Update on the possible causes and management strategies of flesh browning disorders in 'Cripps Pink' apples

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Introduction

The flesh browning disorder of 'Cripps Pink' apples poses a significant threat to the established market identity of this cultivar. Flesh browning in 'Cripps Pink' apples was first observed in 2000 in Australia (Brown et al., 2003; Wilkinson, 2000). Since then flesh browning has become a considerable problem for the cultivar resulting in rejection of export shipments and placing pressure on the Pink Lady™ brand under which high quality 'Cripps Pink' apples are marketed (James & Jobling, 2009). Flesh browning occurrence is unpredictable and intermittent, and could quickly damage the established confidence in the cultivar and erode the developed market advantage (Jobling et al., 2008). Substantial economic losses were incurred by South African producers in 2006 and 2007. This problem was for the greater part alleviated after industry pack houses changed their storage regimes based on recommendations made from research results generated mostly in Australia and other global production areas. However, these recommendations need to be confirmed for South African growing conditions since browning does still occur.

Classification of the flesh browning disorders

Flesh browning of 'Cripps Pink' apples was initially described as a single disorder thought to be related to delayed harvest maturity and controlled atmosphere (CA) storage (Brown et al., 2003; Wilkinson, 2000). However, contrasting visual symptoms suggested that the disorder could be classified into three distinct disorders with different physiological causes (Jobling et al., 2004; James et al., 2005). Based on visual assessment of the location of the browning and a specific type of physiological damage, diffuse browning, radial browning and CO_2 injury were distinguished (James et al., 2005) (Figures 3 A, B and C). A fourth kind of flesh browning called bulge browning was later identified (Jobling et al., 2008) (Figures 3 D and E).

Diffuse browning (Figure 3 A)

Diffuse browning is characterized by browning of the cortex tissue, caused by the collapse of cells, with the vascular tissue remaining unaffected (James & Jobling, 2009; Jobling et al., 2008). Cortex cells are larger and have thinner cell walls compared to the vascular cells and are therefore more prone to collapse resulting from chilling injury which leads to development of the characteristic browning. A higher area of browning is found at the stem and calyx ends of the fruit, with less in the middle of the fruit.

Radial browning (Figure 3 B)

Radial browning is characterized by browning of the vascular tissue, caused by fractured cell walls of the tissue adjacent to the vascular bundles, with the cortex tissue remaining unaffected (James & Jobling, 2009; Jobling et al., 2008). This is the complete opposite pattern of that found in diffuse browning. The small vascular cells are thought to limit the diffusion of CO_2 through the fruit leading to a build up of toxic quantities of CO_2 aggravating the senescent disorder. A higher area of browning is found at the stem end of the fruit, with decreasing levels found towards the calyx end of the fruit.

CO2 injury (Figure 3 C)

CO_2 injury in 'Cripps Pink' apples is expressed in a similar fashion to CO_2 injury in other apples cultivars (James & Jobling, 2008). This disorder was found in 'Cripps Pink' apples when stored under CA conditions with CO_2 concentrations of higher than 1%. Since CO_2 levels are well regulated in South African CA rooms, it is not often seen commercially. CO_2 injury has been identified by the formation of lens shaped pits and cavities throughout the cortex tissue of the fruit.

Bulge browning (Figures 3 D and E)

This type of flesh browning is related to diffuse browning and abnormal pollination and fruit development (Jobling et al., 2008). The swollen area on these asymmetric, misshapen fruit is susceptible to diffuse browning and these fruit can be sorted out in the orchard or on pack lines.

Factors affecting the development of flesh browning disorders

Flesh browning disorders of 'Cripps Pink' apples are the result of a complex combination of factors (James & Jobling, 2008). The susceptibility to developing these disorders during storage can be influenced by pre-harvest conditions, although it is the post-harvest conditions that promote and exacerbate the development of the different disorders. No flesh browning has been observed

Figure 3: *Typical examples of (A) diffuse browning, (B) radial browning (James & Jobling, 2008), (C) CO_2 injury, (D) light bulge browning, (E) more severe bulge browning and (F) peduncular scald*

at time of harvest, indicating that these are storage disorders, but 'Cripps Pink' apples have been shown to develop commercially significant levels from as early as 4 months CA storage (James et al., 2005).

The presence of CO_2 injury in 'Cripps Pink' apples has indicated a susceptibility to this form of flesh browning; however the presence of high CO_2 concentrations in the storage atmosphere is not considered to be the primary cause of either diffuse or radial browning and is considered as an additive factor to their development (James & Jobling, 2009). Storage disorders of apples are often the result of many interacting external and internal factors that impact on cell stability. The susceptibility to chilling injury, senescent disorders and CO_2 injury are dependent on the structure and stability of cell walls, cell membranes and other cell components (Fahn, 1990; Kays and Paull, 2004; Raven et al., 2005). The structural and functional stability of the cell can be influenced by both pre- and post-harvest conditions, such as climatic conditions (Jackson, J., 2003), fruit maturity (Marangoni et al., 1996; Tu et al., 1997), tree crop load (Goffinet et al., 1995, Little and Holmes, 2000, James and Jobling, 2009), mineral nutrition (Neilson & Neilson, 2003, Little and Holmes, 2000, James and Jobling, 2009), rootstocks (Brown et al., 2003), girdling (Wilton and Hornblow, 2003), storage temperature (Fahn, 1990), storage time (James et al., 2005) and storage atmosphere (Schotsmans et al., 2004). Climatic conditions during fruit growth and development can have a large physiological impact on fruit at harvest (James & Jobling, 2008). The level of blush, fruit size and density as well as the number and size of cells within the fruit can all be influenced by climatic conditions. According to literature, the accumulation of growing degree day (GDD) above 10°C during the period from full bloom to harvest had the closest relationship to the type of flesh browning that occurred during storage in 'Cripps Pink' (Jobling et al., 2008).

Factors influencing diffuse browning development

Diffuse browning is classified as a chilling injury that occurs in susceptible 'Cripps Pink' apples when stored at lower temperatures for longer than 4 months. A major factor influencing the development of diffuse browning during storage is the accumulation of growing degree days (GDD - the accumulated heat units over a season) from full bloom to harvest (Jobling et al., 2008) (Figure 1). Diffuse browning in Australia occurs in fruit grown in cooler areas, accumulating less than 1100 GDD from full bloom to harvest. In a season accumulating more than 1100 GDD the likelihood of 'Cripps Pink' apples developing diffuse browning during subsequent storage is relatively low. However, in a cool season where an area has accumulating less than 1100 GDD from full bloom to harvest, the likelihood of occurrence of diffuse browning increases substantially. Italian research also found a correlation with a low incidence of flesh browning in a season with exceptionally high GDD (±1700). It was also found that susceptibility increased when the average temperature was 2°C below the 50 year average (Zanella, 2009). Consequently, the monitoring of seasonal climatic conditions is vital for the deter-

mination of optimal storage conditions and storage duration for the minimization of the development of diffuse browning during storage (Jobling et al., 2008).

Fruit nutrition has also been found to influence the development of diffuse browning in 'Cripps Pink' apples. Incidence of diffuse browning was found to increase with decreasing ratios of Calcium:Potassium and Calcium:Magnesium. Calcium concentration seemed to be the common denominator in diffuse browning occurrence. Calcium plays an important role in strengthening of cell walls and in strengthening the bonds between neighboring cells as well as in membranes integrity (Jackson, 2003). Nitrogen and Nitrogen:Potassium has also been found to play a significant role with the incidence increasing with an increase in Nitrogen and Nitrogen:Potassium ratio (Zanella, 2009).

Diffuse browning has been found to develop after 4-5 months storage, depending on seasonal risk. Incidence of diffuse browning has a strong relationship to storage temperature (Jobling et al., 2008). Fruit stored at lower temperatures (e.g. 0.0°C or -0.5°C) may have a much higher incidence level of diffuse browning compared to fruit stored at higher temperatures (e.g. above 1.0°C), depending on seasonal risk. This indicates that the storage temperature can be modified to reduce the incidence of diffuse browning in 'Cripps Pink' apples. Research shows that storage at 1.0°C or the use of stepwise cooling were found to significantly reduce the incidence of diffuse browning, but these protocols did not sufficiently reduce diffuse browning levels to within acceptable commercial thresholds. One needs to take into consideration that even though storage at a higher temperature might reduce the development of diffuse browning, there could be a compromise of fruit quality with extended storage. Fruit can become greasy or develop scald (peduncular scald; Figure 3 F).

Factors influencing radial browning development

Radial browning is classified as a combination of a senescent and chilling injury disorder that occurs in 'Cripps Pink' apples when they are harvested at an advanced maturity and stored at lower temperatures under CA conditions with higher CO_2 levels, for longer than 4 months. Radial browning occurs in fruit grown in warmer areas, accumulating more than 1100 GDD from full bloom to harvest (Jobling et al., 2008). According to Zanella (2009) radial flesh browning is mainly reported on in Autsralia, whereas diffuse flesh browning is seen in Tasmania (Australia), New Zealand, and Europe.

Similarly to diffuse browning, a major factor influencing the development of radial browning during storage is the accumulation of GDD from full bloom to harvest (Jobling et al., 2008). In a season accumulating more than 1700 GDD, the likelihood of 'Cripps Pink' apples developing radial browning during subsequent storage is relatively low. However, in a cooler season, where an area has accumulating between 1100 and 1700 GDD from full bloom to harvest, the likelihood of radial browning occurrence increases substantially. Consequently, the monitoring of seasonal climatic conditions is vital for the determination of optimal storage conditions and storage duration for the minimization of the development of radial browning during storage.

Fruit nutrition has also been found to influence the development of radial browning in 'Cripps Pink' apples. Incidence of radial browning was found to increase with decreasing crop load and an associated decrease in calcium concentration.

Harvest maturity is another important orchard factor that can influence the development of radial browning. As late harvested fruit have been shown to result in an increased incidence of radial browning during storage, it is important to monitor orchards with regular samples to accurately predict optimum harvest maturities.

The browning of the vascular tissue observed in association with radial browning becomes more severe with increased time in storage and also at CO_2 concentrations of 1% and higher (James et al., 2005). Radial browning has been found to develop after 4-5 months storage, depending on seasonal risk. Incidence of radial browning has a strong relationship to storage temperature (Jobling et al., 2008). Fruit stored at lower temperatures (e.g. 0.0°C or -0.5°C) may have a much higher incidence level of radial browning compared to fruit stored at higher temperatures (e.g. above 1.0°C), depending on seasonal risk. This indicates that the storage temperature can be modified to reduce the incidence of radial browning in 'Cripps Pink' apples. One needs to take into consideration that even though storage at a higher temperature might reduce the development of radial browning there could be a compromise of fruit quality with extended storage. Fruit can become greasy or develop scald (peduncular scald; Figure 3 F).

Storing susceptible 'Cripps Pink' apples under CA conditions with elevated CO_2 concentrations (≥ 1%) will increase the incidence of this disorder during storage.

Managing the flesh browning disorders (Figures 1 & 2)

Prevention of diffuse browning, radial browning and CO_2 injury development

- It is recommended that calcium levels are adequate during fruit growth and development and that the tree crop load is maintained at an adequate level (Jobling et al., 2008).
- It is recommended that the 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 recommended that the concentration of CO_2 in controlled atmosphere storage is kept below 1% to reduce the likelihood of the fruit developing internal CO_2 injury.
- In years of higher suspected risk (≤ 1100 GGD) fruit should not be stored for longer than 4 months under CA conditions.
- It has become standard practice for South African producers to store 'Cripps Pink' at above 0.0°C, sometimes with the addition of SmartFresh℠.

Climatic conditions

Seasonal data could be used to predict the incidence of flesh browning by relating the seasonal GDD to the incidence of actual occurrence of the disorders (Jobling et al., 2008). However, growing area and orchard cut off points would need to be determined from historic or collected data relating incidence to accumulated GDD for that growing area or orchard. The progress of the season can be plotted and the potential risk can be seen prior to harvest

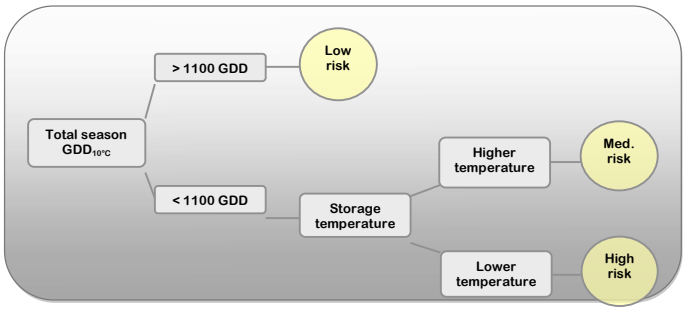

Figure 1: *Summary of risk factors and management strategies for the development of diffuse browning of 'Cripps Pink' apples during storage (Jobling et al., 2008)*

and appropriate marketing and storage strategies put in place.

Calculation of growing degree days

Research has shown a correlation between the risk of flesh browning development and the accumulated heat units over a season (Jobling et al., 2008). GDD values vary depending on area and season. The calculation of growing degree days (GGD) was done by taking the average of daily maximum and minimum temperatures compared to a base temperature (10°C). As an equation:

$$GDD = \frac{T_{max} + T_{min}}{2} - T_{base}$$

The total seasonal GDD is the sum of the daily GDD from full bloom until harvest. The GDD referred to in this study was calculated on a base temperature (Tbase) of 10°C.

South African research results on managing flesh browning disorders

Initial findings from a project funded jointly by Fruitgro[science] and the Postharvest innovation fund (PHI) have confirmed some results presented by Dr. Jenny Jobling of Australia. The study, conducted by ExperiCo and the Department of Horticultural Science at the University of Stellenbosch, looked at several factors thought to influence flesh browning, namely, storage temperature, cooling rate, harvest maturity and storage duration as well as biochemical antioxidant and membrane factors.

- It was confirmed that storage at higher temperatures (e.g. 2.0°C) compared to -0.5°C for extended storage periods under CA, retarded the development of flesh browning, but seemed to increase the risk of greasiness and peduncular scald (Figure 3 F) development.
- The influence of cooling rate on the development of flesh browning was inconclusive. However, fruit subjected to step cooling were generally firmer, with a more intense pink skin colour, compared to fruit subjected to rapid cooling. Step cooled fruit also exhibited a more advanced skin ground colour.
- A direct relationship was found between flesh browning development potential and harvest maturity. Higher levels of flesh browning were exhibited in fruit harvested at an advanced maturity, compared to optimum maturity fruit, regardless of growing area and this can be seen in membrane and antioxidant results which indicates more advanced senescence and higher stress levels as a result. Producers should thus refrain from waiting too long for colour development.

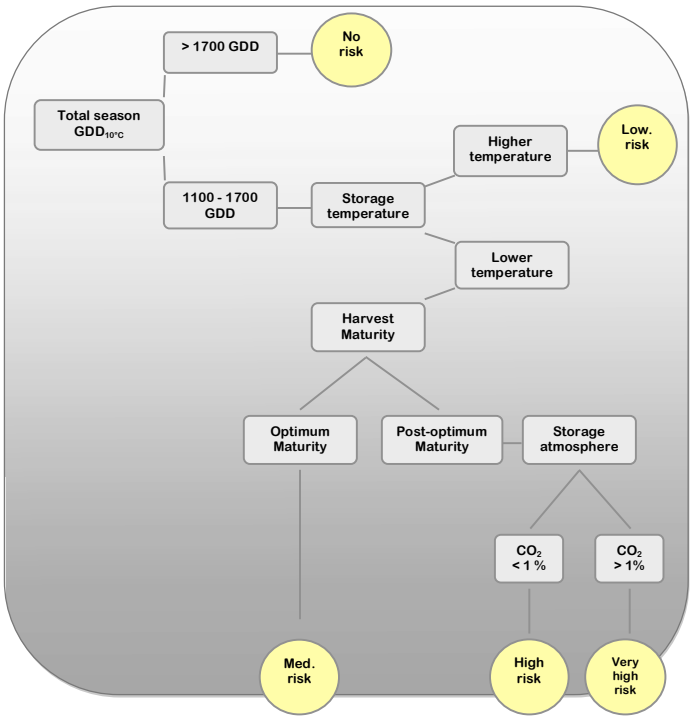

Figure 2: *Summary of risk factors and management strategies for the development of radial browning of 'Cripps Pink' apples during storage (Jobling et al., 2008)*

- A clear trend was found between the occurrence of flesh browning and the storage duration. Flesh browning only occurred in fruit stored under CA for a total period of 5 months or longer. Biochemical data confirmed that fruit that were stored for longer periods were more stressed.
- Diffuse browning was the main form of flesh browning exhibited throughout the 2010 trial.
- In 2009, flesh browning was mainly found in asymmetric fruit. This type of browning is termed bulge browning. These fruit should be eliminated during packing.

Conclusion

While good progress has been made in this research thus far, it is important to note that these results are still preliminary and findings must be comprehensively tested on various populations and in various growing areas prior to full commercial application. Furthermore, none of the pre-harvest factors mentioned in literature have been described for South African conditions and further research should be done to define their involvement.

Acknowledgements

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