gram of fresh tissue.

REPS	P µg/g	N(NH4) µg/g	N(NO3) µg/g	K mg/g	Ca µg/g	Mg µg/g	B µg/g	Zn µg/g	Mn µg/g	Fe µg/g
1	104.3	< 0.48	7.90	1.412	57.2	34.3	4.76	0.67	0.29	26.8
2	117.1	“	32.71	1.431	58.5	46.8	4.88	0.58	0.39	22.6
3	112.7	“	2.57	1.216	57.3	34.4	4.77	0.86	0.26	22.4
4	127.0	“	12.85	1.353	58.6	46.9	4.88	0.68	0.68	25.7
5	149.2	“	2.62	1.446	37.8	56.7	4.72	0.47	0.47	19.8

## **PROJECT COMMUNICATION ACTIVITIES**

- Regular telephone hook-ups of the Pink Lady collaborators
- Jenny attended the Pink Lady Europe meeting in Nimes, France in May. She gave a presentation outlining the projects aims and results to date. It was an excellent opportunity to meet Tony Portman from AgWa as well as Angelo Zanella from South Tyrol, Italy. The Italians are keen to join the project and the proposal is being prepared. There was a lot of interest and support for our collaborative research effort.
- A summary of the conference is attached to this document.
- Summary of flesh browning observed in experimental Pink Lady apples (HAL AP 01036) in the UK, September 2002 to the AFFCO AGM in Adelaide, October 2002.
- Summary of IHD, Knoxfield's component of the HAL project AP 02009 and results of a short survey on probable links to flesh browning in Pink Lady apples were presented at the AFFCO / APAL flesh browning workshop December, 2002.

## SUMMARY OF INTERNATIONAL TECHNICAL SYMPOSIUM PINK LADY™

22 May 2003 – ATRIA Nimes, France

Prepared by Jenny Jobling, Sydney Postharvest Laboratory

- *Points from Pink Lady™ Europe's Presidents welcome – D Crabos.*

Production figures for 2002.

Country	Area planted (ha)	Total Volume	Volume Pink Lady
Europe	2600	57000	40000
Australia	2000	65000	35000
USA	1520	42500	20000
South Africa	1500	45000	10000
Chile	550	17500	7500
New Zealand	170	8100	3750
Brazil	240	9100	1000
Argentina & Uruguay	4000	9000	500
World Wide	10380	289700	127750

The world wide volume of Pink Lady™ is still quite small compared to other varieties. Pink Lady™ is difficult to grow in terms of reaching the “Pink Lady™” specifications but the challenge is to increase the percentage of fruit produced to that reach the Pink Lady™ specifications.

- *Understanding the Tree – JM Lespinasse*

Can't prune all varieties the same way. In Europe the trees are trained to have a weeping habit. The terminal flowers prevent apical growth. PL has a strong tendency to grow the bottom of the tree but this must be slowed to develop a balanced tree. Aim to develop the main stem with lots of buds and reinforce top branching rather than low branching. Keep top branches as PL spurs fruit year after year. In this system the central part of the tree is cleared to produce a chimney so let some light into the centre of the tree.

Avoid high nitrogen to reduce vigour and thin the fruit 40 Dafb to achieve the market requirements in terms of colour and size. As the tree ages fruiting wood needs to be replaced and the nutrition of the tree needs to be maintained.

- *Tree management in Australia – Tony Portman and Geoff Godley*

Domestic market wants 65% block colour there is an enormous range from 40 – 80% colour. 2003 season the colour quality was low as there was low diurnal temperature variation at harvest in WA.

Pink Lady™ needs good even light distribution. The central leader system didn't work as PL is basal dominant. Revised system is to develop the central pole in the first 2 – 3 years

to fill 80% of the row space eg. 4m row = tree height 3m. Then develop light fruiting wood. It is important to delay fruiting to get the structure right.

In WA dormancy breakers are used in areas with low chill units. The chemical that is used is called Waiken and is applied 50 days before full bloom. This chemical also compresses the bloom period.

It is important not to over thin. Don't use primary thinners secondary thinners are better and then hand thin.

Summer pruning is best done pre Christmas, same time as hand thinning. At this time you remove unwanted wood, especially basal wood and removal any upright shoots. Summer pruning suppresses tree vigour.

Higher nitrogen fertilization linked to lower colour. It is best to aim for very low vigour trees. Fruit with low nitrogen begin to colour earlier than fruit with high nitrogen.

- *Quality and harvest parameters in Europe – Claude Tronel*

Ctfl starch scale correlates well with the % cortex area clear of starch. For long term storage they recommend a starch score of 4.5. The specifications are Starch 4 to 5, 8Kg firmness, background colour of F5 (ctfl swatches) and 50% blush. They have found that picking directly into the bin rather into picking bags reduces the rate of bruising.

- *Storage of Pink Lady™ apples in Europe – J Mazollier*

Pink Lady™ in Europe are commonly stored 6 – 8 months. French PL have had diffuse browning (Type 1 as reported in HAL project summary). Young orchards have a higher incidence of the disorder. They propose that this type of browning is related to chilling injury and propose to keep CA at warmer temperatures and their results show that the texture and firmness is maintained if the temperature is at 4°C. This is very high and I have data that doesn't support this result. PL apples stored at 3°C in CA were significantly softer than fruit held in CA at 0°C.

Spraying with Ethephon increased browning and this suggests ethylene and maturity are factors in the development of the disorder.

Propose that it would be good to combine the use of 1-MCP and 4°C CA storage to prevent diffuse browning.

High CO<sub>2</sub> is used to maintain the green colour of Golden Delicious apples this is not so critical for Pink Lady™ apples and so the CO<sub>2</sub> level could be less than the level of oxygen.

- *South Tyrol Experience – A Zanella*

Postharvest problems included bruising, greasiness, superficial scald and internal flesh browning. Bruising can be reduced by harvesting at the correct maturity, and not harvesting when the fruit are wet and cold, good fruit nutrition also helps.

Greasiness is related to over maturity at harvest. Harvest at the correct time and this problem will be reduced. PL is susceptible to superficial scald. 800ppm DPA prevents scald.

They observed Type 1 browning – Diffuse symptoms. They noted that the texture of the damaged flesh has a dry texture. They noted that fruit from 2001 developed more symptoms than fruit from 2002. Although there were still symptoms but the severity was less. The time course for the development of the symptoms was similar. The first symptoms developed after 5 months in storage.

Their work has shown that maturity is not as critical as district in terms of severity. Districts seem to have the same sensitivity to the disorder but the intensity of the symptoms varies from season to season. They have done an orchard survey similar to that carried out in Australia and no correlations could be found from survey data.

Fruit nutritional analysis was interesting. They used a binary linear logistic regression and found that the level of nitrogen and the ratio of nitrogen and potassium were correlated to the incidence of flesh browning. Nitrogen has a negative effect on quality and this could be a forecast system. It is proposed that high nitrogen reduces the rate of colour development and so this could be linked to fruit maturity at harvest. So high nitrogen fruit are harvested later and could be more susceptible to senescent breakdown. Although the work suggests that maturity is not as critical as district.

They use NIR to test flesh browning and the correlation is very good. They found that the symptoms intensify after the storage period and they assess fruit after 7 days at 20°C.

Their research looks at step wise cooling seemed to reduce the incidence of browning. The recommended temperature in Italy is 1.3°C and 2.5°C seemed to reduce the incidence of browning. In Australia fruit is stored close to 0°C and we don't see the Type 1 symptoms? Higher temperatures caused more scald but this could be controlled with the use of 1-MCP.

Their work also showed that 1-MCP reduced the incidence of browning and high CO<sub>2</sub> caused higher browning. Delayed CA did not reduce the incidence of browning.

Pink Lady<sup>TM</sup> have a rate of ethylene production that is similar to other varieties but its respiration rate is 30% higher than Golden Delicious. They found that the permeability of the fruit increased as the fruit ripened to compensate for this increased respiration rate. Treatment with 1-MCP could slow respiration and reduce the severity of browning.

The conference proceedings are available on CD Rom.

- The next meeting of Pink Lady<sup>TM</sup> Europe is proposed for 2005 and there is an opportunity for the researchers to meet again at the International Postharvest Conference in Verona, Italy in June 2004.