The Flesh Browning Disorder of 'Pink Lady'™ Apples

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Figure 1. Radial flesh browning disorder (RFB) of 'Pink Lady'™ apples in Australia.

The 'Cripps Pink' apple has become a popular variety both in Australia where it was developed and internationally due to the distinctive flavor characteristics and attractive appearance of the fruit. To establish a strong position among new and popular cultivars, the 'Cripps Pink' apple has been extensively marketed under the trademarked name 'Pink Lady™'. The 'Pink Lady'™ trademark is owned by Apple and Pear Ltd Australia and a royalty of $1 US per carton of apples covers licensing and marketing costs associated with trademark branding and development. The success of this international marketing resulted in the 'Pink Lady'™ apple cultivar becoming popular in international markets, especially throughout Europe where a price premium of up to four times that of other apple cultivars was received (Studdert, 2002).

"Our research in Australia has shown that the flesh browning disorder of 'Pink Lady'™ apples is three distinct disorders (radial flesh browning, diffuse flesh browning and CO_2 injury.) Radial flesh browning was found to occur in warm growing districts and is characterized by browning of the vascular tissue of the fruit, with the cortex tissue remaining clear and relatively unaffected. Diffuse flesh browning was found to occur in cool growing districts and is characterized by browning throughout the cortex tissue of the fruit, with the vascular tissue remaining clear and relatively unaffected. CO_2 injury is characterized by the appearance of lens shaped pits and cavities throughout the cortex of the fruit and occurs when the fruit are stored in controlled atmosphere storage with the level of CO_2 above 1%."

To use the 'Pink Lady'™ trademark, 'Cripps Pink' apples must meet strict quality guidelines including a minimum area of pink blush on the skin, flesh firmness, total soluble solids, titratable acidity and absence of disorders. However, in 2000, the quality of exported 'Pink Lady'™ apples was found to have declined. This was likely due to the fruit being harvested late (Wilkinson, 2000) as growers attempted to meet the strict blush guidelines. Shipments arriving in the United Kingdom from Australia following five weeks of air storage and a six week sea freight voyage were found to be below the acceptable limits for firmness and had also developed an unacceptable level of skin greasiness prompting stricter quality guidelines to be enforced. In addition, a flesh browning disorder was detected; in 2003, 35 containers of 'Pink Lady'™ apples that had been exported from Australia to the United Kingdom were rejected due to the presence of the flesh browning disorder, representing a large economic loss to Australian apple growers and the potential loss of the reputation of the 'Pink Lady'™ brand.

The flesh browning disorder of 'Pink Lady'™ apples was found to be sporadic in nature with symptoms not being present in every season or growing district. This unpredictability rapidly undermined the established trade confidence in the cultivar and began to erode the market advantage that had been developed. Anecdotal reports suggested that a range of factors might be responsible for the development of the disorder. These factors included fruit maturity at harvest, fruit nutrition, storage conditions such as the storage temperature and atmosphere as well as orchard climatic conditions.

In order to determine the pre- and postharvest factors that were responsible for the development of the flesh browning disorder during storage, a research project facilitated by Horticulture Australia Ltd in partnership with Apple and Pear Australia Ltd was established. The project was collaborative between researchers in Australia, New Zealand, Italy and the United States.

Classification of the Flesh Browning Disorder

The flesh browning disorder of 'Pink Lady'™ apples was originally thought of as a single disorder. However, this research project has demonstrated that it can be classified into three distinct disorders (radial flesh browning, diffuse flesh browning and CO_2 injury) based on contrasting physiology as well as the visual appearance of the browning within the fruit.

1. Radial flesh browning disorder: Radial flesh browning (RFB) is a senescent disorder, aggravated by late harvest, low temperature storage and high CO_2 in the storage atmo-

sphere. Radial flesh browning was found to occur in warm growing districts and is characterized by browning of the vascular tissue of the fruit, with the cortex tissue remaining clear and relatively unaffected (Figure 1). It is thought that the small size of the vascular cells restricts the movement of CO_2 throughout the fruit leading to the buildup of toxic quantities of CO_2 in these areas resulting in cell death and the characteristic pattern of browning. Radial flesh browning has a high area of browning at the stem end of the fruit, decreasing in area at the calyx end of the fruit. In fruit with mild RFB, symptoms may sometimes be observed only at the stem end of the fruit.

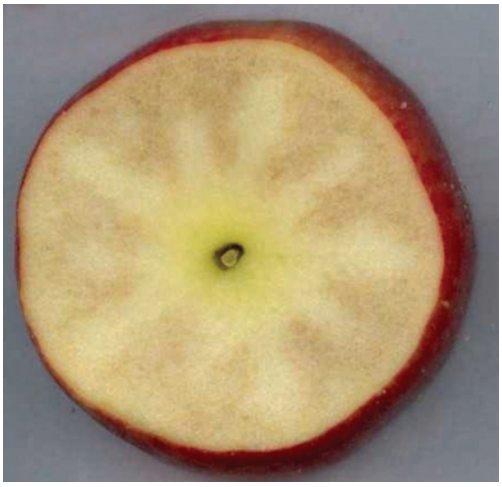

Figure 2. Diffuse flesh browning disorder (DFB) of 'Pink Lady'™ apples in Australia.

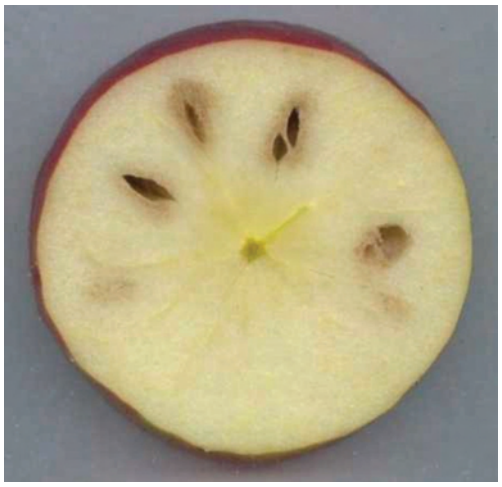

Figure 3. CO_2 injury to the flesh of of 'Pink Lady'™ apples in Australia.

2. Diffuse flesh browning disorder: Diffuse flesh browning (DFB) is a chilling injury, occurring when susceptible 'Pink Lady'™ apples are stored below 38°F. Diffuse flesh browning was found to occur in cool growing districts and is characterized by browning throughout the cortex tissue of the fruit, with the vascular tissue remaining clear and relatively unaffected (Figure 2), thus in direct contrast with RFB. The cortex cells of the fruit are much larger than the vascular cells and also have much thinner cell walls. It is thought that these larger cells are more prone to collapse due to membrane damage resulting from chilling injury than the smaller and thicker walled vascular cells. Diffuse flesh browning has a high area of affected tissue at the stem and calyx ends of the fruit, with a lower area of browning in the middle section of the fruit.

3. CO_2 injury: CO_2 injury of 'Pink Lady'™ apples was expressed in a similar fashion to CO_2 injury that has been observed in other apple cultivars. The disorder is characterized by the appearance of lens shaped pits and cavities throughout the cortex of the fruit. CO_2 injury of 'Pink Lady'™ apples was only found to occur when the fruit were stored in controlled atmosphere storage with the level of CO_2 above 1%.

Factors influencing the development of the flesh browning disorder

The flesh browning disorders of 'Pink Lady'™ apples are the result of a complex combination of factors. In this research, those factors can broadly be categorized as preharvest (orchard) and postharvest (storage) conditions.

1. Postharvest conditions

The susceptibility of 'Pink Lady'™ apples to developing RFB, DFB or CO_2 injury during storage can be influenced by pre-harvest conditions, however it is the postharvest conditions that promote and exacerbate the development of the different disorders. No incidence of RFB, DFB or CO_2 injury has been observed in 'Pink Lady'™ apples at harvest, indicating that these are storage disorders.

Over three consecutive seasons beginning in 2004, a number of storage conditions were examined in order to determine their influence on the development on RFB and DFB of 'Pink Lady'™ apples during storage.

In the 2004 season, optimal (starch index = 3.5) and late (starch index = 8.5, on a scale of 1 to 10) harvested 'Pink Lady'™ apples were stored at 32°F in either air or air + 1% CO_2 for up to seven months. Following four months of storage, the incidence of RFB was higher in fruit stored with 1% CO_2 than in air stored fruit (Figure 4). In this season, the incidence of DFB was extremely high (between 92 and 100% of fruit were affected). However, as shown in Figure 4, the incidence of DFB was lower in fruit that were stored with 1% CO_2 than in fruit that were stored in air. This difference between the response of RFB and DFB to added CO_2 in the storage atmosphere indicates the contrasting physiology between the two disorders. For both RFB and DFB, fruit that were harvested late had a higher incidence of browning than fruit that were harvested at the optimal maturity for long-term storage. A similar pattern of browning was observed following seven months of storage.

In the 2005 season, optimal (starch index = 3.5) and late (starch index = 8.5) harvested 'Pink Lady'™ apples were stored in air at either 32°F or 38°F for seven months. The incidence of RFB was reduced by half by storing the fruit at 38°F (Figure 5). The incidence of DFB however was reduced from as high as 74% down to 4% by storing the fruit at 38°F. This shows that the DFB disorder of 'Pink Lady'™ apples is a chilling injury that can be reduced to within commercial threshold levels by storing the fruit at 38°F. However, the incidence of RFB was not reduced to commercial levels, suggesting that further factors are involved in the development of this disorder. As had been observed in the previous season, fruit that were harvested late had a higher incidence

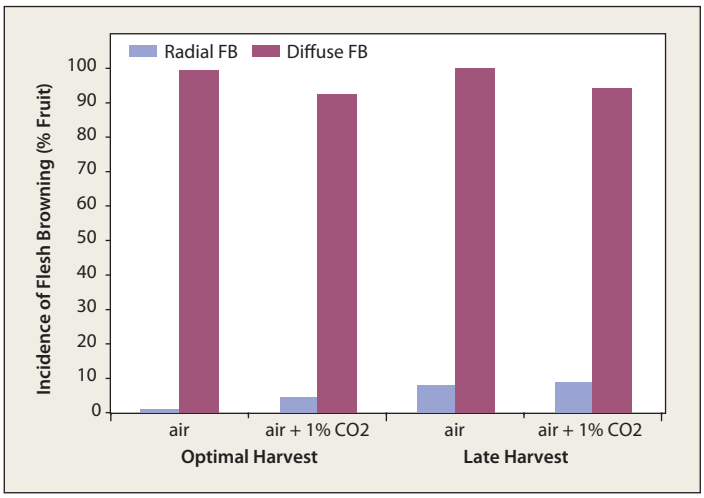

Figure 4. Incidence of flesh browning in 2004 of 'Pink Lady'™ apples in Australia as influenced by harvest date and storage conditions.

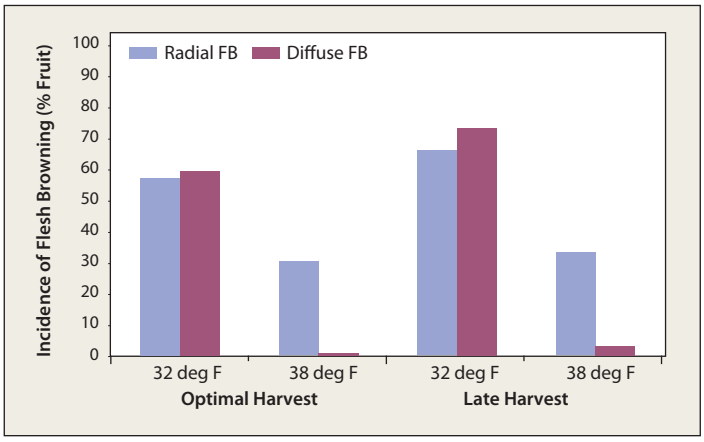

Figure 5. Incidence of flesh browning in 2005 of 'Pink Lady'™ apples in Australia as influenced by harvest date and storage conditions.

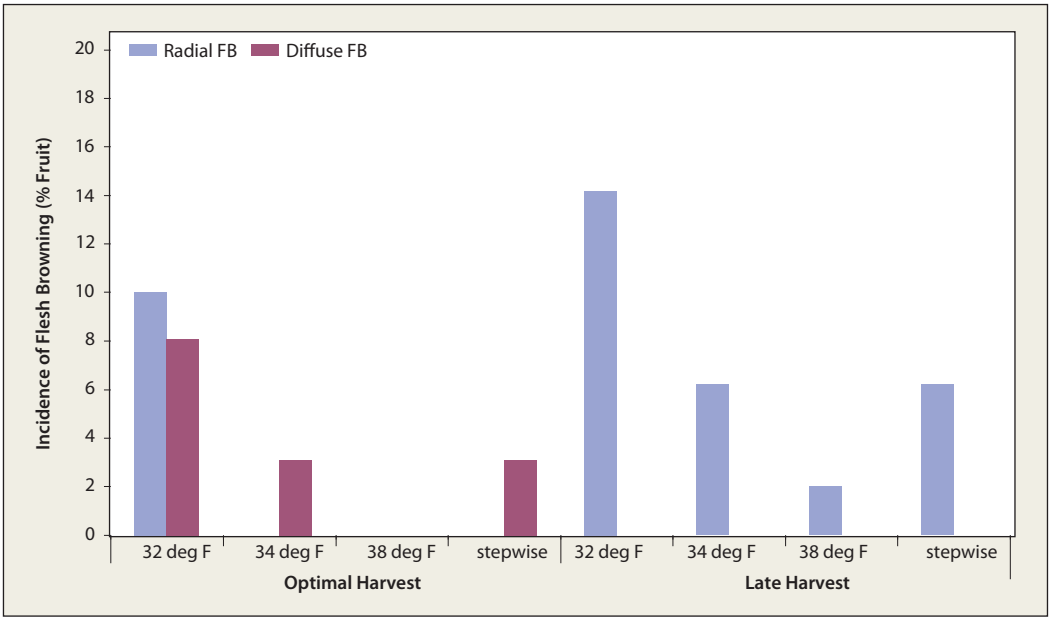

Figure 6. Incidence of flesh browning in 2006 of 'Pink Lady'™ apples in Australia as influenced by harvest date and storage conditions

of browning than fruit that were harvested at the optimal maturity for long-term storage.

While storage at 38°F was shown to significantly reduce the incidence of both disorders, storage at this temperature was shown to reduce the length of effective storage time. Storage at 38°F was found to increase the rate of softening and also to increase the development of skin greasiness. Both of these quality attributes would have resulted in fruit that were not able to meet quality guidelines for export.

In the 2006 season, fruit of optimal and late harvest (RFB only) were stored in air at 32°F and 38°F as well as at 34°F and a stepwise cooling regime (two weeks at 38°F, followed by two weeks at 36°F then storage at 34°F), in order to determine if a compromise between the incidence of flesh browning and the maintenance of fruit quality could be found. The 2006 season had a lower incidence of both RFB and DFB in comparison to the other seasons, however it was shown that storage at 32°F or the stepwise cooling treatment were effective for reducing the incidence of RFB to below commercial threshold levels, when the fruit were harvested at the optimal maturity (Figure 6). If fruit were harvested late, storage at 38°F was the only treatment that was found to reduce the incidence of RFB to within commercial limits. For the development of DFB however, storage at 38°F was the only treatment that was effective for reducing the incidence to within commercial limits. This indicates that fruit with a susceptibility to developing DFB have a higher sensitivity to the storage temperature.

2. Pre-harvest conditions

The high variation in the incidence of both RFB and DFB between regions and seasons suggested that one or more pre-harvest factors were involved in the development of both disorders. Mineral nutrition, crop-load and climatic conditions during fruit growth and development were examined in order to determine their influence on the development of both RFB and DFB during storage.

Table 1 shows the mineral composition of flesh samples taken at harvest for four different growing regions and up to three growing seasons. A significant variation was found in the concentration of minerals between regions and seasons. No significant correlations between the incidence of RFB and any mineral or mineral ratio were found, however a significant negative correlation was found between the

1. Mineral composition of 'Pink Lady'™ apple flesh at harvest, grown in Batlow (NSW), Huon Valley (Tas), Goulburn Valley (Vic) and Manjimup (WA).

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

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

Table 2. Mineral composition of 'Pink Lady'™ apple flesh at harvest from 5 orchard blocks located in Batlow, NSW.

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

*,** Significant at P<0.05 or 0.01 respectively

incidence of DFB and the ratios of calcium to potassium (R2=0.999) and calcium to magnesium (R2=0.998).

Variation in the incidence of RFB and DFB was not only observed between seasons and regions, but was also found within an orchard. Table 2 shows the mineral composition, crop load and incidence of fruit with RFB for five orchard blocks. A significant variation in the incidence of RFB was found between the orchard blocks and the concentrations of boron, calcium, potassium and phosphorus as well as mineral ratios between calcium and phosphorus and calcium and potassium. A trend of increasing incidence of RFB with decreasing crop load and calcium concentration was observed, however no significant correlation between any mineral or mineral ratio and the incidence of RFB was found. Fruit from block 5, the block with the lowest crop load, were found to have the lowest concentrations of calcium and potassium, both of which are associated with cell wall strength and stability. Conversely, fruit from block 1, the block with the highest crop load, had the highest concentration of calcium. Block 1 was also found to have the lowest incidence of fruit with RFB, suggesting that it is likely that calcium plays a role in the development of RFB.

Mineral composition was shown to remove some of the variability in the incidence in RFB and DFB between seasons and regions, however a large variation still existed. Climatic conditions during fruit growth and development were examined in an attempt to reduce this variation further. Climatic conditions during fruit growth and development can have a large influence on fruit physiology at harvest. The level of blush on the fruit, fruit size and density as well as the number and size of cells within the fruit can all be influenced by climatic conditions. The different expressions of flesh browning in 'Pink Lady'™ apples were found to occur in contrasting climatic conditions. Radial flesh browning was shown to consistently occur in warm growing regions, whereas DFB was shown to develop only in cool growing regions. Several climatic conditions were examined and it was found that the accumulation of growing degree days (GDD) above a base temperature of 50°F between the period of full bloom and harvest had the closest relationship to the type of flesh browning developing as well as the incidence and severity of symptoms.

The RFB disorder of 'Pink Lady'™ apples was found to occur in regions or seasons accumulating between 1100

Table 3. Relationship between growing degree days (GDD) from full bloom to harvest and the incidence of Radial and Diffuse types of flesh browning in 'Pink Lady'™ apples

Type of FB	GDD	Incidence of FB
Diffuse	888	95.37
	888	99.38
	904	75.70
	904	76.00
	930	8.08
Radial	1462	57.50
	1462	54.21
	1567	27.78
	1641	10.00
	1679	0.00

and 1700 GDD between full bloom and harvest (Table 3). As the accumulation of GDD increased above 1100 (indicating a warmer season), the incidence of RFB was found to decrease. So that the greater the accumulation of GDD above 1100 GDD, the lower the risk of developing RFB during storage. A region or season accumulating greater than 1700 GDD between full bloom and harvest would have little, or no, risk of developing RFB during storage.

The DFB disorder of 'Pink Lady'™ apples was found to occur in regions or seasons accumulating less than 1100 GDD between full bloom and harvest (Table 3). As the accumulation of GDD decreased below 1100 (indicating a cooler season), the incidence of DFB was found to increase. So that, the lower the accumulation of GDD below 1100 GDD, the greater the risk of developing DFB during storage.

Table 4. Classification, causes and control of Radial and Diffuse types of flesh browning in 'Pink Lady'™ apples

	Diffuse flesh browning	Radial flesh browning
Classification	chilling injury	senescent breakdown
Climatic range	<1100 GDD	>1100 GDD[z]
Maturity	SPI[y] 3.5	SPI 3.5
Storage temperature	3°C[x]	1°C or stepwise cooling[w]
Storage atmosphere	<1% CO_2	<1% CO_2
Orchard management	ensure Ca levels adequate	best commercial practice[v]

[z] Insufficient data in the climatic range of 1100-1400 growing degree days (GDD) >50°F 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 38°F will prevent the development of DFB, however storage at 34°F will reduce symptoms. Storage at 3°C will reduce the period of storage time before the loss of quality occurs.

[w] Storage at 34°F 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 38°F followed by 2 weeks at 36°F then the remainder of the storage period at 34°F.

[u] Best commercial practice for the management of crop load and fruit nutrition are recommended.

Managing the flesh browning disorder of 'Pink Lady'™ apples

Climatic conditions during fruit growth and development were found to be the key factor resulting in the pre-disposition of 'Pink Lady'™ apples developing RFB or DFB during storage. It is likely that with future seasons of research to validate the climatic data that a model may be generated for use to predict the seasonal risk for the development of both RFB and DFB. With the seasonal variation accounted for, the additive storage risks of the composition of the storage atmosphere and the storage temperature can then be adjusted in order to maintain fruit quality while managing the incidence of flesh browning to within commercial threshold levels.

1. Diffuse flesh browning. For optimal long-term storage and control of the DFB disorder, 'Pink Lady'™ apples grown in regions accumulating less than 1100 GDD should be stored at 38°F for a maximum period of four months. Harvest maturity has not been found to consistently increase the risk of developing DFB, however it is recommended that best commercial practices for harvest maturity are employed and that fruit are harvested and placed under ideal storage conditions prior to the ethylene climacteric. It is also recommended that calcium levels are adequate during fruit growth and development. The addition of CO_2 to the storage atmosphere was not found to detrimentally effect the development of the DFB disorder, however it is recommended that the concentration of CO_2 in the storage atmosphere is kept below 1% in order to reduce the likelihood of the fruit developing internal CO_2 injury. These recommendations are summarized in Table 4.

2. Radial flesh browning. For optimal long-term storage and control of the RFB disorder, 'Pink Lady'™ apples grown in regions accumulating between 1100 and 1700 GDD should be stored at 34°F for a maximum period of nine months. Harvest maturity was shown to increase the risk of developing RFB during storage, consequently it is recommended that best commercial practices for harvest maturity are employed and that fruit are harvested and placed into ideal storage conditions prior to the ethylene climacteric. It is also recommended that calcium levels during fruit growth and development are adequate and that tree crop load is maintained at an optimal level. The addition of CO_2 to the storage atmosphere was found to detrimentally effect the development of RFB. It is recommended that the concentration of CO_2 in the storage atmosphere be kept below 1% in order to reduce the likelihood of the fruit developing both RFB and internal CO_2 injury. These recommendations are summarized in Table 4.

With only three complete seasons, this research project has identified and classified Australian regions that are susceptible to RFB and DFB and it has determined the pre and postharvest factors that are involved in the development of both disorders. It is important to note that these results are still preliminary and future seasons of research are required to validate these findings and to determine the effectiveness of the strategies outlined in years where the risk of developing flesh browning is high. The success of this project has been the result of the effective collaboration between the scientific partners and commercial growers and exporters. This work has gone a long way to providing a practical management strategy for the flesh browning disorders of 'Pink Lady'™ apples by first gaining an understanding of the underlying physiology of the disorder.

References

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This article reports part of Hannah James' PhD thesis. Hannah has a postdoctoral position in Chris Watkins' laboratory in Ithaca where she is investigating flesh browning in McIntosh and Empire apples. Hannah's research is supported by the New York Farm Viability Institute and the New York Apple Research and Development program.