Harvest maturity, storage conditions and tree age influencing internal browning and fruit quality of 'Rosy Glow' apple (*Malus domestica* Borkh.).

by

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Declaration

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SUMMARY

'Rosy Glow' being a limb/bud sport variety of 'Cripps' Pink' apple, is also regarded prone to internal flesh browning (IFB) similar to its parent cultivar. IFB in 'Cripps' Pink' apples has been reported to be influenced by both pre-harvest and post-harvest factors, such as harvest maturity, tree age, mineral nutrition, storage temperature, and duration. The application of chemicals, such as 1-methylcyclopropene (1-MCP) and diphenylamine (DPA), also influences the development of IFB. This study investigated the effect of tree age, harvest maturity, storage temperature, 1-MCP treatment, and storage duration in controlled atmosphere (CA) on IFB development in 'Rosy Glow' apples over two seasons (2014/2015 and 2015/2016).

Fruit were harvested at <40% and >50% starch breakdown (SB) for the harvest maturity trial (Trial 1) and <40% SB for the storage duration, temperature, 1-MCP (Trial 2), and tree age trial (Trial 3). Trial 1 and Trial 3 fruit were stored for 7 months in CA (1% CO_2 and 1.5% O_2) plus 6 weeks in regular atmosphere (RA) at -0.5 °C and 7 days at 20 °C and evaluated after each period. Trial 2 fruit, treated with or without 1-MCP, were stored at -0.5 °C or 2 °C and evaluated after 3, 5, and 7 months in CA plus 6 weeks in RA and a 7-day shelf-life period. Fruit were evaluated for IFB, SB, firmness, background colour, total soluble solids (TSS), titratable acidity (TA), greasiness, and blush colour at the end of each storage period.

The results showed that diffuse browning (DB), radial browning (RB), combination browning (CB), and CO_2 browning (CO_2B) were the types of IFB observed in all three trials. Optimum harvested fruit exhibited a lower susceptibility to IFB in general in both seasons (2015 and 2016), comparative to fruit harvested post-optimum. 1-MCP treated fruit had a lower IFB incidence and no tree age effect was observed in this trail. DB and RB was first observed after 5 months in CA plus 6 weeks RA at -0.5 °C.

DB was the main type of browning present. Harvest maturity (>50% SB) played a significant role in 'Rosy Glow' IFB development. Fruit quality was better retained at -0.5 °C than at 2 °C, while 1-MCP treated fruit quality was better maintained than control fruit over time. An orchard influence was observed on 'Rosy Glow' IFB and requires further investigation.

OPSOMMING

‘Rosy Glow’, wat 'n tak/knop sport variëteit is van ‘Cripps’ Pink’ appels, word ook beskou as geneig tot interne vlees verbruining (IVV), soortgelyk aan die ouerkultivar. Daar word beweer dat IVV in ‘Cripps’ Pink’ appels beïnvloed word deur beide voor-oes- en na-oes faktore, soos oesrypheid, boomouderdom, minerale voeding, opbergingstemperatuur en duur. Die toediening van chemikalieë, soos 1-metielsiklopropeen (1-MCP) en difenielamien (DPA), beïnvloed ook die ontwikkeling van IVV. Hierdie studie het die effek van boomouderdom, oes volwassenheid, opbergingstemperatuur, 1-MCP behandeling en opbergingsduur in beheerde atmosfeer (BA) op IVV-ontwikkeling in ‘Rosy Glow’ appels gedurende twee seisoene ondersoek (2014/2015 en 2015/2016). Vrugte is geoes teen <40% en> 50% styselafbraak (SA) vir die oesrypheidsproef (proef 1) en <40% SA vir die opbergingsduur, temperatuur, 1-MCP (proef 2) en die boomouderdomsproef (Proef 3). Proef 1 en Proef 3-vrugte is vir 7 maande in BA gestoor (1% CO_2 en 1,5% O_2) plus 6 weke in gewone atmosfeer (GA) by -0,5 °C en 7 dae by 20 °C, en na elke periode geëvalueer. Proef 2 vrugte, behandel met of sonder 1-MCP, is by -0,5 °C of 2 °C gestoor en na 3, 5 en 7 maande in BA plus 6 weke in GA en 'n 7-dae rakleeftydperk geëvalueer. Vrugte is geëvalueer vir IVV, SA, fermheid, agtergrondkleur, totale oplosbare vastestowwe (TOVS), titreerbare sure (TS), vetterigheid en bloskleur aan die einde van elke opbergtydperk. Die resultate het getoon dat diffuse verbruining (DB), radiale verbruining (RB), kombinasie-verbruining (CB) en CO_2-verbruining (CO_2B) die tipes interne IVV was wat in al drie die proewe waargeneem is. In beide seisoene (2015 en 2016) het die vroeg tot optimaal geoesde vrugte 'n laer vatbaarheid vir IVV in die algemeen gehad, vergelykend met vrugte wat na-optimum oesrypheid (>50% SA) geoes is. 1-MCP behandelde vrugte het 'n laer IVV-voorkoms gehad en geen boomouderdomseffek is in hierdie studie waargeneem nie. DB en RB is eers na 5 maande in BA plus 6 weke GA by -0.5 °C waargeneem. DB was die belangrikste tipe IVV teenwoordig. Oesrypheid (> 50% SA) het 'n belangrike rol gespeel in die ontwikkeling van ‘Rosy Glow’ IVV. Die vrugkwaliteit is beter behou by -0,5 °C as by 2 °C, terwyl 1-MCP behandelde vrugkwaliteit beter gehandhaaf is as kontrole-vrugte. Die invloed van die boord is op die 'Rosy Glow'-IVV waargeneem en vereis verdere ondersoek.

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GENERAL INTRODUCTION

The 'Rosy Glow' apple (*Malus domestica*) is a mutant variety of the 'Cripps' Pink' apple, which was discovered in an orchard at Masons Road, Forest Range in Southern Australia in 1996. It is a highly and early colouring variety (Mason and Mason, 2003).

The type of mutation that gave rise to the 'Rosy Glow' apple variety is called a limb/bud sport mutation (Janick et al., 1996). According to Janick et al. (1996), a limb/bud sport mutation initiates in a cell and then grows into a bud and thereafter into a shoot, producing usually a single trait difference from the parent plant. Mutants of this nature are easily identified by a change in fruit colour, usually red sports (depicting increased anthocyanin levels). Being a limb/bud sport, 'Rosy Glow' is assumed to be genetically very similar to 'Cripps' Pink' apple. The ability to assess the genetic difference between the two is beyond the scope of current technology (Langford, personal communication). Thus, besides the difference in fruit colour, there is most likely an insignificant difference between the two varieties.

Fruit sold as Pink Lady® apples are 'Cripps' Pink' and its sports usually have a pink blush of more than 40% and meets the standards set by the International Pink Lady® Association (IPLA). 'Rosy Glow' bears the Pink Lady® trademark and it is considered an improved selection by the IPLA (Dall, 2007; De Castro et al., 2007). Fruit quality, regarding the flesh browning disorder, remains a very important threat to the Pink Lady® apples in and after controlled atmosphere (CA) storage (Jobling, 2002; Bergman et al., 2012).

The need to keep Pink Lady® apples over a longer period makes CA storage inevitable. Volz et al. (1998) and Castro et al. (2005) reported that CA storage prolongs storage life and maintains the quality of Pink Lady® apples. Nonetheless, CA storage is associated with physiological disorders like flesh browning, in 'Fuji' and 'Braeburn' apples as reported by Volz et al. (1998) and Lau (1998), respectively. Long-term storage is also known to cause 'Superficial scald' in apples (Bramlage et al., 1996).

Jobling and James (2008) reported that there are four types of flesh browning in 'Cripps' Pink' apples, namely diffuse browning (DB), radial browning (RB), bulge browning (BB), and CO_2 browning (CO_2B). The type of browning is classified based on where in the flesh it was found, the type of damage done physiologically and the appearance of the damage. 'Rosy Glow' has been predicted by Dall (2008) to be more prone to the flesh browning disorder than its parent cultivar, 'Cripps' Pink'. Thus, there is the need to investigate this prediction. Kupferman (2002) outlined many factors, both pre-harvest (crop load, harvest maturity etc.) and post-harvest (CA,

CO_2 concentrations etc.) that influence the susceptibility of apples to browning. Low crop load tree fruit develop cavities and are more susceptible to internal browning (IB). Similarly, fruit harvested in a more advanced stage tends to develop browning more often than those harvested at an optimum stage. Lowering CO_2 levels just after harvest, as well as delaying CA storage helps reduce incidence of IB (Kupferman, 2002).

This study sought to determine whether pre-harvest factors such as harvest maturity and tree age will influence the susceptibility of 'Rosy Glow' apples to flesh browning. The onset and extent of flesh browning during CA storage at two different temperatures (−0.5 °C and 2 °C) and the effect of 1-methylcyclopropene (1-MCP) on the quality of the fruit after CA storage were assessed. The aim of this study was also to determine the period for which 'Rosy Glow' apples can be stored in CA, while still maintaining their quality according to the Pink Lady® standards. This study was conducted over two seasons (2014-2015) in the Western Cape, South Africa on 'Rosy Glow' apples grown in the Grabouw area.

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Chapter One: LITERATURE REVIEW

Factors affecting apple (M*alus Domestica* Borkh.) internal flesh browning.

1. INTRODUCTION

1.1 'Rosy Glow', an improved selection of the Pink Lady® brand

'Rosy Glow' as an improved selection that supports the success of the Pink Lady® apple as the fruit develops a greater area of pink blush when less mature than 'Cripps' Pink' fruit (Dall, 2008). 'Rosy Glow' apple is described as a highly pink blush coloured fruit even in the shaded parts of the tree (Mason and Mason, 2003).

A selection such as 'Rosy Glow' promotes the longevity of the brand as it curbs 'Cripps' Pink' production problems such as having to delay harvesting for the development of required colour (Dall, 2008). 'Rosy Glow' fruit colours early which enables growers to harvest fruit at optimum maturity for long-term storage (Mason and Mason, 2003). These attributes make 'Rosy Glow' an important cultivar for the Pink Lady® brand.

Dall (2007) observed that there is much focus on planting 'Rosy Glow' rather than 'Cripps' Pink' especially in Europe and South Africa and predicted even more 'Rosy Glow' plantings from the year 2008 and beyond. However, it was also predicted that 'Rosy Glow' may be more prone to browning than 'Cripps' Pink' but further research was recommended to ascertain the veracity of this prediction (Dall, 2007).

1.1.2 Invention of 'Rosy Glow'

The 'Rosy Glow' apple was discovered in an existing orchard in South Australia when highly blushed fruit developed on a particular branch of a 'Cripps' Pink' tree (Mason and Mason, 2003). After making a graft of this branch on an unpatented root stock (Northern Spy) and making sure the colouring characteristics were maintained, 'Rosy Glow' was declared a new sport mutation and patented (US 2003/0226181 P1).

Even though the tissue density of the 'Rosy Glow' apple has not yet been reported on, it is worthy to note that 'Rosy Glow' is likely to be as dense as its parental cultivar, 'Cripps' Pink', or even denser. According to Jobling et al. (2003) the density attribute of 'Cripps' Pink' could contribute to predisposing the fruit to the browning disorder due to the accumulation of CO_2 levels in the flesh. It is therefore predicted that 'Rosy Glow' could be predisposed to the browning disorder as found in 'Cripps' Pink'.

1.2 Flesh browning disorder

1.2.1 Browning: compounds and enzymes

Enzymatic browning in fruit is defined as brown, red or dark pigments or colouration that is observed mainly as a result of the oxidation of natural phenolic compounds to polymerised quinones (Mathew and Parpia, 1971; Mayer, 1986; Murata et al., 1995). Vaughn and Duke (1984) reported that enzymatic browning is a reaction that occurs when polyphenol oxidase (PPO) comes into contact with phenolic compounds. Amiot et al. (1992) observed that factors such as phenolic concentration, activity of PPO and the availability of L-ascorbic acid play important roles in oxidation reactions that lead to enzymatic browning. This enzymatic oxidation reaction is the cause of membrane disintegration, leading to loss of cell integrity when degraded beyond maintenance (Rawyler et al., 1999). Cell membrane integrity disruption is therefore fundamental to development of flesh browning in fruit.

1.2.2 Characterization of flesh browning

The first incidence of flesh browning in 'Cripps' Pink' apples was reported in the year 2000 (Brown et al., 2002) and flesh browning has since been characterized into three types based on the locality of the browning and the type of cells affected (Mitcham et al., 2004). James (2007), indicated that diffuse browning (DB), carbon dioxide browning (CO_2B) also known as CO_2 injury, and radial browning (RB) were the prevalent Pink Lady® browning disorders. Bulge browning (BB) was later on discovered by James and Jobling (2008), who reported that it is related to DB and develops as a result of abnormal pollination and fruit development.

Diffuse browning

Browning of the flesh is said to be diffuse when visual assessment shows browning in the cortex tissues which is caused by the collapse of the cortex cells whereas the vascular tissue is unaffected (James and Jobling, 2008). James and Jobling (2008) indicated the possibility of the vascular cell being structurally more stable due to its thickened cell walls compared to the affected cortex cells. The more browning of this type at the stem and calyx ends than in the middle of the fruit was observed by James and Jobling (2008). According to Bergman et al. (2012), DB results from chilling injury to the thin cell wall of the cortex. Crouch et al. (2014) reported that, DB may be influenced by chilling injury, harvest maturity, and more than 3 months storage in controlled atmosphere (CA). Crouch et al. (2015) indicated that DB is the most prevalent type of browning in 'Cripps' Pink' apples in South Africa.

Radial browning

Contrary to DB, RB was observed as browning close to the vascular tissue. This is attributed to the breaking of the cell walls in the tissue next to the vascular bundles while the cortex was left intact (James and Jobling, 2008). Bergman et al. (2012) attributed this browning type to the improper diffusion of CO_2 through the small sized vascular cell, leading to high CO_2 build-up that hasten their senescence rate, but more evidence is pertinent to the acceptance of this reason. James and Jobling (2008) reported that, RB may be influenced by harvest maturity (i.e. late harvest fruit being more susceptible), growing-degree-days (GDD; i.e. accumulation of GDD above 1100, and above implies reduced susceptibility) and the gas composition of the storage atmosphere (higher incidence of browning with storage atmospheres consisting of 1% CO_2). Moggia et al. (2015) reported that RB may be associated with growing degree hours (GDH) and possibly firmness at harvest.

CO_2 browning

When Pink Lady® apples are stored in CA environments with a CO_2 level of more than 1%, flesh browning known as CO_2 injury is observed (Lau, 1998; Bergman et al., 2012). CO_2B in 'Cripps' Pink' is observed as small elliptical lens shaped cavities in the flesh and the injured tissues were firm, unlike injuries from low temperature (Lau, 1998; Jobling, 2002) and the cavities may be brown, located in the cortex (James and Jobling, 2008). CO_2B may be as a result of elevated CO_2 concentration coupled with low concentrations of O_2 in the storage atmosphere of the apple fruit which may lead to the development of reactive oxygen species (ROS) like H_2O_2, which causes membrane damage (de Castro et al., 2007). This process may cause the membrane to disintegrate, releasing PPO enzymes in the plastids. The PPO reacts with phenolic compounds from the vacuoles and form quinones which gives the brown colour observable in the flesh of the fruit. This disorder differs from RB primarily in the area of fruit affected as well as the type of cell affected. CO_2 injury, as a flesh browning disorder, results from low O_2 conditions triggering fermentation processes which generates ROS to cause membrane damage to the cells of the cortex, unlike RB, which results from the build-up of CO_2 due to small sized vascular tissue cells inhibiting the diffusion of CO_2 and kills the cells adjacent or near the vascular bundles (Majoni, 2012). A more detailed description of the 'Cripps' Pink' browning is done by Jobling (2002).

Bulge browning

According to James and Jobling (2008), BB results from a pollination defect which causes an abnormal fruit development (asymmetric and misshapen). The cortex cells of the abnormal side are enlarged and for that matter, develop weak membranes. This predisposes the fruit to membrane disintegration and thus, internal browning under a stress inducing storage environment. The pattern of browning here is similar to DB where the weaker cortex cells are affected while the vascular cells stay intact and do not brown. Fruits with this type of browning disorder can be identified and sorted out (East et al., 2005; Bergman et al., 2012).

1.2.3 Factors affecting flesh browning

Different studies have related flesh browning disorders in apples to different pre-harvest and post-harvest factors. Pre-harvest factors such as climate, tree age, and crop load (Tough et al., 1996; Ferguson et al., 1999; Hurndall and Fourie, 2003; James and Jobling, 2008), rootstock used (Brown et al., 2002a; Butler, 2015), mineral nutrition (James, 2007) and harvest maturity (Lau, 1998) have been reported to predispose the apple fruit to flesh browning. Post-harvest factors like post-harvest handling and storage conditions have been observed to react with the pre-harvest factors above to cause flesh browning disorders in apples (Lau, 1998; Ferguson et al., 1999; Kader, 2002; James, 2007; James and Jobling, 2008). There are many disorders in fruit that, even though are seen in the fruit during it's post-harvest life, started developing due to factors and conditions prevailing in the orchard long before the fruits are harvested. Pre-harvest disorders such as relating to BB may be determined as early as at the flowering stage (abnormal flowering) (East et al., 2004; James, 2007). Harvest maturity and other post-harvest factors are further discussed in the following paragraphs due to their relevance to this study.

1.2.3.1 Harvest maturity

Maturity of fruit has a relationship with the ripening level of the fruit (James, 2007; Butler, 2015). Maturation is a subjective term, used to mean a stage of development that is desirable to the farmer or consumers mostly for immediate consumption or utilisation and in apples, it is accompanied by a build-up of sugars from the breaking down of starch, making it sweet and more suitable for consumption (James, 2007). Harvest maturity has a different implication all together. For instance, apples may be harvested at a certain stage to favour, colour development, size or even storage ability and not necessarily for immediate utilisation (James, 2007). Harvest maturity therefore is of high importance for optimization of the economics of fruit production (James, 2007).

Extensive research conducted on the correlation between harvest maturity and storability of apples has proven that maturity of fruit plays one of the cardinal roles regarding the development of storage or post-harvest disorders (Beaudry et al., 1993; Jobling and McGlasson, 1995; Blankenship et al., 1997; Fellman et al., 2003; Gross et al., 2004). Brown et al. (2002), Mitcham et al. (2004) as well as Moggia et al. (2015) reported that, fruit harvested late are normally prone to flesh browning disorders, particularly, RB. On the contrary, 'Granny Smith' showed signs of flesh browning development when it was harvested two weeks before optimum maturity (Toivonen, 2008).

Optimal harvest has been defined by James (2007) as a strategic harvesting with a good compromise between what is marketable and is with maximum storage potential. James (2007) recommended that, for highest storage potential, apples may be harvested before the climacteric stage, when the ripening process is yet to start. Fruit for the highest market value needs a good balance between its sensory attributes such as colour, sweetness tartness, aroma, juiciness as well as crispness and this balance is best achieved when fruit is allowed to begin ripening on the tree (James, 2007). Late blush development at advanced maturity of fruit, predisposes said fruit to internal browning development (Jobling, 2002).

Several strategies have been recommended and used for the determination and prediction of the harvest maturity of fruit for optimum market value. Little and Holmes (2000) stated that long term average harvest dates have a reputation of having been historically used very commonly for this purpose. They went on to say that this strategy is most useful when year-long consumer demand for the produce is not of very much concern. This may be since seasonal effects cannot be taken into consideration in this method. Days after full bloom (DAFB) has been reported to be better than long term average harvest dates as it takes elevation, growing region and even seasonal temperatures into consideration (Little and Holmes, 2000). Nevertheless, inconsistencies in defining full bloom have also been identified. For example, it is known as the opening of 50% blossoms in the UK but Australia uses 60 to 80% blossom opening to define the same terminology (Little and Holmes, 2000).

Studies have shown that, to predict the harvest maturity of apples, climatic and maturity historical data are best relied on, but maturity of fruit itself is ascertained by the measurement of various physicochemical properties. These properties as well as the relationship between them and the storage performance of many cultivars have been documented with their acceptable indices in several studies (Blanpied and Little, 1991; Blankenship et al., 1997;

Drake and Eisele, 1997; Little and Holmes, 2000; Zude-Sasse et al., 2001; James, 2007). The properties measured in this study are elaborated on below.

Total soluble solids (TSS) and titratable acidity (TA)

Little and Holmes (2000), describe TSS as acids, soluble carbohydrates and salts found in the fruit cell, while stating that their level varies directly with the ripening level of the fruit. Mainly composed of sugars, TSS more commonly depicts the sweetness of fruits (James, 2007). The standard export requirement of fruit sold under the Pink Lady® trademark for TSS stipulated at an average of 15% with a minimum of 13% (Hurndall and Fourie, 2003). According to Hurndall and Fourie (2003), Pink Lady® does not have problems with acid levels, nonetheless, they recommended levels between 0.4% and 0.8% for long term storage. Organic acids, acting as intermediaries in the citric acid cycle, are vital to the respiratory process. TA are therefore related to the metabolic rate of the fruit (Clark et al., 2003) and this in turn impacts on the susceptibility of the fruit to internal flesh browning (IFB). TA decreases with an increase in duration of storage time (Jan and Rab, 2012). During increased respiration, organic acids and the TA concentration of fruit decreases (Ghafir et al., 2009). In a previous South African study, Butler (2015) indicated that fruit with low TA may be susceptible to the development of RB while high TSS fruit was inclined towards the development of DB.

Watercore of apples has been related to the TSS content of fruit at harvest (Tamura et al., 2003). The water core disorder in apples also affect the vascular bundle area leaving the cortex tissue unaffected like RB. When fruit are not mature enough and pre-harvest night temperatures are low, intercellular spaces may be flooded with sorbitol and flooding of the intercellular spaces with sorbitol disrupts gas diffusion which leads to a build-up of CO_2 in the affected area (Argenta et al., 2002). Advanced cases of watercore could lead to internal breakdown as vascular tissue in affected fruit lacks the sorbitol to fructose conversion capacity thus, fructose can diffuse to other parts of the fruit (Kollas, 1968). It has been hypothesised that increased leaf to fruit ratio increases the incidence of watercore due to the increased feeding of sorbitol to fruit as number of leaves increases (Kollas, 1968)

Peel colour and flesh firmness

There are two different colours that are used to determine the maturity of ‘Cripps’ Pink’ apples namely, background and foreground colour (blush) (Watkins et al., 1992b; Huybrechts et al., 2002; Crouch et al., 2014). The background chlorophyll is making up the green background colour which breaks down to give way to the carotenoids unearth, as the fruit matures. This phenomenon ensures the importance of the background colour in determining fruit maturity (Kays, 1991). The foreground colour or the blush determines whether fruit qualifies under the Pink Lady® standard (Hurndall and Fourie, 2003). The blush colour is made up by anthocyanins which are upregulated by UV light and cooler temperatures prior to harvest and can be negated by temperatures higher than 30 °C (Arakawa et al., 1985; Curry, 1997; Iglesias et al., 2002; Reay, 1999). Blush percentage and intensity has been used to sort fruit into suitability for harvest, marketing and storage (Lau, 1985; Watkins et al., 2003). Forty percent of the total surface area of the fruit must be blushed for it to be classified as a Pink Lady® apple (Hurndall and Fourie, 2003). In warmer growing regions fruit are often hung longer on the canopy in order to wait for a cold front during autumn to attain a high blush colour, which in turn can lead to an advanced harvest maturity and IFB after storage (Crouch et al., 2014).

Firmness of fruit is said to vary inversely with the maturity of fruit due to the cell wall thinning action caused by enzymes (pectinase) as fruit ripens (Kays, 1991). Even though this is subject to seasonality and cultivar differences, fruit maturity has been measured using fruit firmness together with TSS in ‘Delicious’ apples (Little and Holmes, 2000; Watkins et al., 2003). Cripps et al. (1993) recommended a flesh firmness of 8.5 kg cm^{-2} as best for optimum storage, but for the export market a firmness of as low as 7 kg cm^{-2} is allowed (Hurndall and Fourie, 2003). According to Moggia et al. (2015) RB can be mostly associated with the firmness at harvest as well as the GDH accumulated.

Starch conversion

Apples are climacteric fruit, breaking down starch and converting it to sugars as they respire during ripening (Dilley and Dilley, 1985; Kays, 1991). The use of the Hortec starch chart for apples and pears is employed for the purpose of maturity and storability determination in South African apple and pear industries. A starch breakdown (SB) percentage of 15 to 40% is recommended for optimal harvest and desirable storability of ‘Cripps’ Pink’ (Hurndall and Fourie, 2003).

1.2.3.2 Post-harvest factors affecting flesh browning

Postharvest losses were estimated by Kader (2002) to be about 30% of the fresh produce harvested all over the world. Practices involved in handling of produce and subsequent processes as well as methods of storage and storage conditions are therefore factors that have been proven to have a considerable effect on the quality of fruit.

Storage techniques, temperatures and processes like the handling practices contribute to determine the quality of the produce reaching the consumer. Numerous storage methods are reported and prescribed for the preservation of fruits and vegetables depending on their physiology and consumer demand as well as availability of equipment for the purpose (Kays, 1991; Prussia et al., 1993; Little and Holmes, 2000; Kader, 2002; Kitinoja et al., 2002; Barbosa-Cánovas and Nations, 2003; Hurndall and Fourie, 2003). Storage techniques and conditions as well as other postharvest treatments are discussed further below.

Storage techniques and conditions

Browning in fruit, like many other post-harvest disorders, have been reported to be influenced by the storage methods and duration, processes and the temperature in which they are stored (Little and Holmes, 2000; de Castro et al., 2004; de Castro et al., 2007; James, 2007; Bergman et al., 2012). Storage methods, duration and temperature at which fruit are stored vary between fruit types as well as from country to country (Fidler et al., 1973; Ryall and Pentzer, 1982; Prussia et al., 1993; Kitinoja et al., 2002; Barbosa-Cánovas and Nations, 2003). James (2007) reported that different cultivars respond to storage conditions in different ways. 'Gala' and 'Braeburn' for example developed disorders after 3 months of storage, while 'Lady Williams' and 'Democrat' kept up to 9 months without developing storage disorders (Little and Holmes, 2000).

Modified atmosphere techniques: CA, modified atmosphere (MA) and dynamic controlled atmosphere (DCA)

CA, MA, and DCA storage are techniques used to enhance the post-harvest life of produce, especially fruit. Storage duration, temperature, relative humidity, and the levels of O_2, CO_2, as well as ethylene are the most common variables, controlled in the use of different storage techniques (Saltveit, 2003). Manipulation of storage atmosphere gases (O_2 and CO_2) and temperature is the most recommended way for the extension of apple storage life (Zagory and Kader, 1988; Watada et al., 1996; Kader, 2002; Saltveit, 2003). According to Jayas and Jeyamkondan (2002), MA and CA may be used interchangeably depending on the amount of control one exercises over the gases in the storage atmosphere. The composition of gases in CA and DCA are highly manipulated throughout the storage period, normally in automated systems, which are capital intensive and expensive to operate (Fonseca et al., 2002; Jayas and Jeyamkondan, 2002). The MA system on the other hand, mostly involves varying the permeability of fruit packaging or storage structure to gases. Atmosphere in MA may be altered and initial conditions set, but fruit physiology (respiration rate) is allowed to take over the manipulation afterwards (Zagory and Kader, 1988; Jayas and Jeyamkondan, 2002), often, leading to a CO_2 rich and O_2 poor atmosphere (Fonseca et al., 2002).

O_2 and CO_2 levels in the fruit's immediate environment are controlled such that they, in turn, control the rate of respiration of the fruit to prolong fruit storage life (Zagory and Kader, 1988; Kader et al., 1989; Parry, 1993; Solomos, 1994; Yam and Lee, 1995). According to Yam and Lee (1995), this manipulation is mostly a decrease in O_2 content and an increase in CO_2 levels in the storage atmosphere of the fruit. A reduction in the respiration rate is associated with a reduction in the rate of biochemical and metabolic processes e.g. ethylene production in the tissues to enhance the keeping of photosynthetic reserves (Jayas and Jeyamkondan, 2002). Ethylene production hastens the ripening rate of fruit in storage, initiating enzymes for cell wall softening to cause a reduction in fruit firmness. O_2 and CO_2 play an agonistic and antagonistic role, respectively, toward ethylene production (increased CO_2 concentration coupled with low O_2 concentration and *vice versa*). The inverse variation of the concentrations of these two gases (low CO_2 + high O_2) therefore reduces the storage life of the fruit (Wang, 1990; Beaudry, 1999; Fonseca et al., 2002; Jayas and Jeyamkondan, 2002; Saltveit, 2003; Wang et al., 2005; Watkins, 2006a; Johnson, 2009). Johnson (2009) observed a low internal ethylene in CA stored fruit during climacteric development.

These manipulations, notwithstanding their advantages, may also have negative effects on the stored fruit, e.g. development of ROS due to oxidative stress which may result from CA storage where O_2 levels are low and CO_2 levels are comparatively high, leading to CO_2 injury (de Castro et al., 2007; James, 2007). According to de Castro et al. (2007), stress and ROS can result in impairments in the membrane leaking enzymes like PPO to react with phenols from the vacuole to form brown coloured quinones seen as flesh browning. Lau (1998) reported that 'Braeburn' browning disorder, which is characterised by brown patches in the apple flesh and sometimes seen as water-soaked tissues when it is very intense, is aggravated by CA storage where O_2 levels are low and CO_2 is high.

Too low O_2 levels may trigger the fermentation process as an alternative to aerobic respiration which may cause damage to the cell (Beaudry, 1999; Franck et al., 2007). Jayas and Jeyamkondan (2002), as well as Wang (1990), stated that products like aldehydes, lactates, and alcohols are produced *via* the glycolysis pathway in the event of no or too low O_2 in fruits and vegetables. These products can initiate the development of disorders and off flavours which compromise the quality of stored fruit (Taiz and Zeiger, 2010). According to James and Jobling (2008), RB disorder may result from storage atmospheres made up of high CO_2 concentrations coupled with low O_2 levels.

Hurndall and Fourie (2003) recommended an O_2 level of not less than 1.5% and a CO_2 of not more than 1% for CA storage of Pink Lady®. O_2 and CO_2 level for MA was estimated at 2–3% and 1–8%, respectively (Jayas and Jeyamkondan, 2002). Recommendations for DCA gas levels were not found and this may be because there are no specific gas conditions for this technique (Veltman et al., 2003).

Storage duration

The duration for which fruit can store will depend on these manipulations as well as seasonal variation. James and Jobling (2008) reported that depending on the risk level of a season, 'Cripps' Pink' may take up to 5 months to develop diffuse flesh browning disorder. Crouch et al. (2014) confirmed the importance of storage duration's effect on DB development in 'Cripps' Pink' apples under South African conditions. However, they also mentioned that the maturity at which fruit is harvested plays an equally important role as the storage duration to determine the development of the disorder. A duration of 7 months and 4 months are estimated for CA and regular atmosphere (RA) respectively for Pink Lady® (Hurndall and Fourie, 2003). According to Butler (2015) RB developed before DB in 'Cripps' Pink' apples when they compared detection of browning by near-infrared (NIR) at 7 months (7M) to 7 months + 4 weeks + 7 days (7M+4W+7D). They also observed that RB was found in fruit at 7M and DB increased from 7M to 7M+4W+7D, while combination browning (CB) increased from 7M to 7M+4W+7D.

Temperature and relative humidity (RH)

Thermo-genic effect of respiration is reduced by the modified atmosphere techniques with the aid of a reduction in the storage temperature (Jayas and Jeyamkondan, 2002; Franck et al., 2007). Studies have shown that storage temperature plays an important role in the bid to extend the storage life of harvested produce (Prussia et al., 1993; Barden and Bramlage, 1994; Paull, 1999; Fonseca et al., 2002; Brackmann et al., 2005; Maurer and Arts, 2007; Brash, 2007; James, 2007; Shin et al., 2008; Moggia et al., 2009; Taylor et al., 2012). However, temperature has been reported to be associated with RH to the extent that they seem to have an inverse relationship, in this case, with regards to harvested produce. Low temperatures therefore, while reducing the rate of respiration, increases the humidity, which in turn favours the growth of pathogenic microbes (Beuchat and Ryu, 1997; Paull, 1999; Franck et al., 2007; Shin et al., 2008). Paull (1999) gave an extensive elaboration on the relationship between temperature and RH and explained their impact on the vapour pressure deficit, which determines the rate of moisture evaporation from fruit. The uncontrollable nature of RH, except by the control of temperature, was also stated (Paull, 1999). Temperature and RH at different levels impact ofruit differently. High temperature and low RH have been reported to induce high rates of respiration leading to deterioration of produce (Zagory and Kader, 1988; Paull, 1999; Fonseca et al., 2002; Jayas and Jeyamkondan, 2002; James, 2007; Shin et al., 2008). Low temperatures slow down respiration, to enhance storage period, but may also cause chilling injury associated with DB disorder (James, 2007), superficial scald and others (Little and Holmes, 2000; James, 2007; Moggia et al., 2009). Chilling injury affects the structure of the cell membrane converting it from the normal liquid-crystalline state to a solid gel state, thereby impairing its function and causing leakage of the cell contents (Majoni, 2012).

It was therefore recommended that a balance be made between the cost of temperature reduction and that of gas controls to be able to optimise the benefits of modified atmospheres techniques (Jayas and Jeyamkondan, 2002; James and Jobling, 2008).

Other postharvest treatments

The economic importance of postharvest disorders in horticultural production ventures, due to the delicate nature of the produce, has led to the development of certain chemicals to reduce or stop certain disorders in specific produce. Some of these chemicals are 1-methylcyclopropene (1-MCP), aminoethoxyvinylglycine (AVG), and diphenylamine (DPA). Fruit destined for storage may be treated with any of these chemicals to reduce the rate of ripening caused by ethylene during the climacteric phase of development (Serek et al., 1994; Sisler et al., 1996; Sisler and Serek, 2003; Watkins, 2006a) or to induce an antioxidant effect inhibiting NADH oxidase and succinoxidase activities in cell mitochondria (Baker, 1963; Lurie et al., 1989).

The mechanism of operation of these chemicals is further discussed below.

1-MCP (SmartFresh™, manufactured by Agrofresh Inc.)

1-MCP is said to be first made into a complex with γ-cyclodextrin powdery formulation from which other beneficial 1-MCP is released upon dissolution in water (Watkins, 2006a). Patented by Sisler and Blankenship (1996), it was sold under the name EthylBloc® by Floralife, Inc. (Walterboro, SC) when the Environmental Protection Agency (EPA) authorised its use on ornamentals in 1999. It was later improved for use on edible horticultural produce and marketed as SmartFresh™ by AgroFresh, Inc., a subsidiary of Rohm and Haas (Springhouse, PA) (Watkins, 2006a).

1-MCP generally works by binding permanently to the receptor of ethylene to decrease the biosynthesis of ethylene by inhibiting ethylene's access to its receptor (Serek et al., 1994; Sisler and Serek, 1997, 2003; De Ell et al., 2005). Watkins (2006a) reported that the concentration of 1-MCP that may occupy all the receptor-binding sites of ethylene is cardinal to the efficacy of the applied 1-MCP. How long 1-MCP is effective when applied is dependent on the species, cultivar, as well as how ethylene is biosynthesised by the species or cultivar (Watkins, 2006a). However, Blankenship and Dole (2003) stated that 1-MCP use is subject to the consideration of factors like cultivar, stage of development of produce, lag time between fruit harvest and treatment, as well as repeated applications.

The ethylene receptor binding activity of 1-MCP results in several effects depending on the species involved in the treatment. Affected physiological and biochemical activities include a decrease in respiration and ethylene production, pigment breakdown (colour change effects), production of volatiles (effect on aroma and flavour), stabilization acid and sugar levels (effect on taste), decrease in protein and membrane modifications (effect on disorder developments). Factors such as firmness, TAA, and TA have been reported to have been maintained, while diseases and pests are also reportedly reduced by the application of 1-MCP (Fan et al., 1999; Rupasinghe et al., 2000; Watkins, 2006a).

1-MCP has been applied on a wide variety of fresh produce ranging from ornamentals to fruits and vegetables (Watkins and Miller, 2004; Watkins, 2006b). Blankenship and Dole (2003) reported that 1-MCP is very effective at very low concentrations, i.e. from 2.5 nl l^{-1} to 1 µl l^{-1} applied at 68 – 77 °F (20 – 25 °C) for 12 – 24 hours. Due to its full response potential, even at these very low concentration, 1-MCP is tagged as a reduced risk product by the USA EPA, because it leaves no detectable residues on the produce after treatment (Sisler and Serek, 2003).

AVG and DPA

AVG and DPA are also chemicals that have been used to enhance the storage life of fruits, including apples. AVG was endorsed by the EPA of USA in 2001 and is used as a pre-harvest treatment to regulate the growth of horticultural crops like apples and stone fruits (D'Aquino et al., 2010). According to Martínez-Romero et al. (2007), AVG was commercialized as ReTain® by Valent BioSciences Corp. in Libertyville, Illinois, USA. ReTain® contains about 15% (w/w) AVG which can retard and\or stop the biosynthesis of ethylene, both on tree and in storage. Yu and Yang (1979) reported the biosynthetic pathway to produce ethylene as the conversion of methionine to S-adenosylmethionine (SAM) in a reaction catalysed by SAM synthase, then to 1-aminocyclopropane-1-carboxylic acid (ACC) catalysed by ACC synthase (ACS). ACC oxidase (ACO) finally catalyses the conversion of ACC to ethylene *via* the ethylene receptor (Martínez-Romero et al., 2007). In their research, Yu and Yang (1979) observed that AVG blocks ethylene production by obstructing the production and activation of ACS, which mediates the changing of SAM to ACC (Jobling et al., 2003).

AVG is reported to have been used to prolong the storage and shelf life of apples, plums, nectarines, peaches, pears, and other fruits (Martínez-Romero et al., 2007). Retardation of ethylene production by AVG has been reported to result in several physiological interventions. Some of these interventions are a delay of fruit drop before harvest and fruit maturity, retardation of starch degradation and softening, build-up of sugars and ester volatiles, enhanced skin colour and flesh firmness, and a delay in the reduction of acidity (Mir et al., 1999; Khan et al., 2001; Amarante et al., 2002; Bregoli et al., 2002; Huybrechts et al., 2002; Jobling et al., 2003; Schupp and Greene, 2004; Silverman et al., 2004; Torrigiani et al., 2004; Rath et al., 2006).

DPA is reported more specifically to control superficial scald in apples (Smock, 1955; Hall et al., 1961; Lau, 1990; Bauchot and John, 1996; Fan et al., 1999; Wang and Dilley, 1999; Zanella, 2003). It has been reported that natural volatiles like α-farnesene (2,6,10-trimethyl-2,6,9,11-dodecatetraene) and the chemicals they produce under oxidation such as conjugated trienes, conjugated trienols (CTols), and 6-methyl-5-hepten-2-one (MHO), are the reactants which cause superficial scald (Huelin and Coggiola, 1970; Filmer and Meigh, 1971; Watkins et al., 1992a; Du and Bramlage, 1993; Song and Beaudry, 1996; Whitaker et al., 1997). DPA prevents the superficial scald disorder by inhibiting the production of MHOs from α-farnesene (Smock, 1955, 1957; Huelin and Coggiola, 1970; Wang and Dilley, 1999).

DPA has also been observed to prevent soft scald (Wills et al., 1981), core flush (Little and Taylor, 1981), as well as inhibit IFB in apples, by mitigating the effect of ethylene as well as the fruits sensitivity to increased CO_2 concentrations (de Castro et al., 2004). It has however been reported that concerns have been raised on the use of DPA in the treatment of fruits regarding health and safety for human consumption purposes (Lau, 1990; Wang and Dilley, 1999).

1.3 CONCLUSION

Studies have well established the fact that storage disorders including IFB are influenced by factors that need attention at both pre-harvest and post-harvest stages of production (Merritt et al., 1961; Bramlage et al., 1979, 1980; Drake et al., 1979; Wills et al., 1981; Emongor et al., 1994; Volz et al., 1993, 1994; Ferguson et al., 1994, 1999; Lee and Kader, 2000; Kader and Rolle, 2004). Factors such as harvest maturity, mineral nutrition, crop load, climate, root stock, postharvest handling, storage temperatures, and storage treatments have all been associated with the postharvest life, quality, storage duration as well as the marketability of fruits. These factors have been reported to affect the physico-chemical properties of the fruit and, as such, have a great impact on the physiological and biochemical reactions that degrade or enhance the storability, quality as well as the marketing propensity of fruit.

The fruit under study here ('Rosy Glow') has been considered as an improved cultivar that could enhance the value of the Pink Lady® trademark and maintain or even enhance the market value thereof. Advanced maturity of Pink Lady® apples caused by late colour development of 'Cripps' Pink' apples could become a thing of the past as 'Rosy Glow' develops an attractive blush during the early stages of ripening (Dall, 2007). Nonetheless, the prediction of 'Rosy Glow's susceptibility to FB cannot be overlooked. It is therefore, the objective of this study to investigate the assertion of FB susceptibility. An attempt will be made to investigate whether harvest maturity, storage temperature, and 1-MCP treatment has an influence on the development of the disorder herein. This study will also investigate the possibility of tree age affecting the intensity of susceptibility to the FB disorder of 'Rosy Glow'.

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Chapter 2: PAPER ONE

The effect of harvest maturity on postharvest quality with special reference to internal flesh browning incidence of 'Rosy Glow' apple (*Malus Domestica* Borkh.).

ABSTRACT

'Rosy Glow' apples (*Malus domestica* Borkh.) are stored for up to 7 months in South Africa in order to obtain high prices. The aim of this study was to assess the effect of harvest maturity on the internal browning (IB) incidence and postharvest quality of 'Rosy Glow' apples after an extended storage period over two seasons (2014 and 2015). Fruit were harvested at early to optimum maturity (13–40% starch breakdown; SB) with an average firmness of 9.3 kg, 0.66% titratable acidity (TA) and a total soluble solids (TSS) of 12.7% at harvest. Post-optimum maturity (>50% SB) fruit recorded an average firmness of 8.7 kg, a TSS of 13.3% and TA of 0.64% at harvest. IB evaluations as well as physicochemical analysis (background colour (BC), firmness, TSS, TA and SB) were conducted after harvest and after every storage period. All physicochemical parameters were influenced significantly by the interaction of storage period and harvest maturity in both seasons. Optimum harvest maturity fruit either had a significantly higher or similar firmness and TA compared to post-optimum harvest maturity fruit in both seasons. Four types of IB were found in the two seasons: diffuse browning (DB), radial browning (RB), carbon dioxide browning (CO_2B) and combination browning (CB = DB + RB) were observed in the two seasons with DB being the dominant type of browning. Total browning was affected by a harvest maturity and storage period interaction, and correlated negatively with TA, but positively with TSS to varied degrees in both seasons. Total browning weakly positively correlated with BC in the first season, but highly positively in the second season and was weakly negatively correlated with firmness. DB and RB were influenced by harvest maturity in the first season, but by the interaction of harvest maturity and storage period in the second season. Optimum harvested fruit exhibited a significantly higher total browning percentage in the first season, while in the second season, total browning was significantly higher in post-optimum harvested fruit. SB results showed that optimum harvested fruit, on the average, was lower than the recommended SB level (15-40%) in the first season, which may have influenced the high IB recorded for these fruit. There was a low incidence of CO_2B and CB in both seasons. Results from the two seasons suggest that 'Rosy Glow' may be susceptible to DB, RB and CB when harvested post-optimum maturity. Furthermore, DB and RB in post-optimum harvested 'Rosy Glow' apples may be progressive in shelf-life.

Keywords: diffuse browning, maturity indices, Pink Lady®, starch breakdown

1.1 INTRODUCTION

Harvest maturity is an essential factor to consider with regards to effective storage of fruit, be it short, medium or long-term storage (Kader, 1997) and it is influenced by various factors such as season, orchard and fruit properties among others (James, 2007). It is known that harvesting at post-optimum maturity renders apples, specifically 'Cripps' Pink', more susceptible to the internal browning (IB) disorder after a long-term storage at controlled atmosphere (CA) conditions (Lau, 1998; Bergman et al., 2012; Majoni et al., 2012; Crouch et al., 2014). Previous research (Bergman et al., 2012) reports that, 'Cripps' Pink' IB can be grouped into five types depending on the type of tissue affected. These include diffuse browning (DB), radial browning (RB), carbon dioxide browning (CO_2B), combination browning (CB), and bulge browning (BB).

Correlations were observed between the IB types mentioned and some quality or maturity indices in 'Cripps' Pink' apple fruit. Butler (2015) reported that higher total soluble solids (TSS) may significantly influence DB incidence, due to its positive association with advanced fruit maturity. According to Majoni et al. (2013), a higher titratable acidity (TA) and firmness were observed in fruit with optimum harvest maturity, and exhibited less IB. Majoni (2012) observed that fruit's ability to maintain background colour (BC) related to low IB incidence and also recommended optimum maturity at harvest (<40% starch breakdown; SB) for long term CA storage.

Majoni (2012) observed that 'Cripps' Pink' apple fruit harvested more mature (64% SB) could store for only a short period (up to 3 months), and thus recommended that fruit be harvested ealier (34% SB) if intended for a longer storage in CA at -0.5 °C to minimise the susceptibility to IB. According to James (2007) 'Cripps' Pink' apple fruit harvested late may be more susceptible to RB, which is as a result of senescence, even though other IB types may also be observed. Paliyath and Droillard (1992) reported that senescence or stress may lead to the deterioration of cell membrane in plant tissues, which results in increased membrane permeability.

'Rosy Glow' is a limb sport of 'Cripps' Pink' and may, therefore, be susceptible to IB (Dall, 2007). It is however, not known what the susceptibility of optimum and post-optimum harvest and long-term storage is with regards to 'Rosy Glow' quality. Maturity at which fruit is harvested has been amply reported to influence quality of fruit after storage (Blanpied and

Little, 1991; James, 2007; Toivonen, 2008; Majoni, 2012). Majoni (2012) reported optimum harvested fruit as less susceptible to IB, when treated with 1-MCP, due to increased glutathione levels after storage when post-optimum harvested fruit treated in same way did have browning and low glutathione levels.

This trial assessed 'Rosy Glow' apple quality based on the physicochemical properties of the fruit, including their susceptibility to IB, according to two harvest maturities and storage time. The objective of this study trial was to attempt to determine the risk of each harvest maturity and length of storage to IB for 'Rosy Glow' apple produced under South African conditions.

1.2 MATERIALS AND METHODS

1.2.1 Fruit material

'Rosy Glow' fruit were hand-picked from two commercial orchards, Glen Fruin Farm (block 20, 34°11'23.6"S 19°03'10.4"E planted in 2008) in Grabouw (Elgin) and Damar Farm (block 16, 34°03'22.2"S 19°06'54.0"E planted in 2010) in the Vyeboom area in the Western Cape Province of South Africa for season one (2014) and season two (2015), respectively. Fruit trees were managed with current commercial practices and were grown on MM 109 rootstocks. The Glen Fruin orchard was in its 6th leaf at the time of this study while the Damar orchard was in its 5th leaf. Fruit were picked during the commercial harvest period, after a series of iodine tests (SB percentage as a measure of maturity determined with 0.5 M potassium iodide (KI) on fruit cut surface).

1.2.2 Fruit numbers and harvest specifications

Early to optimum maturity fruit were harvested at a SB of 13–40% and post-optimum maturity fruit at >50% SB for both seasons. A total of 640 (2014 season) and 1600 (2015 season) fruit were harvested at random, from the outer canopy on all sides, from four trees and nine trees, in 2014 and 2015 respectively, in the same row, per harvest maturity. Fruit were transported in lug boxes to the Department of Horticultural Science, Stellenbosch University and maturity evaluations were done immediately after harvest. Fruit were then stored in regular atmosphere (RA) at -0.5 °C for one day to remove field heat, before CA storage period commenced.

1.2.3 Fruit storage

Three fruit storage techniques were used in this trial namely CA, RA, and shelf-life (SL). Storage was done in CA at -0.5 °C for 7 months in Janny MT (France) CA plastic bins with sealed lids, hereafter called 'Janny bins', in the 2014 season. The Janny bins were 60 cm deep, 90.5 cm wide and 110 cm long (measuring from the inside of the bin) and contained 10 plastic lug boxes, each containing fruit. Each Janny bin was connected to a pulsing inlet for nitrogen (N_2), oxygen (O_2) and CO_2, as well as a gas overflow. The CO_2 and O_2 concentrations were kept constant at 1% and 1.5%, respectively, using an automatic pulsing system (Janny MT Bins Pulse Gas Control System, Gas At Site (Pty) Ltd., South Africa). In the 2015 season, fruits were stored in the CA facility of the Agricultural Research Council (ARC-Infruitec) due to an increase in the fruit numbers. In South Africa, Pink Lady® can be stored in CA (≥1.5% O_2 and ≤1% CO_2) conditions for 7 months at -0.5 °C (Hurndall and Fourie, 2003). Fruit were moved into RA at -0.5 °C for 6 weeks to simulate packing, shipping, and stock rolling after CA storage. Later, fruit were kept at room temperature (20 °C) for seven days to simulate ripening or the SL period.

1.2.4 Fruit browning evaluation and description

At the end of each storage period, the number of fruit required for evaluation at that time was selected at random from the lot in storage. Individual fruit was cut equatorially but closer to the stem end for IB assessment. IB was assessed based on the pattern of brown colour observed in the flesh of fruit as well as the part of flesh affected as specified by James, (2007). IB type and presence of disorder were recorded for individual fruit after the evaluation was conducted.

1.2.5 Experimental design

The trial was a completely randomised design, analysed in a two-way analysis of variance (ANOVA) with harvest maturity and storage period as factors. Assessments at a storage period consisted of 10 replicates of 8 fruit each in the 2014 season and 20 replicates of 10 fruit each in the 2015 season. Fruit were assessed at four periods namely:

1. at harvest
2. after 7 months (m) of storage in CA (1% CO_2 and 1.5% O_2) conditions at -0.5 °C (7m CA)
3. after 7m CA + 6 weeks (w) of RA storage at -0.5 °C (7m CA + 6w RA)
4. after 7m CA + 6w RA + 7 days (d) of SL at 20 °C (7m CA + 6w RA + 7d SL)

1.2.6 Physicochemical analyses

Maturity and quality indices, as a measure of quality, were conducted after CA, RA, and SL. All assessments were done on each of the fruit for all replications unless otherwise stated.

1.2.6.1 Fruit firmness

A 4-in-1 fruit texture analyzer (FTA, Model GS 20 (Sl. No.: 2007-FTA-409), Güss Manufacturing Ltd., Strand, South Africa) made up of a motorized penetrometer, a scale with a maximum load capacity of 3 kg, a DMF apparatus for determining diameter as well as an electronic calliper with automated reading capturing, was used to assess fruit firmness. Firmness (kg) was determined as the maximum force necessary to push an 11.1 mm diameter probe into the flesh after peeling (±1 mm) two equatorial sides (blushed and unblushed sides) of the fruit with a potato peeler.

1.2.6.2 Background colour

BC of the fruit was measured using the Colour Chart for Apples and Pears (Unifruco Research Services (Pty) Ltd., South Africa, 1991) with a scale of 0.5 – 5.0 (0.5 = dark green and 5.0 = deep yellow). The colour chart index is widely used in industry to record the colour transition from green to yellow during ripening.

1.2.6.3 Greasiness

The ease with which whole fruit will turn while firmly held without slipping and making a sound was used to determine the presence and level of greasiness of fruit skin. Fruits were then scored on a scale of 0 – 5 with 0 no greasiness and 5 highly greasy.

1.2.6.4 Starch breakdown

Fruit were cut in half equatorially and one half used for the assessment of SB percentage using the iodine test. The cut surface of the fruit was covered with a potassium iodide solution (0.5 M KI) and then left for 2 min. SB percentage (unstained area) was determined using the starch conversion chart (Unifruco Research Services, PTY, South Africa) with a scale of 0 to 100%, where 0% was totally stained and 100% representing totally unstained.

1.2.6.5 Total soluble solids and titratable acidity

Equatorial apple slices representing all sides from the fruit (seeds punched out with a metallic 2.5 cm diameter cylinder) were placed in an electric juicer (AEG Electrolux, Type JE-107 no. 91100085/ PNC 950075206, P.R.C.) and the juice was used to determine TSS (%) and TA (%). TSS was measured using a calibrated hand-held electronic refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). TA was measured using an automated titrator (Titrino 719 S and Sample Changer 674, Metrohm (Pty) Ltd., Herisau, Switzerland) by titrating 5 g of juice from each apple sample with 0.1 M sodium hydroxide (NaOH) to a pH of 8.2. In 2014, ten pooled samples from a composite of eight fruit per sample were recorded for each replicate, while in 2015 twenty pooled samples from a composite of ten fruit per sample were recorded for each replicate.

1.2.6.6 Statistical analysis

Physicochemical analyses data were subjected to ANOVA using SAS Enterprise guide version 5.1 (SAS Institute Inc., Cary, NC, USA) and XLSTAT version 2015.5 (Addinsoft Inc., NY, USA). Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means. Pearson's correlation coefficient was generated and used to establish correlations between harvest maturity, storage period and/or quality parameters to IB development at harvest and after storage.

1.3 RESULTS

1.3.1 Prevalence of browning incidence and browning types

1.3.1.1 Season one (2014)

Figure 1A shows that 42 (6.6%) of the total of 640 fruit evaluated for both maturities were affected by IB throughout season one (over all storage periods). Although this percentage seems low it is commercially significant since the maximum allowable incidence is <1% that is stipulated by the International Pink Lady® Association (IPLA) standards. Four types of IB were observed, namely DB, RB, CO_2B, and CB (DB and RB) and the most common type of IB was DB (69%; Fig. 1B).

1.3.1.2 Season two (2015)

Incidence of IB affected 85 fruit (Fig. 2A), which will be about 5.3% of the total number of fruit evaluated for both maturities (i.e. 85 of 1600) throughout season two (over all storage periods). Four types of IB were observed; DB, RB, CO_2B, and CB, with the most common being DB (64.7%; Fig. 2B).

1.3.2 Effect of harvest maturity and storage period on total browning and browning types

1.3.2.1 Season one

The total incidence of IB differed significantly between storage periods for both harvest maturities ($P \leq 0.026$; Table 1). Total browning was highest at 7m CA but did not differ significantly from total browning at 7m CA + 6w RA + 7d SL for optimum maturity fruit (Fig. 3). However, total browning increased with storage period for post-optimum maturity fruit even though there was no significant difference between total browning at 7m CA and at 7m CA + 6w RA (Fig. 3). The highest incidence of total browning for the post-optimum maturity fruit was recorded at 7m CA + 6w RA + 7d SL and it was significantly different from that of the other storage periods. Harvest maturity affected CB incidence differently, at least, at one of the storage periods ($P \leq 0.024$). However, due to low incidence (7 fruit = 16.7%; Fig. 1B) further statistical analysis in the form of multiple comparison was not conducted on CB. Harvest maturity significantly affected DB and RB in season one, $P \leq 0.047$ and $P \leq 0.044$, respectively (Table 1), such that, fruit harvested at optimum maturity, exhibited a higher incidence of DB compared to post-optimum harvested fruit (Fig. 4). However further statistical analysis was not conducted on RB incidence due to low (5 = 11.9%; Fig. 1B) fruit numbers (Fig 1B). None of the factors proved significant in the influence of CO_2B (Table 1).

1.3.2.2 Season two

Table 2 shows the influence of harvest maturity and storage period on IB incidence in 'Rosy Glow' apples. Harvest maturity caused a significant difference in total browning incidence and was influenced by storage period ($P \leq 0.0001$). DB and RB varied significantly with different maturities at different storage periods ($P \leq 0.0001$ for both types of IB; Table 2). CO_2B was not influenced by any of the factors in the season 2015 but CB differed significantly with storage period only ($P \leq 0.011$; Table 2). A higher IB incidence was observed in fruit harvested at post-optimum maturity over all the storage periods compared to fruit harvested at optimum maturity, however, only significantly after the SL storage period (Fig. 5). Generally, the highest incidence of IB was observed after 7m CA + 6w RA + 7d SL with a maximum total browning of 32%, DB of 22% and RB of 10% in the fruit (Fig. 5). Further statistical analysis was not conducted on CB due to low incidence (5 fruit = 5.9%; Fig. 2B).

1.3.3 Effect of harvest maturity and storage period on fruit maturity and quality

1.3.3.1 Season one

Fruit maturity was influenced significantly by the interaction of harvest maturity and storage period ($Pr>F$ <0.0001 for all the parameters; Table 3). Fruit harvested at optimum maturity maintained a similar BC at all storage periods, except that optimum harvest fruit evaluated at 7m CA + 6w RA, was observed to have maintained a greener BC (2.7; Table 3). Post-optimally harvested fruit, on the other hand, exhibited a BC like optimum harvest fruit at harvest, as well as at 7m CA. However, post-optimum harvest fruit evaluated at 7m CA + 6w RA and 7m CA + 6w RA + 7d SL, was observed to have developed a more yellow BC (3.4 and 3.5 respectively; Table 3) comparative to optimum harvest fruit (2.7 and 3.0 respectively; Table 3) at same storage period. SB, even though was significantly different for the two maturities at harvest (13% for optimum harvested fruit and 54% for post optimum harvest fruit), was complete (100%) for fruit harvested at both maturities after the first storage period (7m CA). Firmness was highest (8.0 kg) at harvest in fruit harvested at optimum maturity but was not statistically different from firmness in post-optimum harvested fruit (7.8 kg; Table 3). Fruit harvested at optimum maturity, maintained firmness better than post-optimally harvested fruit at all storage periods in season one (Table 3). However, there was statistically no significant difference between the two maturities at harvest and after 7m CA (Table 3). It is worthy to note that firmness of fruit harvested post-optimum in this season fell below the internationally accepted standard for Pink Lady® export (7.0 kg) at 7m CA + 6w RA and 7m CA + 6w RA + 7d SL. TA was similar for both maturities at storage periods 7m CA + 6w RA and 7m CA + 6w RA + 7d SL, even though they differed significantly at harvest and at storage period, 7m CA (Table 3). Optimum maturity fruit recorded a significantly higher TA at harvest and at 7m CA (0.56 and 0.49 respectively; Table 3) comparative to post-optimum fruit evaluated at the same time (0.54 and 0.46 respectively; Table 3). Fruit harvested at post-optimum maturity maintained a similar TSS at all storage periods and was almost at the recommended 13% level at harvest (12.9%) except, at 7m CA + 6w RA + 7d SL when it scored a significantly higher TSS (13.4%; Table 3). Optimum harvest fruit, however, recorded a slightly lower TSS level of 11.9% at harvest but increased to 13.4% during storage at 7m CA + 6w RA + 7d SL (Table 3). It is worthy to note that TSS, for both harvest maturities, were similar (13.4%) at the end of season one, 7m CA + 6w RA + 7d SL. No greasiness was observed in this season

1.3.3.2 Season two

Results from season two showed a similar trend to season one ($P < 0.0001$ for BC, TA and TSS; $P < 0.003$ for SB and $P < 0.000$ for firmness; Table 4). Optimally harvested fruit kept a greener BC and differed significantly from fruit harvested post-optimally at each storage period (Table 4) even though BC was similar for both maturities at harvest. SB was significantly different for the two maturities at harvest (38% for optimum harvested fruit and 47% for post optimum harvest fruit) but, was complete (100%) for fruit harvested at both maturities by 7m CA. Firmness was highest (9.3 kg) in optimum maturity harvested fruit evaluated at harvest. The firmness of fruit harvested optimally was significantly higher than fruit harvested at post-optimum harvest maturity (8.7 kg). Fruit harvested at optimum maturity maintained similar firmness with increasing storage periods and was only significantly lower than the firmness at harvest. On the contrary, the firmness of post-optimally harvested fruit deteriorated with increasing storage period and was consistently significantly lower than firmness of optimally harvested fruit (Table 4). These differences notwithstanding, firmness of fruit harvested at both maturities remained within the acceptable international export standards for Pink Lady® (>7.0 kg). TA of fruit was observed to be on the decline from harvest to the end of the season for both maturities. Table 4 shows that TA of fruit harvested at optimum maturity recorded a significantly higher acidity (0.66%) at harvest and it differed significantly from that of post-optimum harvested fruit. Optimum harvested fruit consistently recorded a significantly higher TA than post-optimum fruit. Fruit TA level recorded was similar, 0.36 and 0.35% for optimum and post-optimum respectively, at 7m CA + 6w RA + 7d SL (Table 4). TSS of fruit did differ significantly between optimum and post-optimum maturity, right from harvest to 7m CA + 6w RA + 7d SL. Post-optimum harvest fruit consistently exhibited a significantly higher TSS at all the storage periods, reaching up to 14.1% at 7m CA + 6w RA + 7d SL. Fruit harvested at both maturities maintained TSS level above the recommended 13% level at all the storage periods except at harvest, where optimum maturity of fruit was slightly lower (12.7%; Table 4). No greasiness was recorded for this season.

1.3.4 Relation between various types of browning and maturity/quality parameters

1.3.4.1 Season one

RB and CB associated positively with BC, with correlation values as high as 0.941 and 0.918, respectively. Total browning and all the IB types, except CO_2B, exhibited a negative correlation of various strengths with TA, i.e. total browning = -0.932 and CB = -0.829 (Table 5). CO_2B has almost no relationship with TA. Furthermore, RB and CB negatively correlated with firmness. RB had the strongest correlation (-0.889), followed by CB (-0.840) (Table 5). TSS strongly positively correlated with SB (0.916; Table 5).

1.3.4.2 Season two

In the second season, total browning, DB and RB as well as CB were extremely positively associated with BC (0.998, 0.997, 0.995 and 0.981, respectively; Table 6). The correlation between TSS and total browning, DB, RB, and CB were highly positive, valued at 0.880, 0.899, 0.850 and 0.848, respectively (Table 6). TA strongly, positively correlated with firmness (0.905). Firmness also strongly correlated negatively with SB (-0.868; Table 6). TSS strongly positively correlated with BC (0.876) while TA highly, negatively correlated with SB (-0.928; Table 6).

1.4 DISCUSSION

'Cripps' Pink' IB has been studied extensively (James et al., 2007; Bergman et al., 2012; Majoni et al., 2013; Crouch et al., 2014; Butler, 2015; Moggia et al., 2015) and causes of the diverse types of the disorder (DB, RB, CO_2B, CB, and BB) have been reported. DB and RB have been reported to result from chilling injury and senescence and/or cellular stress due to improper gas permeability, respectively (Fidler et al., 1973; Bramlage et al., 1980; Mitcham et al., 2004; James et al., 2007). According to Moggia et al. (2015), BC of fruit at harvest can predict DB incidence and the incidence of RB can be predicted by firmness at harvest. James et al., (2007) also demonstrated that DB related to storage temperature and growing degree-days (GDD) above 10 °C ($GDD_{10\ °C}$), but RB to $GDD_{10\ °C}$, harvest maturity, storage temperature and other factors in 'Cripps' Pink' apples. RB evolves from several factors (crop load, mineral nutrition, GDD, etc.) which concludes on the basic factor being senescence with interactions from chilling injury and high CO_2 injury (James et al., 2007).

Post-optimum harvested fruit showed a higher susceptibility to total browning incidence, especially at 7m CA + 6w RA + 7d SL (Figs. 3 and 5) and browning correlation results showed that fruit with a low level of TA (season 1; TB and CB) and are more yellow (season 1: RB and CB; season 2: TB, DB, RB, CB) are less firm (Season 1: TB and CB) and have a high TSS (season 2: TB) are more likely to develop the IB incidence. These observations are in line with the findings of Moggia et al. (2015) which ascribed high IB susceptibility to low firmness or advanced maturity. According to Kays (1991), loss of firmness in the fruit due to ripening results from cell wall thinning actions caused by enzymes such as pectinase. TA (organic acids) act as intermediaries in the citric acid cycle, making them related and vital to the respiratory process (Jan and Rab, 2012) and the metabolic rate of the fruit (Clark et al., 2003).

DB made up a majority (>50%) of the total IB incidence observed in both seasons, while a similar percentage of fruits were affected with CB. Season two recorded an increase in %RB incidence while the incidence of CO_2B remains the same in both seasons (Figs. 1B and 2B). The variations observed in total IB from one storage period to the other and from season to season may be an indication that the IB disorder in apples is affected by a great number of factors (crop load, mineral nutrition, harvest maturity, storage temperature etc.) as has been well established (De Castro Hernandez et al., 2004; Crouch, 2010; Bergman et al., 2012; Majoni et al., 2013; Crouch et al., 2014, 2015).

1.4.1 Diffuse browning

First season results show higher DB percentage in optimum harvest fruit but DB was significantly higher in post-optimum harvest fruit and was progressive in SL in the second season (Table 1; Fig. 5). The observation made in the first season contradicts the reported harvest maturity influences alluding to the fact that post-optimum harvested fruit exhibit higher IB (Hurndall and Fourie, 2003; Brown et al., 2005; James, 2007; Majoni et al., 2013; Butler, 2015), but corroborates the findings of Toivonen (2008). According to Toivonen (2008) 'Granny Smith' fruit harvested two weeks before the optimal harvest date had a lower SB and showed a higher inclination towards cut surface browning as opposed to fruit harvested thereafter (one week or more after the first harvest). SB in the first season of this study was observed to be moderately positively correlated to IB, but this correlation was not statistically different. Park et al. (1993) reported that 'McIntosh' apple fruit harvested earlier (i.e. before ethylene climacteric), recorded a significantly higher resistance to gas diffusion in the flesh and this phenomenon, according to Majoni (2012), is capable of rendering fruit susceptible to IB, especially RB. Hurndall and Fourie (2003) recommended a SB range of 15-40% for optimum harvested fruit, however, the average SB at harvest for the optimum harvest in the first season of this trial was 13% (Table 3), which is below the minimum (15%) recommended level. It is possible that optimum harvested fruit was harvested too early and this may have caused the flesh of the fruit to pose a high resistance to gas diffusion, hence the high IB percentage observed. Alternatively, at this stage the fruit antioxidant levels were too low to resist extensive cold storage which is the case in superficial scald development in 'Granny Smith' (Toivonen, 2008). There is however no specific work done in this regard on 'Cripps Pink' and therefore no literature to support or debunk this assertion. It is recommended that further investigation should be carried out in quest of more insight into the possible physiological processes leading to high IB development by fruit harvested at a SB percentage less than the 15% recommended by Hurndall and Fourie (2003). Average SB for optimum harvested fruit in the second season was 38% (Table 4) and within the recommended rate which may affirm why the second season results were in line with reports published earlier (Bergman et al., 2012; Majoni et al., 2013; Crouch et al., 2014).

This study, in line with findings from other South African studies (Majoni, 2012; Butler, 2015) where DB was reported to be progressive with period of storage (increased with advancing storage period) in 'Cripps' Pink' apples, revealed that DB in 'Rosy Glow' may be progressive with advancing storage and may be observed more during the SL storage period. The results also confirm the influence of harvest maturity on diffuse browning in both seasons as reported by other studies (Hurndall and Fourie, 2003; East et al., 2004; James, 2007; James and Jobling, 2008; Fig. 5). The extreme increase in IB from 7m CA + 6w RA to after shelf life indicates that the re-activation of the fruit metabolism after long term storage leads to the appearance of IB. IB found after shelf life is, therefore, a symptom of damage caused during long term storage. It can therefore be confirmed, based on the results from the second season, that fruit harvested optimum, stored better (i.e., developed less diffuse browning) than post-optimum harvested fruit.

It is worthy to note that firmness and BC at harvest was reported to affect RB and DB in 'Cripps' Pink' apples in Chile (Moggia et al., 2015). It is clear from this data that, the actual harvest maturities were sufficiently different (SB, TSS, and firmness) to have tested the contrast of harvest maturity. Fruit with yellower BC and higher TSS values, were found to be more susceptible to DB. According to James (2007) and James and Jobling (2009) high SB, TSS and more yellow BC are indicators of advanced maturity which is mostly associated with post-optimum maturity. These factors enhance and/or are as a result of high respiration rates due to the climacteric rise of ethylene and also possibly metabolic degradation (Kays, 1991). Fruit harvested post-optimum may have suffered more DB, compared to optimum harvested fruit especially in the second season, due to higher respiration rates .

1.4.2 Radial browning

Harvest maturity affected the incidence of RB in both seasons, agreeing to what has been reported in previous studies (Brown et al., 2005; James, 2007; Bergman et al., 2012; Crouch et al., 2014; Moggia et al., 2015). Results show that, harvest maturity interacted with storage period such that, the incidence of RB in fruit harvested post-optimum progressed with storage period from 2% at 7m CA + 6w RA to 10 % at 7m CA + 6w RA + 7d SL in the second season of this study. This is contrary to reports that RB is not progressive in 'Cripps' Pink' apples (Crouch et al., 2014). Post-optimum fruit exhibited a significantly higher percentage of the RB during SL (Fig. 5), declining firmness and high %TSS at 7m CA + 6w RA and 7m CA + 6w RA + 7d SL (Table 4). This could be possibly attributed to increased respiration rates due to senescence which, according to Kays (1991), can lead to the colapse of cells due to the thinning of cell walls resulting in the decrease in firmness. This observation is confirmed by existing literature on 'Cripps' Pink' flesh browning research (Lau, 1998; Hurndall and Fourie, 2003; James, 2007; Crouch et al., 2014; Moggia et al., 2015).

According to Butler (2015), RB is influenced by many factors including senescent breakdown (irreversible deterioration, during or after long storage, where cells lose their structure and function; Hodges, 2003), while Moggia et al. (2015) associated RB with growing degree hours (GDH), firmness, as well as BC at harvest. Other studies have associated RB with many other factors such as chilling injury and CO_2 injury (Fidler et al., 1973; Bramlage et al., 1980; Mitcham et al., 2004), which is an indication that RB may result from a complex combination of factors. All these factors may have, in one way or the other, interacted with harvest maturity to make RB progressive in the second season, contradicting the studies mentioned earlier. However, also worthy to note is that the second season had a higher level of RB, which possibly indicates that the previous season and studies did not have sufficient amounts of RB in order to show its progressive nature. It has well been confirmed in this study that harvest maturity plays an important role in influencing 'Rosy Glow' RB and that it may be progressive.

1.4.3 Carbon dioxide and combination browning

CO_2B observed in both seasons was very low (1 fruit in each season; 2.4% and 1.2% for seasons 1 and 2 respectively) and was also not significantly influenced by any of the other factors (Tables 1 and 2). The incidence of combination browning was also low in both seasons (7 fruit = 16.7% and 5 fruit = 5.9% for season one and two respectively), for which reason multiple comparison analysis could not be performed on the data. However, CB was influenced by the interaction of harvest maturity and storage period in the first season, but storage period alone in the second season. In South Africa, CB has been reported to be made up of RB and DB in same 'Cripps' Pink' apple fruit (Butler, 2015). Butler (2015) reported that CB in 'Cripps' Pink' apple was seasonal, progressive in SL and may get worse when fruits are stored for longer storage periods at cold storage. They also found that other preharvest factors such as type of soil in the orchard may affect the susceptibility of fruit to CB disorder in 'Cripps' Pink'. Butler (2015) also stated that the incidence of CB may arise in the presence of factors that render a fruit susceptible to both RB and DB. The Chilean version of CB was reported by Moggia et al., (2015) as mixed IB in 'Cripps' Pink'. They hypothesised that it is more closely related to DB and that it is associated with BC and/or firmness at harvest. Moggia et al. (2015) however, recommended that investigations be made into the ethylene and nutritional content of both fruit and trees for more insight into the causes of this type of browning. Due to the low incidence of this type of browning in this study, no conclusions can be drawn as to the specifics of the harvest maturity effect observed.

1.5 CONCLUSION

The 'Rosy Glow' apple cultivar is susceptible to all types of IB. However, no BB was noted in this study. The incidence of IB in 'Rosy Glow' may be mitigated by harvesting fruit at optimum harvest maturity (30–40% SB) as fruit harvested below or above the optimum maturity is prone to the development of IB. The progressing of RB in the second season needs to be investigated further to determine whether RB in 'Rosy Glow' may be progressive. The difference in browning percentages, from one season to the other, is also acknowledged for which it is recommended that seasonal variations, orchard as well as pre-harvest influences of the IB disorder in 'Rosy Glow' should be investigated.

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1.7 TABLES AND FIGURES

Table 1: P-values indicating significance at the 0.05% level on percentage of the type of browning of 'Rosy Glow' apples for harvest maturity, storage period and the interaction between harvest maturity and storage period. Fruit were harvested at two harvest maturities from Glen Fruin farms in the Elgin region of the Western Cape in the 2014 season.

Source of variation	Total browning	Diffuse browning	Radial browning	Carbon dioxide browning	Combination browning
			Pr>F		
Storage period	0.065	0.288	0.120	0.375	0.111
Harvest Maturity	0.766	0.047	0.044	0.322	0.064
Storage period*Harvest maturity	0.026	0.288	0.120	0.375	0.024

Table 2: P-values indicating significance at the 0.05% level on the percentage of variation type of browning of ‘Rosy Glow’ apples for harvest maturity, storage period and the interaction between harvest maturity and storage period. Fruit were harvested at two harvest maturities from Damar farms in the Vyeboom region of the Western Cape in the 2015 season.

Source of variation	Total browning	Diffuse browning	Radial browning	Carbon dioxide browning	Combination browning
			Pr>F		
Storage period	< 0.0001	< 0.0001	< 0.0001	0.395	0.011
Harvest Maturity	< 0.0001	< 0.0001	0.0001	0.319	0.240
Storage period*Harvest Maturity	< 0.0001	< 0.0001	< 0.0001	0.395	0.248

Table 3: Maturity indices of 'Rosy Glow' measured for two harvest maturities at storage periods: at harvest, after 7 months of controlled atmosphere (CA) storage at -0.5 °C, and after an additional 6 weeks of regular atmosphere (RA) storage simulating the shipping period and 7 days of shelf-life (SL) storage at ambient temperature (≈20 °C). Fruit were harvested at two harvest maturities from Glen Fruin farms in the Elgin region of the Western Cape in the 2014 season.

Storage period	Harvest Maturity	Background colour (chart index)	Starch breakdown (SB; %)	Firmness (kg)[a]	Titratable acidity (TA; %)	Total soluble solids (TSS; %)
At Harvest	Optimum	2.9 b	13 c	8.0 a	0.56 a	11.9 c
	Post-optimum	3.0 b	54 b	7.8 ab	0.54 b	12.9 b
7m CA	Optimum	3.0 b	100 a	7.7 b	0.49 c	12.8 b
	Post-optimum	3.0 b	100 a	7.6 bc	0.46 d	13.0 b
7m CA + 6w RA	Optimum	2.7 c	100 a	7.8 ab	0.44 de	12.9 b
	Post-optimum	3.4 a	100 a	6.2 d	0.43 e	13.0 b
7m CA + 6w RA + 7d SL	Optimum	3.0 b	100 a	7.3 c	0.38 f	13.4 a
	Post-optimum	3.5 a	100 a	6.2 d	0.37 f	13.4 a
Source of variation			Pr>F			
Storage period		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Harvest Maturity		< 0.0001	< 0.0001	< 0.0001	0.149	0.861
Storage period*Maturity		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a]measured with an 11.1 mm penetrometer tip;

Table 4: Maturity indices of 'Rosy Glow' measured for two harvest maturities at storage periods: at harvest, after 7 months of controlled atmosphere (CA) storage at -0.5 °C, and after an additional 6 weeks of regular atmosphere (RA) storage simulating the shipping period and 7 days of shelf-life (SL) storage at ambient temperature (≈20 °C). Fruit were harvested at two harvest maturities from Damar farms in the Vyeboom region of the Western Cape in the 2015 season.

Storage period	Harvest Maturity	Background Colour (Chart index)	Starch breakdown (SB; %)	Firmness (kg)[a]	Titratable acidity (TA; %)	Total soluble solids (TSS; %)
At Harvest	Optimum	3.1e	38c	9.3a	0.66a	12.7f
	Post-optimum	3.1e	47b	8.7b	0.64b	13.3e
7 months CA	Optimum	3.2d	100a	8.4c	0.49c	13.4de
	Post-optimum	3.36c	100a	7.7d	0.46d	13.7bc
7m CA + 6wRA	Optimum	3.2d	100a	8.3c	0.47c	13.5cde
	Post-optimum	3.5b	100a	7.6d	0.42d	13.9ab
7m CA + 6w RA + 7d SL	Optimum	3.5b	100a	8.3c	0.36e	13.6cd
	Post-optimum	4.0a	100a	7.1e	0.35e	14.1a
Source of variation	Pr>F					
Storage period		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Harvest Maturity		< 0.0001	0.027	< 0.0001	< 0.0001	< 0.0001
Storage period*Maturity		< 0.0001	0.003	0.000	< 0.0001	< 0.0001

[a]measured with an 11.1 mm penetrometer tip;

Table 5: Pearson correlation r-value (top) and corresponding p-value (bottom) of the correlation between percentage of total browning, browning types and quality parameters at the 0.05% significance level of 'Rosy Glow' apples. Fruit were harvested at two harvest maturities from Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season.

Variables	Background Colour (Chart Index)	Starch breakdown (%)	Firmness (kg)	Total soluble solids (%)	Titratable acidity (%)	Total Browning (%)	Diffuse Browning (%)	Radial Browning (%)	Carbon dioxide Browning (%)	Combination Browning (%)
Background Colour (Chart index)	**1.000**	0.396	**-0.853**	0.062	-0.607	0.473	-0.019	**0.941**	0.061	**0.918**
Starch breakdown (%)		**1.000**	-0.681	**0.916**	**-0.853**	0.781	0.689	0.444	0.354	0.527
Firmness (kg)			**1.000**	-0.418	0.767	-0.521	-0.106	**-0.889**	0.106	**-0.840**
Total soluble solids (%)				**1.000**	-0.585	0.541	0.621	0.067	0.456	0.146
Titratable acidity (%)					**1.000**	**-0.932**	-0.702	-0.748	-0.071	**-0.829**
Total Browning (%)						**1.000**	**0.868**	0.584	0.297	0.731
Diffuse Browning (%)							**1.000**	0.111	0.393	0.298
Radial Browning (%)								**1.000**	-0.179	**0.976**
Carbon dioxide Browning (%)									**1.000**	-0.032
Combination Browning (%)										**1.000**
Background Colour (Chart index)	**0**	0.436	**0.031**	0.907	0.201	0.343	0.971	**0.005**	0.908	**0.010**
Starch breakdown (%)		**0**	0.137	**0.010**	**0.031**	0.066	0.130	0.378	0.492	0.283
Firmness (kg)			**0**	0.410	0.075	0.289	0.841	**0.018**	0.841	**0.036**
Total soluble solids (%)				**0**	0.222	0.268	0.189	0.899	0.363	0.782
Titratable acidity (%)					**0**	**0.007**	0.120	0.087	0.893	**0.041**
Total Browning (%)						**0**	**0.025**	0.223	0.567	0.099
Diffuse Browning (%)							**0**	0.835	0.441	0.567
Radial Browning (%)								**0**	0.734	**0.001**
Carbon dioxide Browning (%)									**0**	0.953
Combination Browning (%)										**0**

Values in bold are different from 0 with a significance level alpha=0.05

Table 6: Pearson correlation r-value (top) and corresponding p-value (bottom) of the correlation between percentage of total browning, browning types and quality parameters at the 0.05% significance level of 'Rosy Glow' apples. Fruit were harvested at two harvest maturities from Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

Variables	Background Colour (Chart Index)	Starch breakdown (%)	Firmness (kg)	Total soluble solids (%)	Titratable acidity (%)	Total Browning (%)	Diffuse Browning (%)	Radial Browning (%)	Carbon dioxide Browning (%)	Combination Browning (%)
Background Colour (Chart index)	**1**	0.425	-0.707	**0.876**	-0.717	**0.998**	**0.997**	**0.995**	-0.060	**0.981**
Starch breakdown (%)		**1**	**-0.868**	0.277	**-0.928**	0.379	0.383	0.363	0.320	0.331
Firmness (kg)			**1**	-0.612	**0.905**	-0.663	-0.670	-0.642	-0.396	-0.580
Total soluble solids (%)				**1**	-0.518	**0.880**	**0.899**	**0.850**	-0.284	**0.848**
Titratable acidity (%)					**1**	-0.684	-0.683	-0.676	-0.186	-0.654
Total Browning (%)						**1**	**0.999**	**0.998**	-0.110	**0.990**
Diffuse Browning (%)							**1**	**0.994**	-0.128	**0.988**
Radial Browning (%)								**1**	-0.095	**0.992**
Carbon dioxide Browning (%)									**1**	-0.210
Combination Browning (%)										**1**
Background Colour (Chart index)	**0**	0.401	0.116	**0.022**	0.109	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.910	**0.001**
Starch breakdown (%)		**0**	**0.025**	0.596	**0.008**	0.458	0.454	0.479	0.536	0.522
Firmness (kg)			**0**	0.196	**0.013**	0.151	0.146	0.169	0.438	0.227
Total soluble solids (%)				**0**	0.292	**0.021**	**0.015**	**0.032**	0.586	**0.033**
Titratable acidity (%)					**0**	0.134	0.135	0.141	0.724	0.159
Total Browning (%)						**0**	**< 0.0001**	**< 0.0001**	0.835	**0.000**
Diffuse Browning (%)							**0**	**< 0.0001**	0.809	**0.000**
Radial Browning (%)								**0**	0.857	**< 0.0001**
Carbon dioxide Browning (%)									**0**	0.690
Combination Browning (%)										**0**

Values in bold are different from 0 with a significance level alpha=0.05

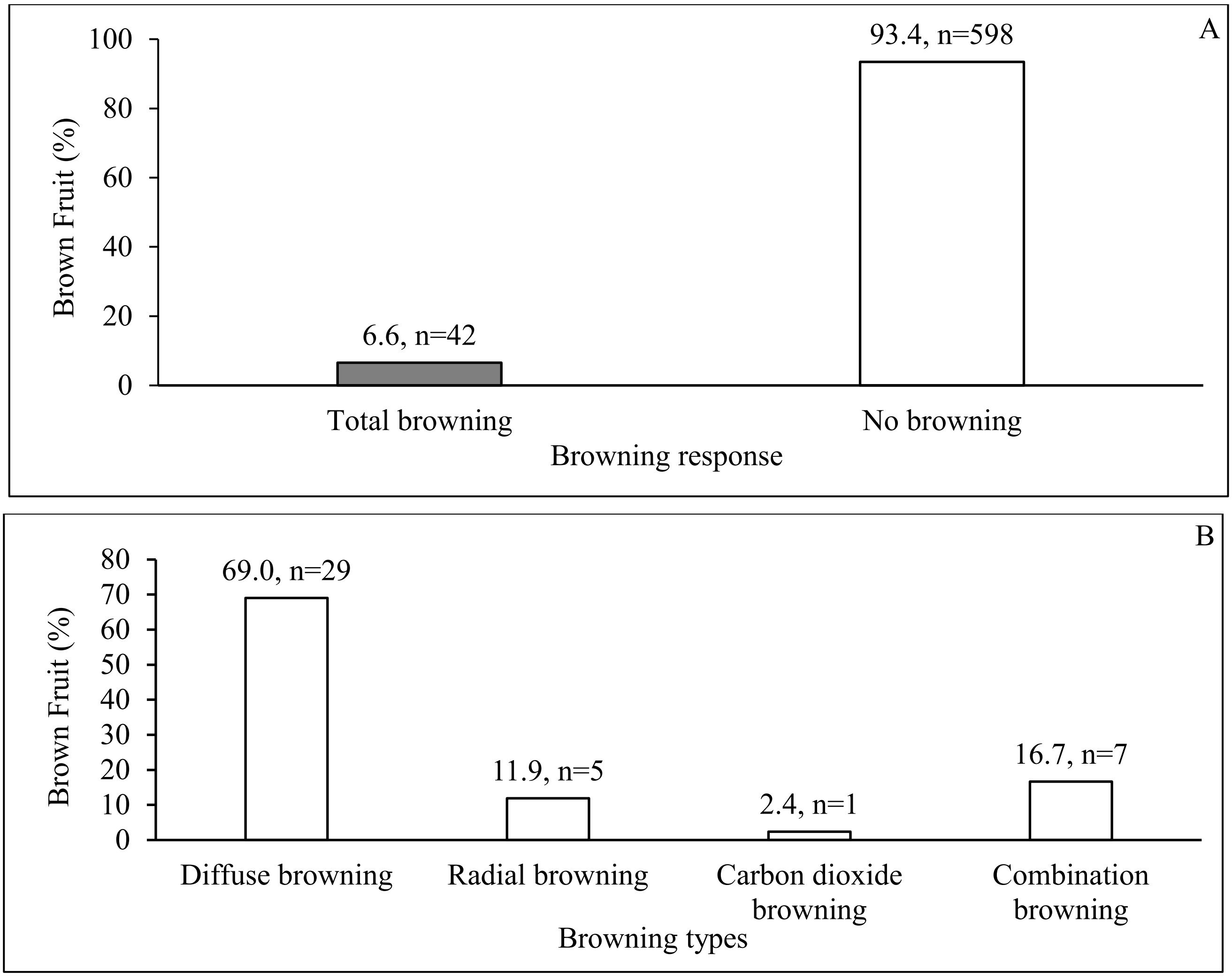

Figure 1: Browning incidence (%) in number of fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples that were harvested in 2014 at two harvest maturities. Measurements were made on fruit that were stored for 7 months in controlled atmosphere, followed by 6 weeks in regular atmosphere at -0.5 °C and a 7-day shelf-life period at ambient temperature as a total for both maturities. Fruit were harvested from Glen Fruin farms in the Elgin region of the Western Cape in the 2014 season.

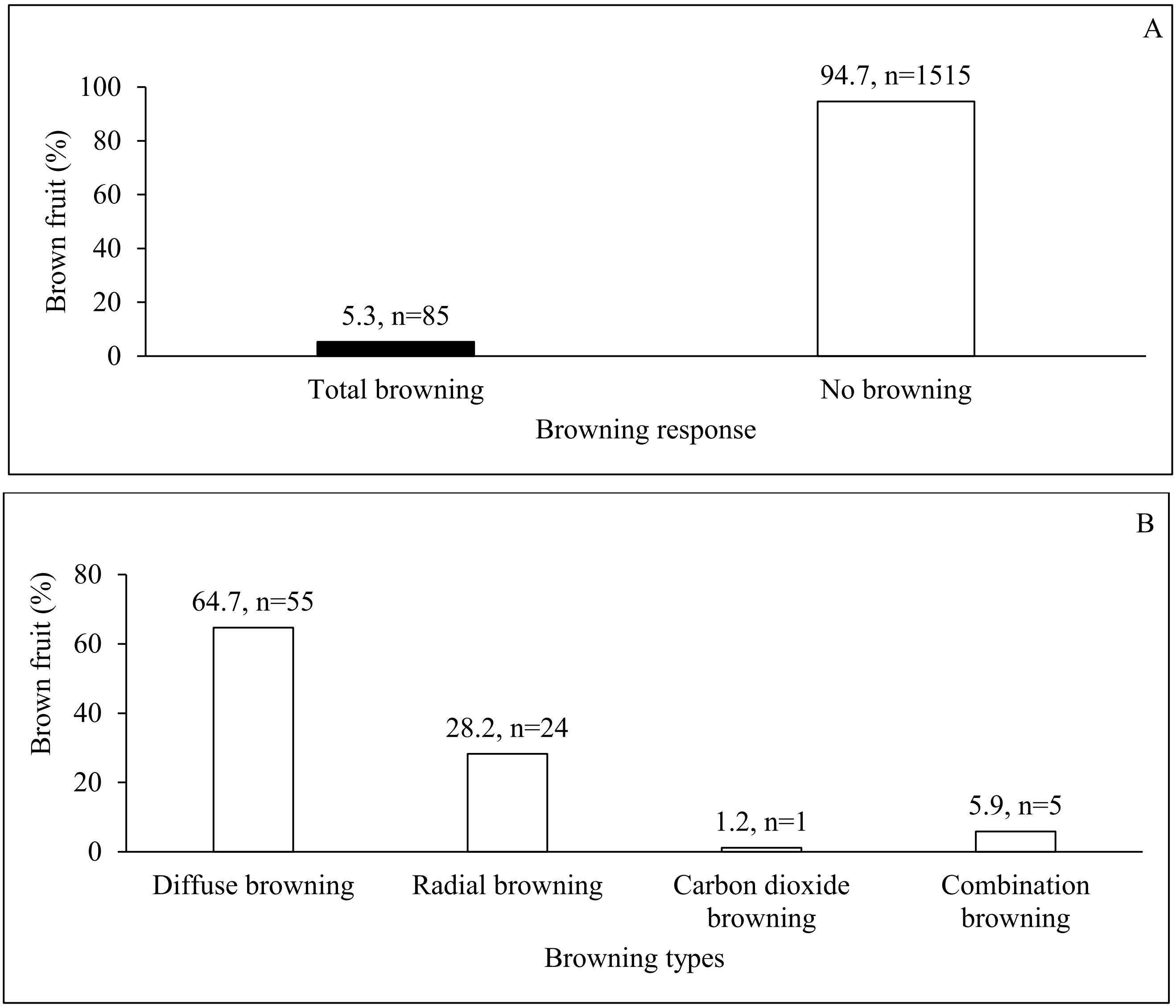

Figure 2: Browning incidence (%) in number of fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples that were harvested in 2015 at two harvest maturities. Measurements were made on fruit that were stored for 7 months in controlled atmosphere, followed by 6 weeks in regular atmosphere at -0.5 °C and a 7-day shelf-life period at ambient temperature. Fruit were harvested from Damar farms in the Vyeboom area of the Western Cape in the 2015 season.

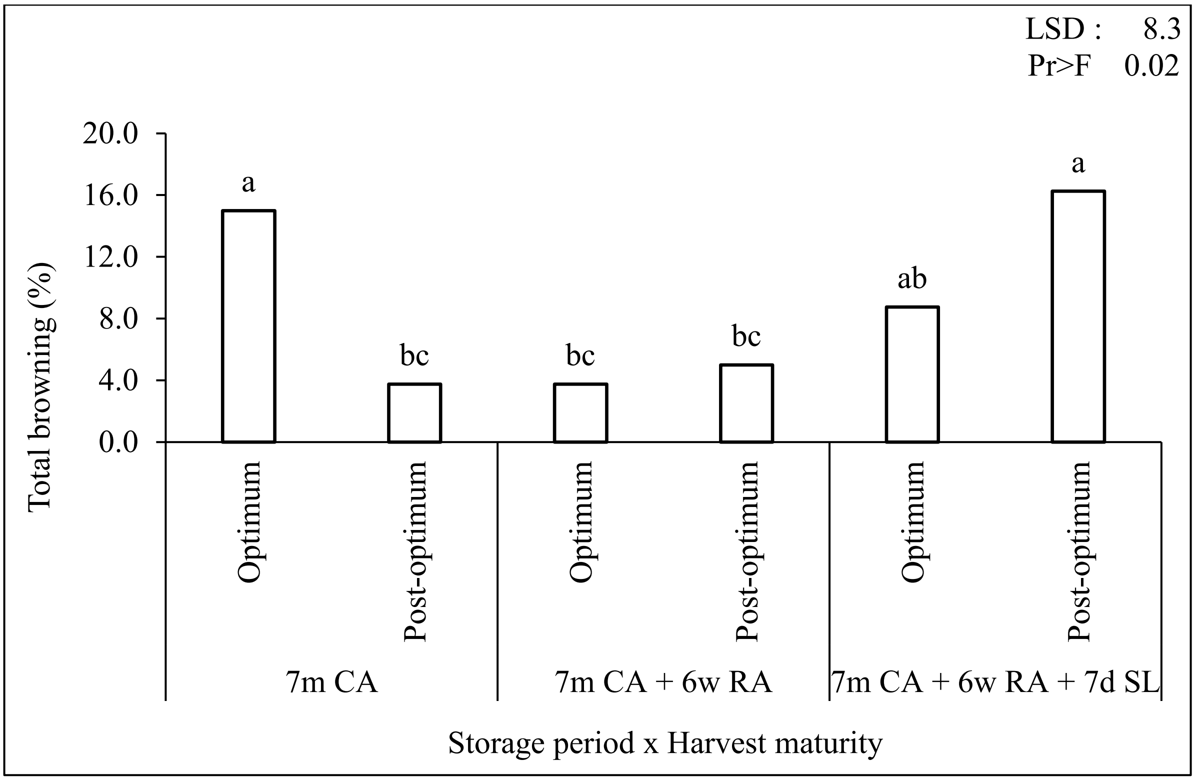

Figure 3: The effects of maturity and storage period on percentage total browning of 'Rosy Glow' apples. Fruit were harvested from Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (CA), followed by 6 weeks in regular atmosphere (RA) at -0.5 °C and a 7-day shelf-life (SL) period at ambient temperature.

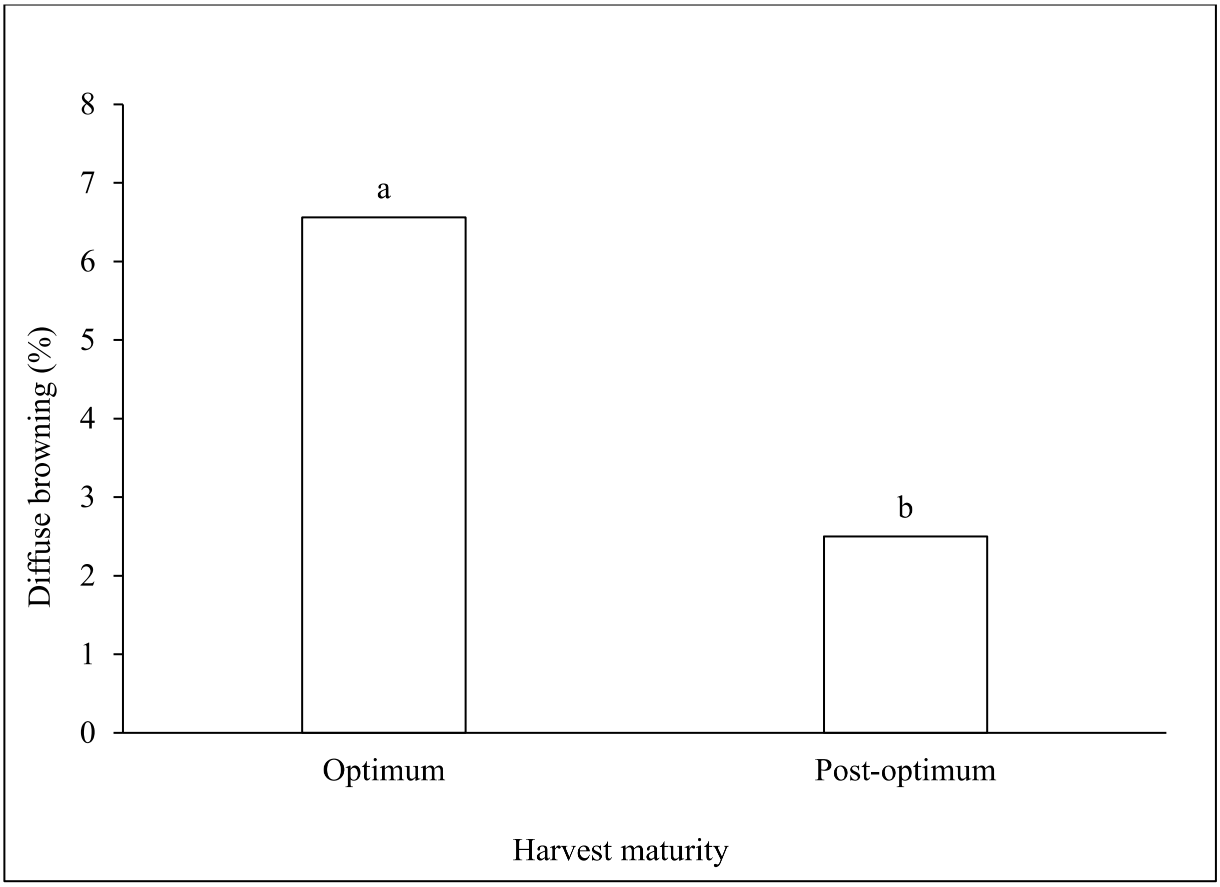

Figure 4: Percentage of diffuse browning for two harvest maturity comparisons of 'Rosy Glow' apples. Fruit were harvested from Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season.

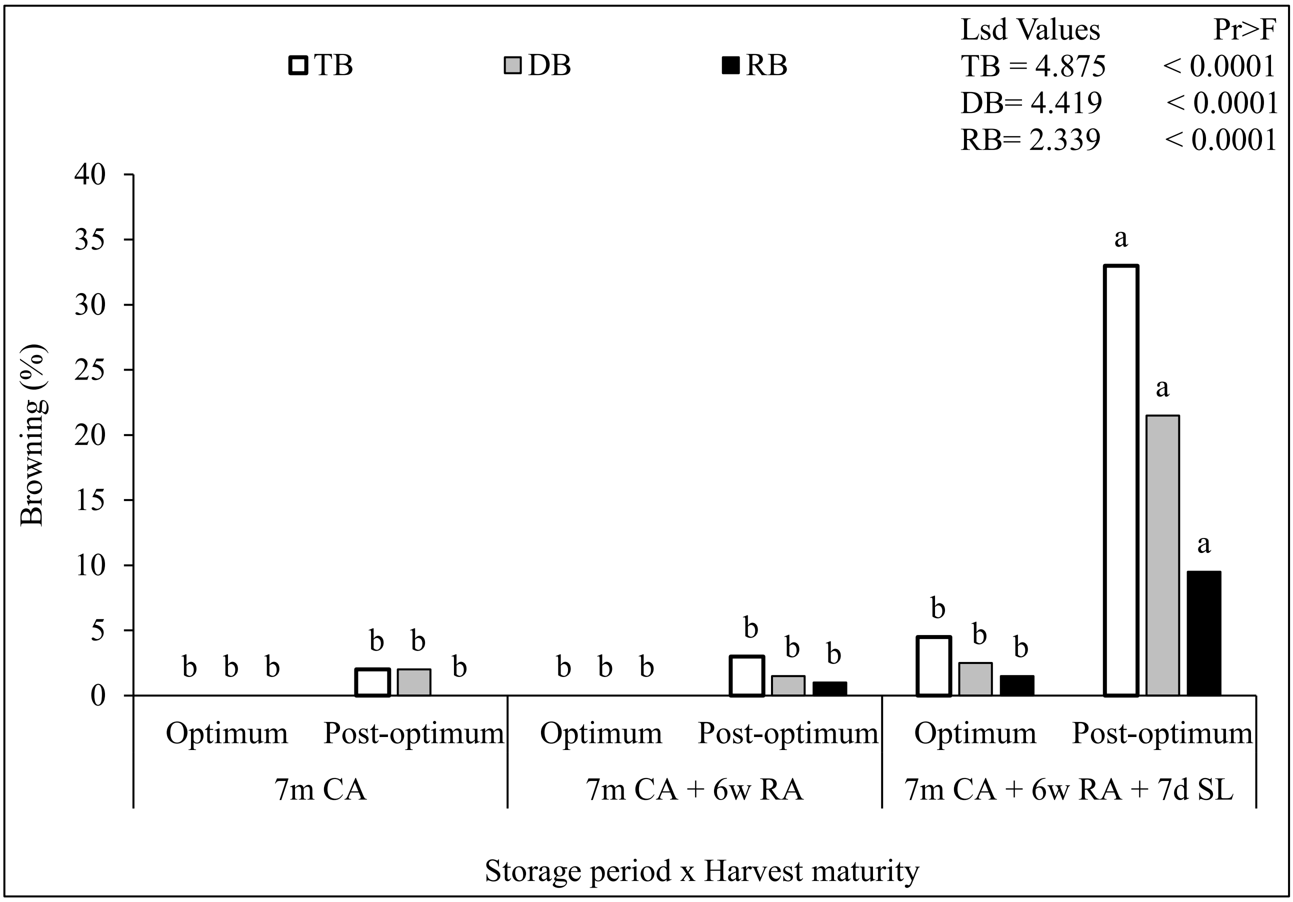

Figure 5: Changes in the percentage total browning (TB), diffuse browning (DB) and radial browning (RB) by interaction between storage period and maturity comparison of 'Rosy Glow' apples. Fruit were harvested from Damar farm in the Vyeboom region of the Western Cape in the 2015 season. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (CA), followed by 6 weeks in regular atmosphere (RA) at -0.5 °C and a 7-day shelf-life (SL) period at ambient temperature.

Chapter Three: PAPER TWO

Internal browning and quality of 'Rosy Glow' apples affected by storage time, 1-MCP and storage temperature.

ABSTRACT

'Cripps' Pink' apples are prone to internal browning (IB) after long term storage. Storage regimes for 'Rosy Glow' a sport of 'Cripps' Pink' should therefore be investigated. This study investigated the effect of 1-methylcyclopropene (1-MCP) and storage temperature on the quality, as well as the IB susceptibility of 'Rosy Glow' apples after long term storage in controlled atmosphere (CA). 'Rosy Glow' apples were harvested at a commercial harvest with a starch breakdown (SB) of 11%, a background colour (BC) of 2.7, a firmness of 9.1 kg, total soluble solids (TSS) of 11% and titratable acidity (TA) of 0.67% in the first season but at 39% SB, 3.2 BC, 9.2 kg firmness, 13% TSS, and 0.63% TA in the second season and treated with or without 1-MCP. Both treatments were stored at temperatures of -0.5 °C and 2 °C for up to 7 months in CA (1% CO_2 and 1.5% O_2), followed by 6 weeks in regular atmosphere (RA) simulating shipping and stock rolling. Fruit were ripened for 7 days at ≈20 °C (shelf life; SL). Fruit were assessed for quality based on BC, firmness, TSS, TA, greasiness, SB, and IB after 3, 5 and 7 months in CA, all followed by RA and SL evaluations over two seasons. Diffuse browning (DB) was the dominant flesh browning type and was first observed after 5 months in CA and 6 weeks in RA. 1-MCP treated fruit had a better quality and lower internal browning compared to untreated control fruit (No 1-MCP). Higher levels of DB were observed in fruit stored at -0.5 °C in the first season, while fruit stored at 2 °C exhibited more DB in the second season. DB was, however, not statistically influenced by temperature in the first season. A smaller sample size and browning incidence in the first season may have resulted in this difference in these two seasons.

Keywords: 1-MCP, controlled atmosphere, diffuse browning, radial browning, *Malus domestica*, storage duration.

2.1 INTRODUCTION

Apples are climacteric fruit, which produce and respond to ethylene (Harada et al., 2000). This production and response to ethylene influences the quality and optimum storage of apples, including 'Rosy Glow'. It is well established that the regulation and control of the level of ethylene aids to ensure optimum storability of apples (Bramlage et al., 1980; Lau, 1990; Amiot et al., 1995; Fan et al., 1999a; Harada et al., 2000; Jobling, 2002; Andreotti et al., 2004; Moggia et al., 2009; Bergman et al., 2012; Majoni, 2012; Crouch et al., 2014).

The development of storage technologies (controlled atmosphere (CA), dynamic controlled atmosphere (DCA), and modified atmosphere (MA) storages systems) and chemical treatments (1-methylcyclopropene (1-MCP), aminoethoxyvinylglycine (AVG) etc.) reduce or delay the biosynthesis and action of ethylene before, during and after storage (Serek et al., 1994; Sisler et al., 1996; Sisler and Blankenship, 1996; Beaudry and Watkins, 2001; Sisler and Serek, 2003; Watkins, 2006a). 1-MCP was patented in 1996, authorized for use on ornamentals in 1999 and then further developed for use on edible horticultural produce (Sisler et al., 1996; Sisler and Serek, 1997, 2003; Watkins, 2006a). Watkins (2006a) indicated that 1-MCP binds to ethylene receptors in fruit and the response of fruit to ethylene is mitigated, if not prevented, thus ensuring prolonged and better storage. 1-MCP application within 7 days of harvest have been evaluated extensively on 'Cripps' Pink' apples. Reports state that 1-MCP aided the extension of 'Cripps' Pink' fruit shelf life (SL) quality after storage (Bergman et al., 2012; Majoni et al., 2013; Crouch et al., 2014).

Many other factors impact the quality and optimum storability of climacteric fruit. Storage temperature is reported to play the most important role in climacteric fruit storage due to its effect on respiration rate and ethylene production and, the activity of many related metabolites and therefore ripening (Li and Li, 2008). A lower storage temperature of fruit leads to a decreased fruit response to ethylene (Kays, 1991; Jobling and McGlasson, 1995). However, these studies also observed that beyond a certain threshold fruit tend to produce ethylene due to stress. Hurndall and Fourie (2003) recommended a storage temperature of -0.5 °C for 'Cripps' Pink' apple, while Majoni et al. (2013) hypothesised that, fruit SL may increase when fruit are treated with 1-MCP and stored at a higher temperature (>-0.5 °C), due to 'Cripps' Pink' being prone to chilling injury in the form of diffuse browning (DB) (James, 2007).

The aim of this study was to determine whether 2 °C could be used as a higher storage temperature for 'Rosy Glow', a branch sport of 'Cripps' Pink', in combination with 1-MCP to help maintain the fruit quality and reduce the internal browning (IB) in particular, DB after long-term CA storage.

2.2 MATERIALS AND METHODS

2.2.1 Fruit material

'Rosy Glow' apples were hand-picked from two commercial orchards, Glen Fruin (Block 20, 34°11'23.6"S 19°03'10.4"E) in the Elgin area and Damar (Block 16, S34'03.371" E0 91'06.866") in the Vyeboom area of the Western Cape in South Africa for season one (2014) and season two (2015), respectively. Fruit trees were grown and managed with current commercial practices and were grown on MM109 rootstocks. The Glen Fruin and Damar orchards were in their sixth and fourth leaf, respectively, at the time of this study (2014). Fruit were picked during the commercial harvest period at a starch breakdown (SB) of between 11% and 40%.

2.2.2 Fruit numbers and harvest specifications

A total of 1920 fruit were evaluated in the 2014 season and in the 2015 season, 4800 fruit were evaluated. Fruit were harvested at shoulder height on the outside of the tree canopy of one orchard row from trees with an even height and shape and transported in lug boxes to the Department of Horticultural Sciences, Stellenbosch University, Stellenbosch, South Africa. Maturity evaluations were done immediately after harvest and field heat was removed by storing remaining fruit at -0.5 °C for one day. On the following day, fruit were placed in CA containers for further storage after the fruit had reached the required temperatures.

2.2.3 Fruit treatment and storage

Fruit were divided into two equal parts according to two storage temperatures (A = -0.5 °C and B = 2 °C). One-half of the fruit from each temperature (A/2 and B/2) were treated with 1-MCP (SmartFresh™, at the commercially recommended rate) for 24 h, while the second half were left untreated (control, No 1-MCP). CA (1.5% O_2 and 1% CO_2) and regular atmosphere (RA) storage techniques were used to store fruit of the various treatments. Fruit of the respective temperatures were simultaneously stored for 3, 5 or 7 months in CA in Janny MT plastic bins with sealed lids ('Janny bins') at both temperatures (-0.5 °C and 2 °C). Fruit were taken out after each CA period and stored for further 6 weeks under RA conditions (simulating packing, shipping and stock rolling), still at respective temperatures (-0.5 °C and 2 °C). The Janny bins were 57 cm deep, 90 cm wide and 110 cm long, with the carrying capacity of 10 lug boxes each full of fruit. Each Janny bin was connected to a pulsing inlet for N_2, O_2 and

CO_2, as well as a gas overflow. The CO_2 and O_2 concentrations were kept constant at 1 and at 1.5%, respectively, by an automatic pulsing system (CA Janny bin Pulse Gas Control System**,** Gas at Site (PTY) LTD., South Africa). Fruit were thereafter ripened for 7 days at ≈20 °C simulating the SL and ripening period.

2.2.4 Fruit browning evaluation and description

At the end of each storage period, the number of fruit required for evaluation at that time was selected at random from the lot in storage. Individual fruit were cut above the equator closer to the stem end for IB assessment. IB was assessed based on the pattern of brown colour observed in the flesh of fruit, as well as the part of flesh affected as specified by (Jobling, 2002b) and Butler (2011). Browning type and presence of disorder were recorded for every individual fruit after the evaluation was conducted.

2.2.5 Experimental design

The experiment was a completely randomised design, analysed in a three-factorial analysis of variance (ANOVA) with 1-MCP, temperature and evaluation time as factors. Evaluation at each evaluation time consisted of six replicates of eight fruit per replicate in the 2014 season and twelve replicates of ten fruit in the 2015 season. Fruit were evaluated at ten evaluation times namely:

1. at harvest
2. after 3 months of storage in CA (1.5% O_2, 1% CO_2) at -0.5 °C or 2 °C (3m CA)
3. after 3m CA + 6 weeks of RA storage at -0.5 °C or 2 °C (3m CA + 6w RA)
4. after 3m CA + 6w RA + 7 days of SL at 20 °C (3m CA + 6w RA + 7d SL)
5. after 5 months of storage in CA (1.5% O_2, 1% CO_2) at -0.5 °C or 2 °C (5m CA)
6. after 5m CA + 6 weeks of RA storage at -0.5 °C or 2 °C (5m CA + 6w RA)
7. after 5m CA + 6w RA + 7 days of SL at 20 °C (5m CA + 6w RA + 7d SL)
8. after 7 months of storage in CA (1.5% O_2, 1% CO_2) at -0.5 °C or 2 °C (7m CA)
9. after 7m CA + 6 weeks RA storage at -0.5 °C or 2 °C (7m CA + 6w RA)
10. after 7m CA + 6w RA + 7 days of SL at 20 °C (7m CA + 6w RA + 7d SL)

2.2.6 Physicochemical analyses

Maturity and quality indices were conducted after harvest, CA, RA and SL storage. All assessments were done on each fruit for all replications unless otherwise stated.

Firmness

A 4-in-1 Fruit Texture Analyzer (Güss Manufacturing, Strand, South Africa, model number GS-20 and serial number 2007-FTA-409) made up of a motorized penetrometer, a scale (maximum load capacity of 3 kg), a DMF (diameter, mass and firmness) apparatus for determining diameter and an electronic calliper, was used to assess firmness. Fruit firmness (kg) was measured as the maximum force necessary to push an 11.1 mm diameter probe into the flesh after peeling two equatorial sides with a potato peeler.

Background colour (BC)

BC was measured using the colour chart index for apples and pears (Unifruco Research Services [Pty] Ltd., 1991) with a scale ranging between 0.5–5.0 (0.5 = dark green and 5.0 = deep yellow). The colour chart index is widely used in industry to note the colour transition from a green to yellow ground colour change with ripening.

Total soluble solids (TSS) and titratable acidity (TA)

Equatorial apple slices representing all sides from the fruit (seeds punched out with a metallic 2.5 cm diameter cylinder) were placed in an electric juicer (AEG Electrolux, Type JE-107 no. 91100085/ PNC 950075206, P.R.C) and the juice was used to determine the TSS and TA pooled per replicate. TSS was measured using a calibrated hand-held refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). TA was measured using an automated titrator (Titrino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland) by titrating 10 g of juice from each apple sample with 0.1 M NaOH to a pH of 8.2. TSS was expressed as % Brix while TA was expressed as % malic acid. Six pooled sample replicates from a composite of eight fruits per replicate in 2014 and 12 pooled sample replicates from a composite of 10 fruits per replicate in 2015 were recorded.

Starch breakdown (SB)

Fruit were cut in half equatorially and one half was used for the assessment of SB percentage using the iodine test. The cut surface of the fruit was covered with an iodine (0.5 M potassium iodide) solution and then left for 2 min. SB % (unstained area) was determined using the starch conversion chart (Unifruco Research Services, South Africa) with a scale ranging from 0 to 100% (0% = totally stained and 100% = totally unstained).

Greasiness

The ease with which whole fruit will turn while firmly held without slipping and making a sound was used to determine the presence and level of greasiness of fruit skin. Fruits were then scored on a scale of 0–5, with 0 coding no greasiness and 5 coding highly greasy.

2.2.7 Statistical analysis

Physicochemical analyses data were subjected to ANOVA using SAS Enterprise guide version 5.1 (SAS Institute, 2006, Cary, NC 27513, USA) and XLSTAT version 2015.5.01.23373 (Addinsoft 1995-2016 XLSTAT). Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means. Pearson's correlation coefficient was generated and used to establish correlations between 1-MCP, temperature and/or quality parameters to browning development at harvest and after storage.

2.3 RESULTS

2.3.1 Prevalence of browning incidence and browning types

A. Season one (2014)

IB was observed in a total of 42 (2.2%) out of 1920 fruit (Fig. 1A). Although this percentage seems low it is commercially significant since the maximum allowable incidence is <1%, that is stipulated by the International Pink Lady® Association (IPLA) standards. The first incidence was observed at evaluation time 5m CA + 6w RA (12 fruit; Table 1). Figure 1B demonstrates that three types of the IB disorders were observed namely DB (Fig. 2A), radial browning (RB; Fig. 2B) and CO_2 browning (CO_2B; Fig. 2C), the most common being DB (30 fruit = 71%; Fig. 1B). RB was present in 26% (11 fruit) of the fruit and only one fruit was observed with CO_2 B (Fig. 1B, 2C). There was no incidence of combination browning (CB), a combination of DB and RB (Fig. 2E).

B. Season two (2015)

A total of 84 (1.8%) out of 4800 fruit (Fig. 3A) were affected by IB in the 2015 season, which is slightly lower than the observation in the first season. The first incidence of IB in the 2015 season was also at evaluation time 5m CA + 6w RA (14 fruit; Table 1). Three types of the IB disorders were observed namely DB, CO_2B and CB, the most common again being DB (65 fruit = 77%: Fig. 2A, Fig. 3B). CO_2B (Fig. 2C) was present in 16% (13 fruit; Fig. 3B) of the fruit and two types of CB (RB+CO_2B and DB+RB) (Fig. 2D, E) was observed to have affected 6 fruit, which contributed 7% of the total number of fruit that suffered from IB (Fig. 3B). There was no RB visible in this season, but only part of CB (DB+RB) in 5.8% of fruit affected by IB.

2.3.2 Effect of 1-MCP, temperature and evaluation time on total browning and browning types

A. Season one (2014)

Temperature by 1-MCP treatment interactions as well as evaluation time by 1-MCP treatment interactions were significant for total browning ($P \leq 0.05$) (Table 2). A higher incidence of total browning was found in the 1-MCP untreated control fruit (No 1-MCP) ($P \leq 0.05$) compared to 1-MCP treated fruit for evaluation times 5m CA + 6w RA and 7m CA + 6w RA (Fig. 4). Figure 5 shows that the overall incidence of IB in 1-MCP untreated control fruit (No 1-MCP) was higher than for 1-MCP treated fruit and significantly so at -0.5 °C. At 2 °C IB was too low to differ significantly between treatments. 1-MCP treated fruit did not differ in IB at -0.5 °C and 2 °C (Fig. 5). 1-MCP untreated control fruit (No 1-MCP) exhibited a significantly ($P \leq 0.05$) higher level of the IB incidence at -0.5 °C than at 2 °C (Fig. 5). Thus, the incidence of IB was significantly influenced by storage temperature, as a higher browning incidence occurred at lower temperatures.

DB and RB were first observed at evaluation time 5m CA + 6w RA in 1-MCP untreated control fruit (No 1-MCP), with RB being the dominant browning type at that time (Table 3). Table 2 indicates that DB was significantly influenced by the evaluation time and 1-MCP treatment interaction. Multiple comparison conducted revealed that, 1-MCP untreated control fruit (No 1-MCP) exhibited a significantly higher DB (17%) at evaluation time 7m CA + 6w RA, compared to the 1-MCP treated fruit (2%) at the same evaluation time (Fig. 4). There was no significant difference between 1-MCP treated fruit and 1-MCP untreated control fruit (No 1-MCP) at any other evaluation times with regards to DB (Fig. 4).

RB occurred mostly at evaluation time 5m CA + 6w RA in 1-MCP untreated control fruit (No 1-MCP; Table 3). According to Table 2, its occurrence was different at different temperature levels, 1-MCP treatments and evaluation times (i.e. Pr>F of Temp*1-MCP*Time <0.0001). Further statistical analysis could not be conducted for RB due to the low total incidence (11 fruit). It can be noted that the highest incidence of RB (9.4%; Table 3) was at evaluation time 5m CA + 6w RA of 1-MCP untreated control fruit (No 1-MCP).

B. Season two (2015)

For total browning incidence the three-way interaction of storage temperature, 1-MCP treatment and evaluation time was significantly different (Table 4). Figure 6 shows that total browning was highest at evaluation time 7m CA + 6w RA + 7d SL in 1-MCP untreated control fruit (No 1-MCP) stored at 2 °C. This was significantly higher than the total browning at all other evaluation times. Total browning was significantly higher in 1-MCP untreated control fruit (No 1-MCP) stored at 2 °C at the onset of browning (5m CA + 6w RA) but, at evaluation time 7m CA + 6w RA, 1-MCP untreated control fruit (No 1-MCP) stored at -0.5 °C exhibited higher total browning. Total browning was high in both 1-MCP treated and 1-MCP untreated control fruit (No 1-MCP) stored at both temperatures at evaluation time 7m CA + 6w RA + 7d SL, except that, 1-MCP treated fruit stored at -0.5 °C exhibited no IB at this same evaluation time (Fig. 6). However, 1-MCP untreated control fruit (No 1-MCP) stored at 2 °C exhibited a significantly higher total browning incidence, compared to all other treatment combinations during evaluation time 7m CA + 6w RA + 7d SL. On average, total browning occurrence was higher in 1-MCP untreated control fruit (No 1-MCP; Fig. 6).

DB dominated the second season and exceeded the maximum allowable incidence (<1%) that is stipulated by the International Pink Lady® Association (IPLA) standards. Figure 3B shows that 65 fruit were affected by DB, which is 1.4% of the total number of fruit evaluated in the season. DB disorder was highest in 1-MCP untreated control fruit (No 1-MCP) stored at 2 °C during evaluation time 7m CA + 6w RA + 7d SL and it was significantly higher than DB at all other evaluation times (Fig. 6). At 5m CA + 6w RA no DB was observed in the 1-MCP untreated control fruit (No 1-MCP) and 1-MCP treated fruit stored at -0.5 °C. 1-MCP untreated control fruit (No 1-MCP) stored at -0.5 °C developed a significantly higher DB during 7m CA + 6w RA, compared to 1-MCP treated fruit at -0.5 °C and 2 °C. 1-MCP treated fruit exhibited DB in at 2 °C, but not at -0.5 °C at 7m CA + 6w RA + 7d SL, while no DB was observed in 1-MCP treated fruit stored at -0.5 °C, the highest incidence of DB was seen in 1-MCP untreated control fruit (No 1-MCP) at 2 °C at this same time (Fig. 6). The incidence of DB was variable over the season, but for the clear difference at the last evaluation time where on average, fruit at 2 °C exhibited a higher DB compared to the lower storage temperature (-0.5 °C) in 2015.

CO_2B was also identified as one of the browning types in the season. According to Figure 3B, 13 fruit were affected by this type of browning in 2015 out of the total of 4800 fruit that were evaluated. The incidence of CO_2B was affected by an interaction between evaluation time and 1-MCP treatment (Table 4). Fruit treated with 1-MCP were not as susceptible to CO_2B compared to fruit that were not treated with 1-MCP (control fruit). Further statistical analysis was not conducted on the data, even though, the difference in incidence of CO_2B between 1-MCP treated and 1-MCP untreated control fruit (No 1-MCP) increased with evaluation time.

More CO_2B (10 fruit) was observed in 1-MCP untreated control fruit (No 1-MCP) than in 1-MCP treated fruit (3) throughout the season. Data is not shown due to the low incidence.

There were a few incidences of combination browning (Fig. 3B). It was observed at evaluation time 7m CA + 6w RA + 7d SL, in 1-MCP treated fruit stored at 2 °C (Fig. 6). Two variations of this browning type were observed: RB + CO_2B in one fruit (Fig. 2D) as well as RB + DB in one fruit (Fig. 2E). A combination of RB and CO_2B in one fruit is new and unreported; however, it was observed in only one fruit. Further statistical analysis was not conducted on CB due to the low fruit number. No RB was observed in the second season.

2.3.3 Effect of 1-MCP, storage temperature and evaluation time on fruit maturity and quality

A. Season one (2014)

To place maturity indices after storage into context, the harvest maturity indices are shown in Table 5. Fruit were harvested at pre-optimum SB (11%), where the optimum ranges from 15 - 40% (Hurndall and Fourie, 2003). The TSS levels were at 12% and had not reached the export required 13%. However, fruit firmness was 9.2 kg which, according to Hurndall and Fourie, (2003) was acceptable for export purposes when they recommended that fruit could be shipped at > 7.2 kg. Acidity range recommended for export is 0.4-0.8% and at harvest, fruit in this season exhibited a 0.67% acidity level being within the export required range. Fruit BC at harvest in this season was 2.7 being pale green according to the colour chart used as described in the methods above.

Background colour

During storage, BC (chart index), was influenced by the interaction of storage temperature, 1-MCP treatment and evaluation time (Table 5). After 6 weeks RA, both 3- and 7-months CA stored fruit differed, with 2 °C fruit being more yellow (Fig. 7). 1-MCP treated fruit were greener at -0.5 °C for 3m CA + 6w RA stored fruit and for 2 °C stored fruit after 7m CA + 6w RA as well as the shelf-life period for both temperatures at this time (Fig. 7). Differences were statistically significant. Fruit stored for 3m CA + 6 w RA at 2 °C and 7m CA + 6w RA at -0.5 °C did not show a difference between 1-MCP treatment and 1-MCP untreated control fruit (No 1-MCP; Fig. 7). After 5m CA + 6w RA there was no difference between temperature or 1-MCP treatment in yellowing. Therefore, looking at the storage time and temperature combinations, 1-MCP treated fruit exhibited less yellowing than the 1-MCP untreated control fruit (No 1-MCP; Fig. 7). It also clear that 1-MCP treated fruit maintained their BC at -0.5 °C.

Even though there were many differences, BC in general remained within the acceptable export range (Fig. 7), (scale of 2.5 – 4.0 as extrapolated from the colour chart) according to the IPLA standards (APAL Pink Lady® Exporters Hub, 2017).

Firmness

Firmness of fruit was affected by the interaction of storage temperature and evaluation time (Table 5). Fruit stored at 2 °C had a lower firmness compared to fruit stored at -0.5 °C and this difference became greater with advancing evaluation time (Fig. 8). Firmness of fruit was also affected by the main effect of 1-MCP (Table 5). 1-MCP treated fruit were significantly firmer than 1-MCP untreated control fruit (No 1-MCP; Fig. 9). Fruit firmness remained acceptable for export at all the evaluation times, since they were higher than 7.2 kg for both 1-MCP treated and 1-MCP untreated control fruit (No 1-MCP), as well as temperature and evaluation time combinations (Fig. 8).

Total soluble solids

TSS of fruit was affected by the interaction of 1-MCP application, storage temperature and evaluation time (Table 5). The 1-MCP untreated control fruit (No 1-MCP) and 1-MCP treated fruit generally exhibited an increase in TSS at both temperatures with advancing evaluation times (Fig. 10). However, 1-MCP treated fruit on average had a significantly lower percentage of TSS, when compared within storage temperature, at most of the evaluation times. TSS was highest in 1-MCP untreated control fruit (No 1-MCP) at 7m CA + 6w RA + 7d SL at 2 °C (14.9%) and the lowest in 1-MCP treated fruit after 3m CA at -0.5 °C (12.5%; Fig. 10). TSS levels below 13% are unacceptable for export according to Hurndall and Fourie, (2003).

Titratable acidity

TA was significantly influenced by the interaction of 1-MCP application, temperature and evaluation time (Table 5). TA levels were maintained within acceptable levels (0.4 – 0.8%) as per export standards (Hurndall and Fourie, 2003), throughout the trial (Fig. 11). TA generally decreased consistently from 3m CA to 7m CA + 6w RA + 7d SL for both 1-MCP treatments at both temperatures (Fig. 11). Even though 1-MCP treated fruit generally maintained TA better than 1-MCP untreated control fruit (No 1-MCP) at most of the evaluation times, it can also be observed that there were times when the TA did not significantly differ between the two treatments at the same temperature (e.g. 2 °C at 3m CA + 6w RA, both temperatures at 3m CA + 6w RA + 7d SL, -0.5 °C at 5m CA + 6w RA, both temperatures at 5m CA + 6w RA + 7d SL and 2 °C at 7m CA; Fig. 11).

Starch breakdown

Fruit were harvested at commercial harvest, yet considering SB was lower than the optimum required standard (15 to 40%) for export at harvest, fruit are considered pre-optimum maturity (Hurndall and Fourie, 2003). According to Table 5, the temperature at which fruit was stored influenced SB at different evaluation times. SB was significantly higher in fruit stored at 2 °C from 3m CA to 5m CA until starch was completely broken down at both temperatures at 5m CA + 6w RA and all evaluations after (Fig. 12).

1-MCP application also influenced SB at different evaluation times (Table 5). SB was significantly higher in 1-MCP untreated control fruit (No 1-MCP) at 3m CA and 3m CA + 6w RA + 7d SL compared to 1-MCP treated fruit (Fig. 13). Similar SB levels were observed at the other evaluation times for both 1-MCP treatments (Fig. 13). SB was complete at evaluation time 5m CA + 6w RA, regardless of 1-MCP treatment.

Greasiness

No incidence of greasiness was observed after any storage evaluations for the entire season.

B. Season two (2015)

The maturity at harvest in the 2015 season was more towards the end of the optimum harvest maturity window and more mature than that of the 2014 season but all within the export range (Hurndall and Fourie, 2003; Table 6). The background colour was 3.2, firmness 9.2 kg, TSS 13%, TA 0.63% and starch breakdown at 39%. Table 6 also shows that the 1-MCP untreated control fruit (No 1-MCP) and 1-MCP treated fruit interacted with temperature and evaluation time for all maturity indices, except for SB, which was influenced only by the interaction of evaluation time and 1-MCP.

Background colour

Table 6 shows that the 1-MCP treatment interacted with temperature and evaluation time for BC. At harvest, BC of fruit were not significantly different between treatments (Fig. 14). After extended storage and ripening (at 7m CA + 6w RA + 7d SL), the 1-MCP untreated control fruit (No 1-MCP) were more yellow, especially after storage at 2 °C, while 1-MCP treated fruit-maintained a less yellow BC at both temperatures. 1-MCP treated fruit generally were less yellow for both temperatures compared to the 1-MCP untreated control fruit (No 1-MCP) or retained a similar colour over time (Fig. 14). All fruit evaluated in this trial remained within acceptable limits (pale green but not yellow on the colour chart scale of 2.8 – 3.8) as recommended by Hurndall and Fourie (2003), except for the 1-MCP untreated control fruit (No 1-MCP) stored at 2 °C at the end of the trial (7m CA + 6w RA + 7d SL).

Firmness

For firmness, the 1-MCP untreated control fruit (No 1-MCP) and 1-MCP treated fruit interacted with temperature and evaluation time (Table 6). Fruit had a similar firmness at harvest (Fig. 15). 1-MCP treated fruit exhibited a significantly higher firmness over 1-MCP untreated control fruit (No 1-MCP) at both storage temperatures at all evaluation times, except at 3m CA and 5m CA, where fruit firmness was similar for both 1-MCP treatments at -0.5 °C (Fig. 15). Fruit exhibited similar firmness within same 1-MCP treatment, across storage temperatures at same evaluation times (Fig. 15). The firmness of all the fruit met the acceptable standards (>7.2 kg; at shipping from South Africa, Hurndall and Fourie, 2003) and >6.5 kg when sold in Europe (APAL Pink Lady® Exporters Hub, 2017) in this season as in the previous.

Total soluble solids

1-MCP treatment interacted with temperature and evaluation time for TSS (Table 6). 1-MCP treated fruit stored at both temperatures, exhibited consistent lower levels of TSS significantly different from that of 1-MCP untreated control fruit (No 1-MCP) from 3m CA through to 7m CA. TSS percentage recorded for 1-MCP untreated control fruit (No 1-MCP) and 1-MCP treated fruit at both storage temperatures were similar for the rest of the evaluation times, except for 1-MCP treated fruit stored at -0.5 °C at evaluation 7m CA + 6w RA and 1-MCP untreated control fruit (No 1-MCP) stored at 2 °C at evaluation 7m CA + 6w RA + 7d SL, which were significantly different from one another (Fig. 16). TSS percentage recorded for this season remained within acceptable limits (13%) for export according to Hurndall and Fourie (2003), but for 1-MCP treated fruit at 3m CA, which exhibited a slightly lower level of TSS (Fig. 16).

Titratable acidity

1-MCP treatment interacted with temperature and evaluation time for TA (Table 6). From harvest to the end of 7m CA + 6w RA + 7d SL storage fruit gradually decreased in TA from ≈0.65% to 0.4-0.35%. The biggest decline was noted within the first three months in storage (i.e. from about ≈0.63% to ≈0.53%; Fig. 17). TA percentage was not significantly different in fruit evaluated after 3m CA between 1-MCP treated and 1-MCP untreated control (No 1-MCP) at the same temperature, but also across temperatures. There was also no significant difference between 1-MCP untreated control fruit (No 1-MCP) and 1-MCP treated at 3m CA + 6w RA and 3m CA + 6w RA + 7d SL. At 5m CA, TA observed at 2 °C in 1-MCP untreated control fruit (No 1-MCP) was significantly lower than TA observed in 1-MCP treated fruit at both temperatures, as well as 1-MCP untreated control fruit (No 1-MCP) at 2 °C (Fig. 17). TA level exhibited in 1-MCP untreated control fruit (No 1-MCP) was consistently significantly lower for the remaining evaluation times in the season, regardless of storage temperature. It is interesting to note that, TA levels observed for either of the 1-MCP treatments, were similar across temperatures at evaluation times 5m CA + 6w RA, 5m CA + 6w RA + 7d SL and at 7m CA (Fig. 17).

1-MCP treated fruit, at both temperatures, maintained significantly higher TA levels, especially after the longer storage and evaluation times (i.e. from 5m CA + 6w RA to the end), compared to the 1-MCP untreated control fruit (No 1-MCP). Also, 1-MCP treated fruit remained within the IPLA acceptable export standard (0.4 - 0.8%) throughout the trial, except for 2 °C stored fruit evaluated after 7m CA + 6w RA + 7d SL, while 1-MCP untreated control fruit (No 1-MCP) from both temperatures fell below 0.4% TA after 7m CA to the end (Fig. 17).

Starch breakdown

SB at harvest in this season was at the upper limit (40%) for fruit at harvest according to IPLA standards (APAL Pink Lady® Exporters Hub, 2017). In this season SB of fruit differed significantly with regards to 1-MCP treatment at different evaluation times (Table 6). Figure 18 shows that SB at 3m CA for 1-MCP treated fruit was significantly lower than to 1-MCP untreated control fruit (No 1-MCP). However, both treatments had a very high and similar SB at this time (90-92%), which may not be commercially significant. SB was complete in both 1-MCP treatments by 3m CA + 6w RA.

Greasiness

No incidence of greasiness was encountered in this season.

2.3.4 Relationship between browning and maturity/quality parameters

The relationship (correlations) between the various parameters and factors are discussed from Tables 7 and 8 for season one and two, respectively.

A. Season one (2014)

Total browning observed in this season weakly negatively but significantly associated with firmness (-0.54; Table 7). DB also weakly negatively but significantly associated with firmness (-0.58) and significantly weakly positively associated with BC (0.58). DB strongly positively and very significantly associated with total browning (0.86; Table 7). CO_2B interestingly, very strongly positively correlated with RB (0.96) and was highly significant ($p<0.0001$). TA associated extremely negatively with BC (-0.94) and highly negatively with SB (-0.78), but extremely positively with firmness (0.93; $p \leq 0.001$). TSS related highly positively with SB (0.76), while firmness correlated extremely negatively with background colour (0.99) and moderately negatively with SB (0.60). Finally, SB moderately positively correlated with BC (0.67; Table 7).

B. Season two (2015)

Total browning and DB moderately negatively correlated with TA at -0.75 and -0.72, respectively (Table 8). DB extremely positively with total browning (0.99). Furthermore, CO_2B weakly negatively (-0.57), but significantly associated with TA. TA also correlated moderately negatively with SB (-0.67) while TSS correlated highly negatively with SB (-0.82; Table 8).

2.4 DISCUSSION

It is noteworthy that, while total browning was significantly influenced by a two-way interaction of either 1-MCP application and evaluation time or 1-MCP and temperature in the first season (2014; Table 2), total browning in the second season was significantly influenced by a three-way interaction of 1-MCP, temperature and storage time. DB was first observed at evaluation time 5m CA + 6w RA in both seasons. While DB dominated the observation made at evaluation time 5m CA + 6w RA in the second season, RB dominated the browning observed at the same evaluation time in the first season, recording 9% compared to 1% for DB in the 1-MCP untreated control fruit (No 1-MCP; Table 3). DB was the dominant browning type present in both seasons and increased to 18% in the 1-MCP untreated control fruit (No 1-MCP) after a longer storage time, 7m CA + 6w RA in the first season and 7m CA + 6w RA + 7d SL in the second season. According to Figures 4 and 6, 1-MCP untreated control fruit (No 1-MCP) exhibited significantly higher amounts of DB, with or without the interactive effect of temperature.

Studies have been conducted on 1-MCP's inhibition of ethylene and as well as mitigation of IB and retention of firmness in 'Gala' and 'Redchief' apples (Serek et al., 1994; Sisler et al., 1996; Sisler and Blankenship, 1996; Sisler and Serek,1997, 2003; Fan et al., 1999b; Watkins et al., 2000; Beaudry and Watkins, 2001; Mir et al., 2001; Jobling, 2002; Argenta et al., 2005; Watkins, 2006a, b). Majoni et al. (2013) indicated that 1-MCP mitigated the incidence of IB in 'Cripps' Pink' apples harvested at 64% SB and stored under CA at -0.5 °C for 3 months followed by 7 weeks in RA at -0.5 °C and then kept for 7 days under SL at 20 °C. In that study, fruit treated with 1-MCP also had lower internal CO_2 levels. It therefore seems that senescence and internal CO_2 may have played a role. In a review by Watkins (2006a), the specific effects of 1-MCP on fruits perception of ethylene was clearly outlined. Among many other benefits, Watkins (2006a) mentioned that 1-MCP constrains respiration rates and fruit ripening, it maintains firmness and BC and reduces the rate of loss of TA. Furthermore, TSS dynamics with 1-MCP are not certain as 1-MCP treated fruit may record higher, similar or lower TSS levels than 1-MCP untreated control fruit (No 1-MCP) (Watkins, 2006a). TSS in this study generally was higher in 1-MCP untreated control fruit (No 1-MCP) over time. However, there were certain evaluation times when TSS recorded were similar for both treatments of 1-MCP regardless of storage temperature (Figs. 10 and 16). Except for SB, all the quality and maturity parameters measured in this study were influenced by the interaction of 1-MCP and evaluation time, in at least two different evaluation times (Tables 6 and 7) in both seasons. Regardless of the storage temperature effect, one can safely say that fruit treated with 1-MCP generally performed better than the 1-MCP untreated control fruit (No 1-MCP) with regards to total browning, BC, TA, as well as firmness in the two seasons over time (Figures 6 to 17). The benefit of 1-MCP is mainly present after 5m CA + 6w RA, which is when the browning disorder was initiated. Majoni et al. (2013) reported that 1-MCP can keep 'Cripps' Pink' apples, stored for 3 months and longer, free from browning and scald incidence. In this study, 1-MCP treated fruit maintained quality better than the 1-MCP untreated control fruit (No 1-MCP) and this was even more evident in fruit assessed from evaluation time 5m CA to the end of the study. As mentioned earlier, 1-MCP inhibits the fruits perception of ethylene by binding to ethylene receptors, thereby preventing or delaying ethylene action (Sisler and Blankenship, 1996; Sisler and Serek, 1997, 2003; Watkins et al., 2000; Beaudry and Watkins, 2001; Watkins, 2006a, b). Similarly, in this study, 1-MCP may have inhibited the perception of 'Rosy Glow' to ethylene, thereby maintaining the quality and reducing IB in the treated fruit.

According to Paull (1999), the best temperature to store apples for between 30 to 365 days is -0.5 °C at a relative humidity of 90 - 95%. Likewise, Hurndall and Fourie (2003) recommended that 'Cripps' Pink' apples should be stored at -0.5 °C in CA for optimum storage results. James (2007) recommended storage temperatures specific to IB type and concluded on among others, storing fruits at 3 and 1 °C for the mitigation of DB and RB, respectively in 'Cripps' Pink' apples. Majoni et al. (2013) also concluded that, 'Cripps' Pink' apple could be stored at -0.5 °C only for up to 4 months in CA to reduce the risk of IB development.

Watkins (2006a), as well as Jobling (2002), reported that chilling injury is one of the fundamental causes of the IB disorder and it especially leads to DB. This assertion was confirmed by Majoni et al. (2013) and Butler (2015), who conducted studies in South Africa. They found that fruits are prone to chilling injury when stored at lower temperatures for a prolonged period. For the mitigation or prevention of chilling injury and thus DB, Jobling (2002) suggested storing fruit at higher temperatures, especially above the threshold where the injury occurs. Jobling (2002) observed that increasing the storage temperature from 0 to 3 °C resulted in 'Cripps' Pink' fruit developing less DB, which is the dominant browning type in South African 'Cripps' Pink' apples. Bergman et al. (2012) reported that a lower storage temperature made 'Cripps' Pink' apple fruit susceptible to both DB and RB. Even though there were many other contributing factors, they confirmed that increasing the storage temperature could reduce the flesh browning incidence.

For longer-term storage in South Africa, 'Cripps' Pink' apples used to be stored in CA at -0.5 °C. This low temperature storage may explain the susceptibility of South African 'Cripps' Pink' apples to the DB. In the present study 'Rosy Glow' apples were stored at two different storage temperatures to ascertain the role played by storage temperature in the susceptibility to IB. Figures 6 to 17 show that, a distinct conclusion cannot be made on the effect of storage temperature. A significantly higher amount of DB was observed in fruit stored at -0.5 °C in season one. However, DB observed at 2 °C in season two was significantly higher. Temperature interacted with 1-MCP in season two to influence IB over time, but this did not happen in season one. DB in the first season was not influenced by storage temperature, contrary to the findings of Jobling (2002), while in the second season DB was influenced by the interaction of temperature and 1-MCP with time. This difference may be due to the difference in total fruit number and IB incidence which increased in the second season.

One factor that could also have influenced the effect of temperature is the gaseous composition (CO_2 and O_2) of the storage atmosphere of the fruit. Chilling injury is not only influenced by storage temperature, but also by the internal concentration of O_2 and CO_2, as well as fruit cultivar and sensitivity (Jobling, 2002). Low O_2 levels and high CO_2 levels are known to contribute to the susceptibility of fruit to chilling injury under CA (Jobling, 2002). The CA technique used in this study may have influenced the effect of temperature or inconsistent results as the fruit maturity was so different before storage, which may have influenced the internal O_2 and CO_2 at the time. Harvest maturity is a well-established factor to play a role in internal browning incidence and needs to be considered in future research (Butler, 2015).

The incidence of RB, CO_2B and CB observed in both seasons were low and no inferences can therefore be made from the data. However, the factors that may have influenced their development are beyond the scope of this study. According to Tables 2 and 4, both RB and CB were influenced by 1-MCP, storage temperature and time. Both browning types have been reported to be influenced by a complex interaction of factors (Jobling, 2002; Bergman et al., 2012; Crouch et al., 2014; Butler, 2015). This makes it difficult to draw any conclusions for these browning types in this study, especially since they were not repeated in two seasons. CO_2 browning, as well as a new type of CB made up of CO_2 and RB was observed in this study. This new type of browning has never been reported before and it was observed in only one fruit in the second season of this study. Due to their low incidence, these browning types need to be investigated further to see whether 'Rosy Glow' is prone to these types of browning disorders.

2.5 CONCLUSION

1-MCP has been established to maintain apple fruit quality and inhibit or even prevent disorders associated with long period storage, especially under CA where the level of O_2 is low and that of CO_2 is high. In this study 1-MCP treatment interacted with either temperature or evaluation time or both, to maintain 'Rosy Glow' quality. However, it was observed, that in general, 1-MCP treated fruit performed better in storage, mainly after 5m CA, compared to 1-MCP untreated control fruit (No 1-MCP), but using different storage regimes also significantly reduced IB

The effect of temperature was not very clear since the two storage temperatures used in the study gave dissimilar results in the two different seasons, perhaps due to the difference in fruit number and incidence or harvest maturity. Especially interesting is that, DB was not statistically affected by temperature in season one, perhaps highlighting that a certain number of fruit is needed in order to get statistical differences. Reports from previous studies conducted are not strict on which temperature should be used. It therefore seems that storage temperature may be influenced by many other factors, for example, composition of storage atmosphere. Further investigation is required to draw a distinct conclusion on the optimum storage temperature for 'Rosy Glow' apples.

DB was undoubtedly the dominant browning type observed, implying that 'Rosy Glow' may be more susceptible to DB. All the other browning types observed (RB, CO_2B and CB) need further investigation. The principal factor reported to influence DB development is chilling injury but also, harvest maturity and storage duration is important. However, it was also reported that pre-harvest factors such as growing degree days (GDD), soil, climate and maturity may influence the susceptibility of 'Cripps' Pink' apples to IB. According to Paper 1, which investigated the harvest maturity factor, harvesting 'Rosy Glow' at optimum maturity is very likely to reduce their susceptibility to DB. It is therefore possible that some pre-harvest factors played a role in the susceptibility of 'Rosy Glow' to the IB observed. It is recommended that future research investigate other pre-harvest factors that may have influenced the DB observed in this study.

The incidence of total browning in both seasons was very low. Data should also be seen with caution as it seems that IB in the first season occurred, levelled and then decreased again after a longer period of storage for DB. This phenomenon is also present after SL in 'Cripps' Pink' apples. In contrast, we see more DB during the SL period in the second season, when the number of fruit used in 'Rosy Glow' browning was increased. Second season trials were also conducted in locations more likely to develop higher IB incidence, due to the low incidence of IB in the first season.

'Rosy Glow' apple fruit is susceptible to IB but more specifically, DB. Even though other browning types were observed in this study, there is the need for further investigations into the factors and conditions under which they occur. 1-MCP proved to mitigate incidence of 'Rosy Glow' apple fruit IB in this study, especially in fruit stored for more than 5m CA regardless of storage temperature, generally retaining better firmness, BC and TA while slowing SB. It is therefore recommended that South Africa 'Rosy Glow' apple fruit destined for long term CA storage be treated with 1-MCP, especially if storage period will exceed 5m CA.

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2.7 TABLES AND FIGURES

Table 1: Incidence of internal browning occurring in 'Rosy Glow' apples with number of brown fruit (out of a total of n= 320 per evaluation time) and dominant browning type per evaluation time in controlled atmosphere (CA) and regular atmosphere (RA) simulated shipping and stock rolling period and shelf life (SL) ripening at ambient temperature. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season and orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

Season:	**2014**		**2015**	
Evaluation time	**Number of brown fruit***	**Dominant browning type#**	**Number of brown fruit***	**Dominant browning Type^**
5 months CA	0	-	0	-
5 months CA + 6 weeks RA	12	Radial	14	Diffuse
5 months CA + 6 weeks RA + 7 days SL	2	-	0	-
7 months CA	3	Diffuse	8	Diffuse
7 months CA + 6 weeks RA	20	Diffuse	19	Diffuse
7 months CA + 6 weeks RA + 7 days SL	5	Diffuse	43	Diffuse

*Data represents the mean of fruit stored at -0.5 °C and 2 °C during CA and RA for all browning types.

#Refer to Fig. 1 for detail on browning types.

^Refer to Fig. 2 for detail on browning types.

Table 2: P-values of incidence of total browning, diffuse browning (DB), radial browning (RB) and CO_2 browning (CO_2B) types in 'Rosy Glow' apples as influenced by storage temperature (°C, Temp), 1-MCP treatment (1-MCP), evaluation time (Time) and the two- and three-way interactions of these treatments. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season.

Source	**Total browning**	**Diffuse browning**	**Radial browning**	**CO_2 browning**
		Pr>F*		
Temp	0.323	0.785	0.036	0.319
1-MCP	0.000	0.007	0.007	0.319
Time	< 0.0001	< 0.0001	< 0.0001	0.441
Temp*1-MCP	**0.049**[#]	0.276	0.007	0.319
Temp*Time	0.669	0.951	< 0.0001	0.441
Time*1-MCP	**< 0.0001**	**< 0.0001**	< 0.0001	0.441
Temp*1-MCP*Time	0.319	0.881	**< 0.0001**	0.441

*P>0.05 is considered non-significant

*Three factorial ANOVA

P-values in bold are discussed.

Table 3: Mean total browning, diffuse browning and radial browning percentage of 'Rosy Glow' apples after 5 and 7 months (5m and 7m, respectively) of controlled atmosphere (CA) storage, 6 weeks under regular atmosphere (RA) and 7 days at ambient for 1-MCP treated (Yes) and untreated (No) fruit. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season.

1-MCP	Evaluation time	Total browning (%)*	Diffuse browning (%)*	Radial browning (%)*#
Yes	5m CA	0.0c	0.0b	0.0b
No	5m CA	0.0c	0.0b	0.0b
Yes	5m CA+6w RA	1.1c	1.1b	0.0b
No	5m CA+6w RA	11.5b	1.0b	9.4a
Yes	5m CA+6w RA+7d SL	0.0c	0.0b	0.0b
No	5m CA+6w RA+7d SL	2.1c	2.1b	0.0b
Yes	7m CA	2.1c	1.0b	1.0b
No	7m CA	1.0c	1.0b	0.0b
Yes	7m CA+6w RA	2.1c	2.1b	0.0b
No	7m CA+6w RA	18.8a	18.8a	0.0b
Yes	7m CA+6w RA+7d SL	1.0c	1.0b	0.0b
No	7m CA+6w RA+7d SL	4.2c	4.2b	0.0b

*Data represents the mean of fruit stored at -0.5 °C and 2 °C, under CA and RA as it was not a significant factor in this interaction.
#Two factorial data presented to show the make-up of Total browning which was not influenced by the third factor storage temperature.

Table 4: P-values of incidence of internal browning in ‘Rosy Glow’ apples as influenced by storage temperature (Temp), 1-MCP treatment (1-MCP), evaluation time (Time) and the two- and three-way interactions of these treatments. Fruit were harvested from orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

Source	**Total browning**	**Diffuse browning**	**Combination browning**	**CO_2 browning**
			Pr>F	
Temp	0.003	0.022	0.011	0.142
1-MCP	0.023	0.119	0.011	0.142
Time	< 0.0001	< 0.0001	< 0.0001	0.001
Time*Temp	< 0.0001	< 0.0001	< 0.0001	0.116
Time*1-MCP	0.005	0.099	< 0.0001	**0.013**
Temp*1-MCP	0.023	0.054	0.011	0.769
Time*Temp*1-MCP	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.063

*Fruit stored at -0.5 °C and 2 °C, under CA and RA.

P-values in bold are discussed.

Table 5: Maturity indices at harvest and significance measured for 'Rosy Glow' apples by main and interactive effects of the treatments: temperature (-0.5 °C and 2 °C), 1-MCP (SmartFresh™) and evaluation time (3 months, 5 months and 7 months in controlled atmosphere (CA), each followed by 6 weeks in regular atmosphere (RA) and a 7-day shelf life period at ambient temperature). Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season.

Source:	**Background colour (chart index)**	**Firmness (kg)**	**TSS (%)**	**TA (%)**	**Starch breakdown (%)**
Maturity at harvest	2.7	9.1	12	0.67	11
			Pr>F*		
Temp	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
1-MCP	< 0.0001	**< 0.0001**	< 0.0001	< 0.0001	0.032
Time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Temp*1-MCP	0.824	0.674	0.002	0.101	0.193
Temp*Time	< 0.0001	**< 0.0001**	< 0.0001	< 0.0001	**< 0.0001**
1-MCP*Time	< 0.0001	0.246	< 0.0001	< 0.0001	**0.039**
Temp*1-MCP*Time	**< 0.0001**	0.882	**< 0.0001**	**< 0.0001**	0.098

*P>0.05 is considered non-significant

*Three factorial ANOVA

P-values in bold are discussed.

Table 6: Maturity at harvest and significance measured for 'Rosy Glow' apples by main and interactive effects of the treatments: temperature (-0.5 °C and 2°C), 1-MCP and evaluation time. Fruit were harvested from Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

Source:	**Background colour (chart index)**	**Firmness (kg)**	**TSS (%)**	**TA (%)**	**Starch breakdown (%)**
Maturity at harvest	3.2	9.2	13	0.63	39
			Pr>F*		
Time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Temp	0.588	0.023	0.001	< 0.0001	0.399
1-MCP	< 0.0001	< 0.0001	0.002	< 0.0001	0.089
Time*Temp	0.009	< 0.0001	< 0.0001	< 0.0001	0.749
Time*1-MCP	< 0.0001	< 0.0001	< 0.0001	< 0.0001	**0.037**
Temp*1-MCP	0.030	0.409	0.028	< 0.0001	0.308
Time*Temp*1-MCP	**< 0.0001#**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.143

*P>0.05 is considered non-significant

#P-values in bold are discussed.

Table 7: Pearson correlation r-value (top) and corresponding p-value (bottom) of the correlation between quality parameters, percentage total browning, and browning types at the 0.05% significance level for ‘Rosy Glow’ apples harvested at optimum maturity from Glen Fruin farm during the 2014 season.

Variables	Background colour (chart index)	Starch breakdown (%)	Firmness (kg)	TSS (%)	TA (%)	Total browning (%)	Diffuse browning (%)	Radial browning (%)	CO_2 browning (%)
Background colour (chart index)	**1**	**0.665**	**-0.988**	0.104	**-0.936**	0.518	**0.577**	-0.013	-0.010
Starch breakdown (%)		**1**	**-0.603**	**0.755**	**-0.779**	0.270	0.212	0.151	0.139
Firmness (kg)			**1**	-0.052	**0.929**	**-0.538**	**-0.575**	-0.028	-0.019
TSS (%)				**1**	-0.269	-0.052	-0.207	0.269	0.256
TA (%)					**1**	-0.483	-0.484	-0.083	-0.075
Total browning (%)						**1**	**0.862**	0.423	0.412
Diffuse browning (%)							**1**	-0.095	-0.090
Radial browning (%)								**1**	**0.964**
CO_2 browning (%)									**1**
Background colour (chart index)	**0**	**0.010**	**< 0.0001**	0.724	**< 0.0001**	0.058	**0.031**	0.965	0.973
Starch breakdown (%)		**0**	**0.023**	**0.002**	**0.001**	0.351	0.467	0.605	0.637
Firmness (kg)			**0**	0.861	**< 0.0001**	**0.047**	**0.031**	0.925	0.949
TSS (%)				**0**	0.352	0.861	0.477	0.352	0.378
TA (%)					**0**	0.080	0.079	0.777	0.799
Total browning (%)						**0**	**< 0.0001**	0.132	0.143
Diffuse browning (%)							**0**	0.746	0.759
Radial browning (%)								**0**	**< 0.0001**
CO_2 browning (%)									**0**

Values in bold are different from 0 with a significance level alpha=0.05

Table 8: Pearson correlation r-value (top) and corresponding p-value (bottom) of the correlation between quality parameters, percentage total browning and browning types at the 0.05% significance level for 'Rosy Glow' apples harvested at optimum maturity from Damar farm during the 2015 season.

Variables	Background colour (chart index)	Starch breakdown (%)	Firmness (kg)	TSS (%)	TA (%)	Total browning (%)	Diffuse browning (%)	CO_2 browning (%)	Combination browning (%)
Background colour (chart index)	**1**	0.058	0.150	-0.333	-0.271	0.171	0.182	-0.103	0.262
Starch breakdown (%)		**1**	0.463	**-0.815**	**-0.667**	0.235	0.222	0.261	0.122
Firmness (kg)			**1**	-0.216	-0.108	-0.039	-0.012	-0.400	0.199
TSS (%)				**1**	**0.903**	**-0.644**	**-0.631**	-0.451	-0.468
TA (%)					**1**	**-0.745**	**-0.722**	**-0.565**	**-0.545**
Total browning (%)						**1**	**0.997**	0.458	**0.871**
Diffuse browning (%)							**1**	0.391	**0.899**
CO_2 browning (%)								**1**	-0.017
Combination browning (%)									**1**
Background colour (chart index)	**0**	0.844	0.608	0.245	0.348	0.560	0.534	0.727	0.365
Starch breakdown (%)		**0**	0.095	**0.000**	**0.009**	0.419	0.446	0.368	0.677
Firmness (kg)			**0**	0.457	0.714	0.896	0.966	0.157	0.495
TSS (%)				**0**	**< 0.0001**	**0.013**	**0.015**	0.106	0.091
TA (%)					**0**	**0.002**	**0.004**	**0.035**	**0.044**
Total browning (%)						**0**	**< 0.0001**	0.100	**< 0.0001**
Diffuse browning (%)							**0**	0.167	**< 0.0001**
CO_2 browning (%)								**0**	0.953
Combination browning (%)									**0**

Values in bold are different from 0 with a significance level alpha=0.05

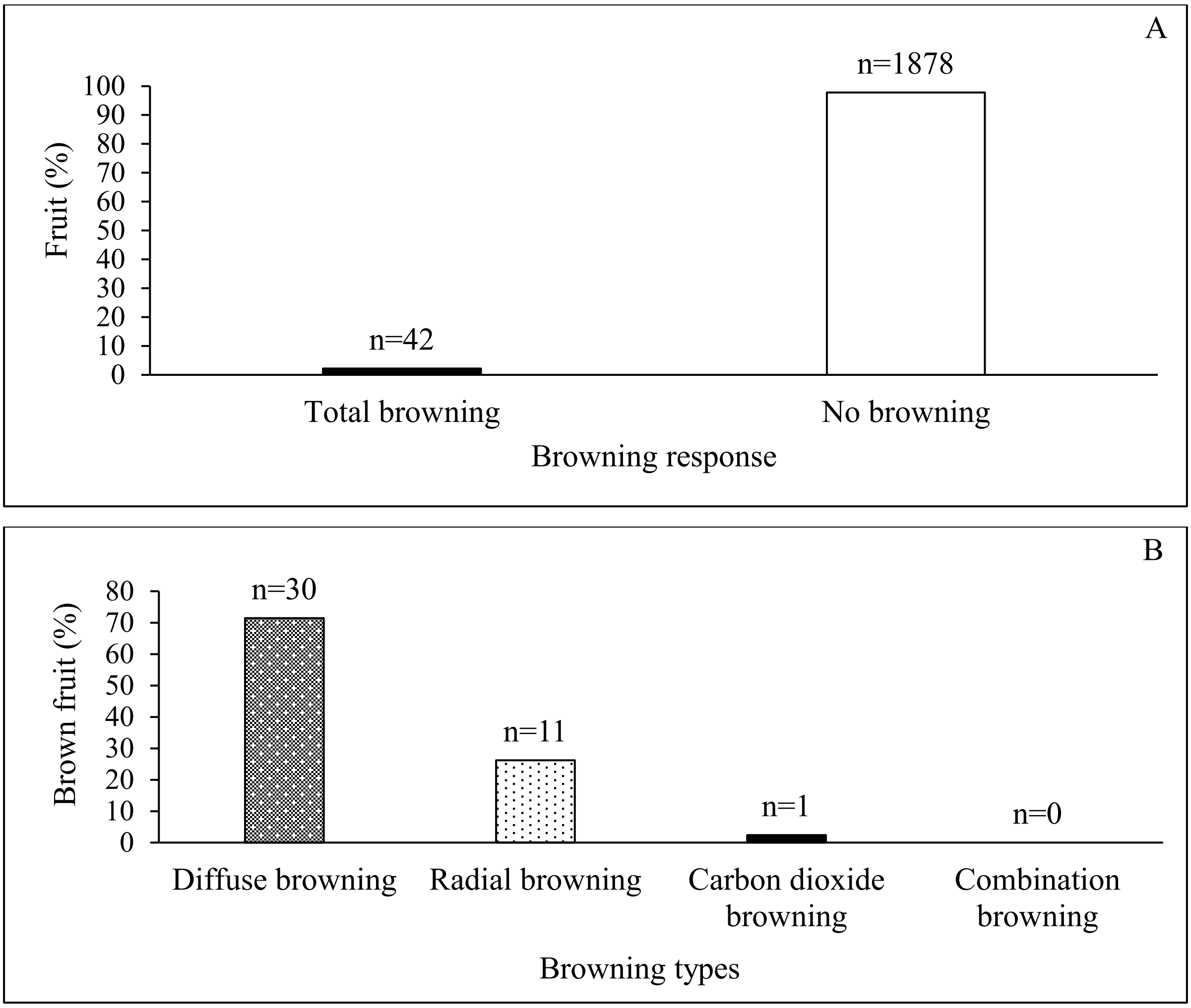

Figure 1: Total browning incidence (%) in a total of 1920 evaluated fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples harvested at optimum maturity during the 2014 season on Glen Fruin farm. Measurements were made on fruit that were stored for 3 months, 5 months and 7 months in controlled atmosphere (CA), each followed by 6 weeks in regular atmosphere (RA) at -0.5 °C and a 7-day shelf life period at ambient temperature.

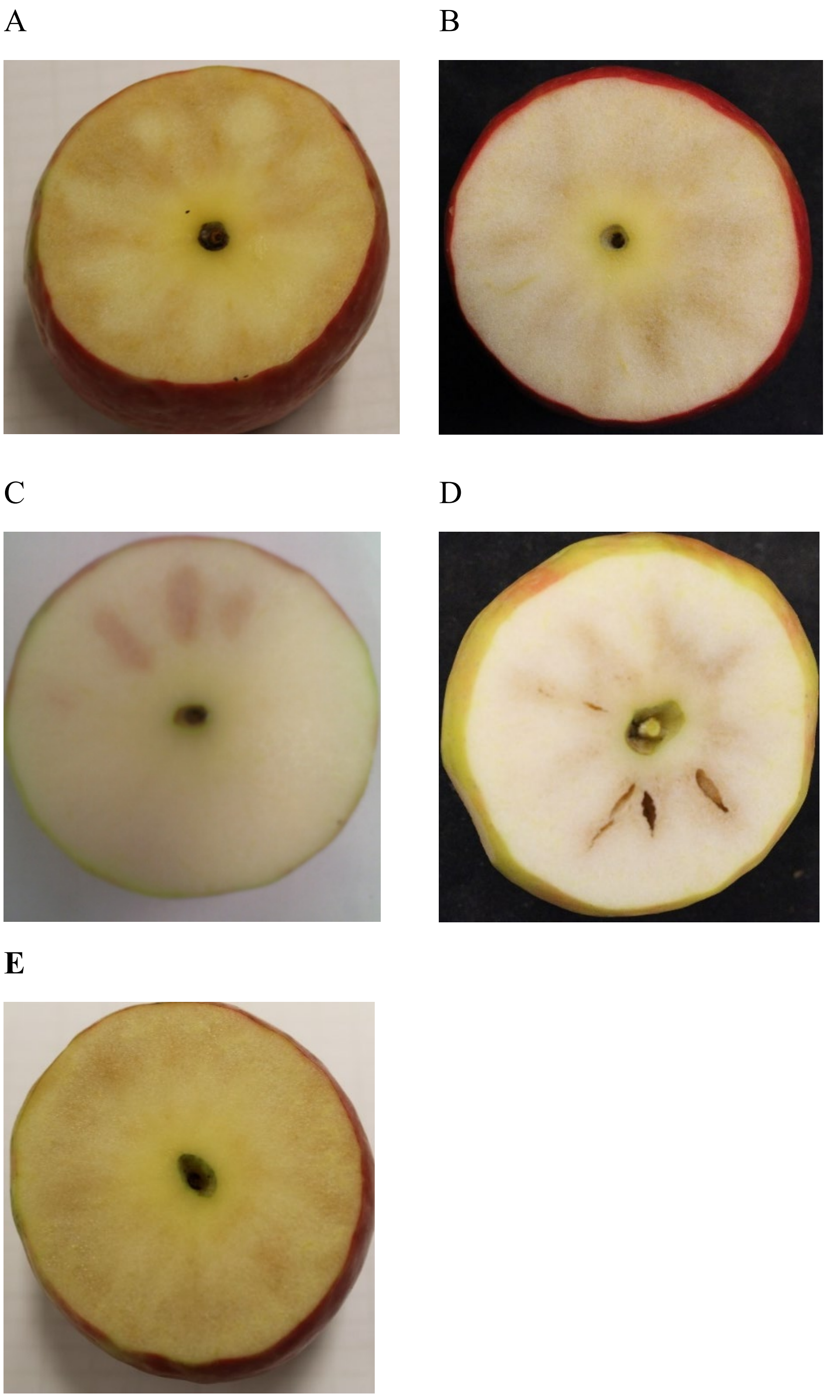

Figure 2: A) Diffuse browning, B) radial browning, C) CO_2 browning, D) combination browning (radial browning + CO_2 browning), E) combination browning (diffuse browning + radial browning) observed after storage (7 months in controlled atmosphere (CA) at -0.5 °C + 6 weeks regular atmosphere (RA) at -0.5 °C and a subsequent 7-day shelf life (SL) period at ambient temperature) of ‘Rosy Glow’ apples that had no 1-MCP treatment. Fruit were harvested in 2014 (A-C) and 2015 (D-E) at optimum maturity from orchard 20 on Glen Fruin farms in the Elgin region (A-C) or orchard 16 on the Damar farm in the Vyeboom region (D-E) of the Western Cape.

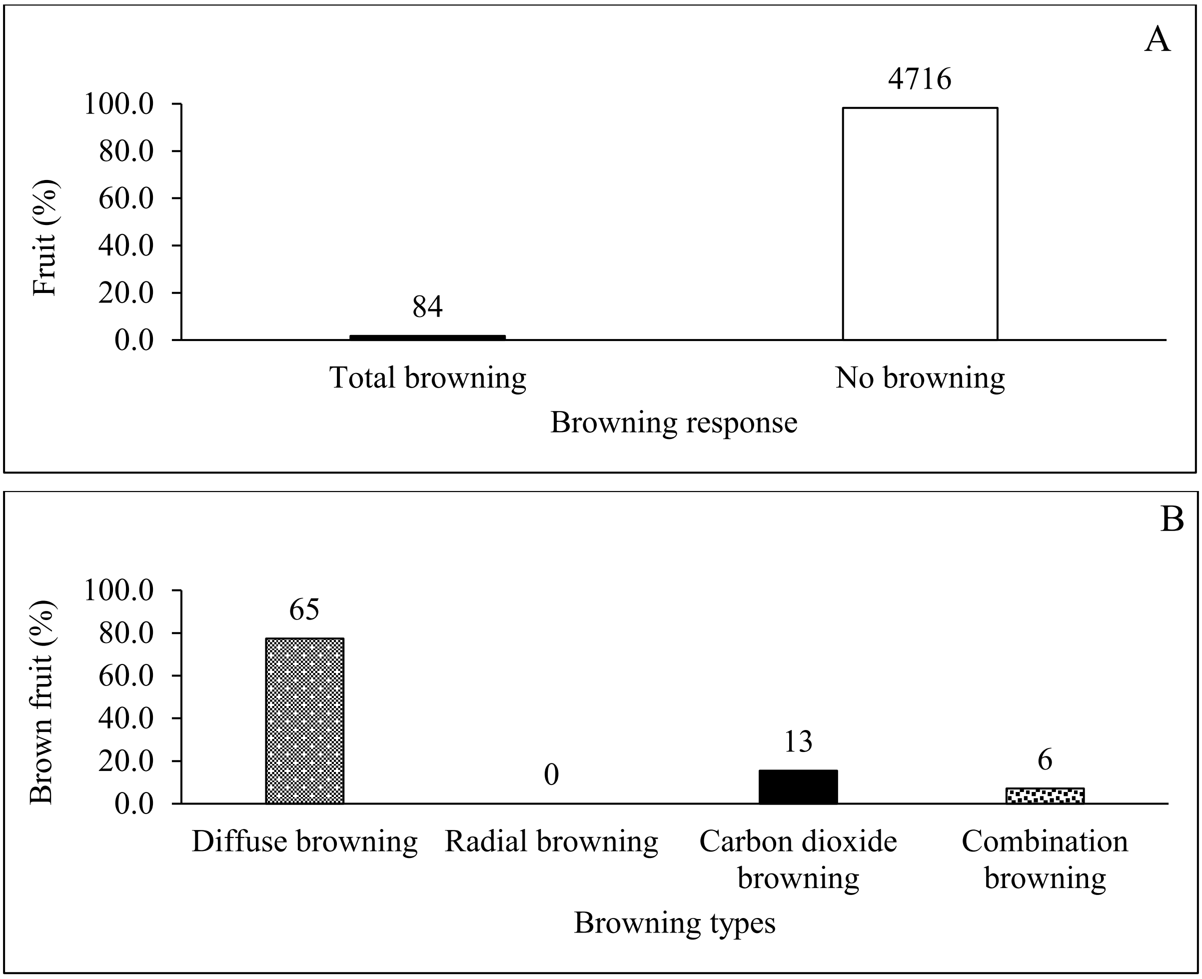

Figure 3: Total browning incidence in a total of 4800 evaluated fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples harvested at optimum maturity during the 2015 season on Damar farm (Combination browning = diffuse browning + radial browning). Measurements were made on fruit that were stored for 3 months, 5 months and 7 months in controlled atmosphere (CA), each followed by 6 weeks in regular atmosphere (RA) at -0.5 °C and 7-day shelf life period at ambient temperature.

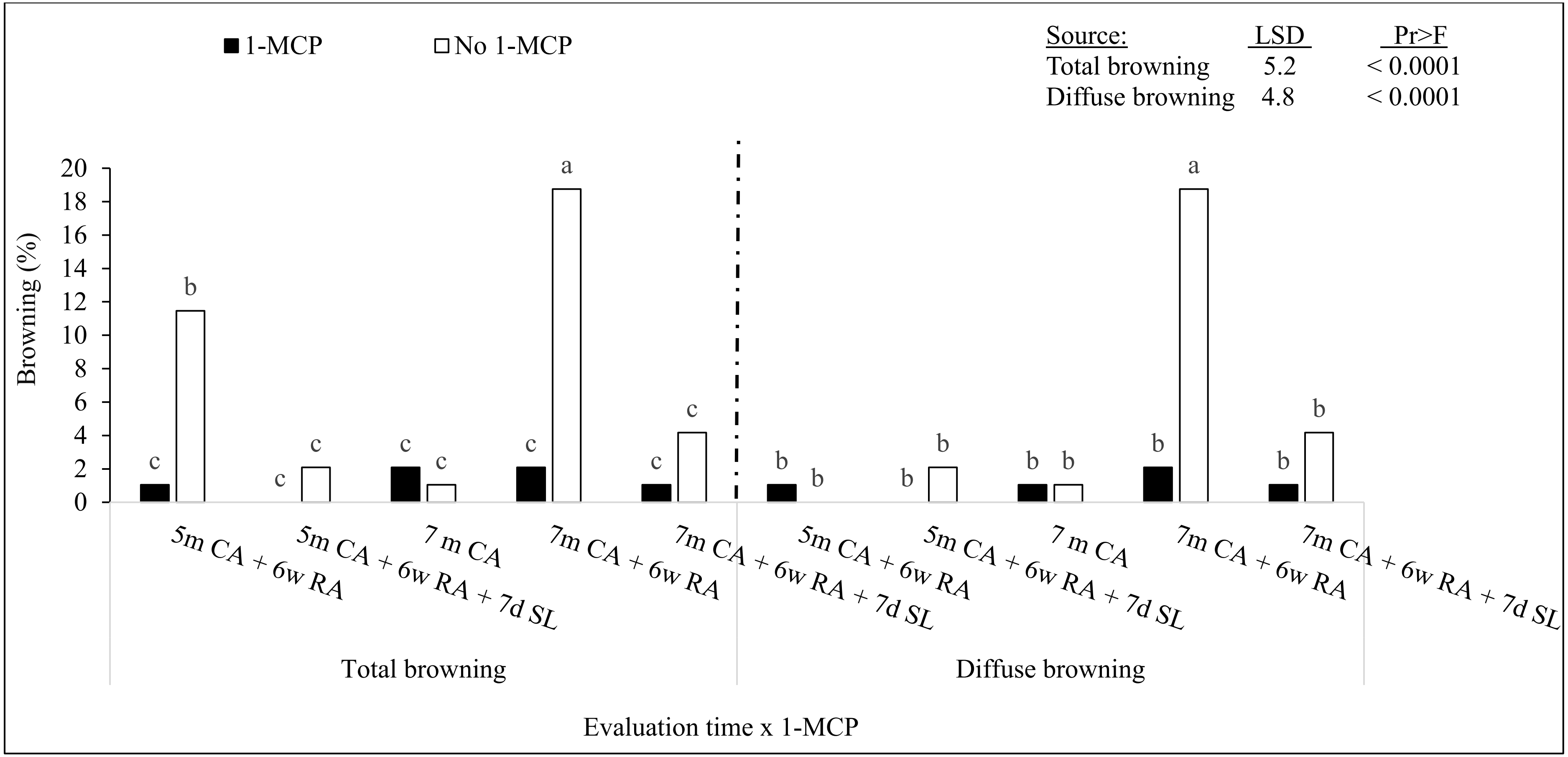

Figure 4: Percentage total and diffuse browning by interaction between evaluation time and 1-MCP treatment for 'Rosy Glow' apples. Measurements were made on fruit that were stored for 5 months and 7 months in controlled atmosphere (CA), each followed by 6 weeks in regular atmosphere (RA) at -0.5 °C and 7-day shelf life period at ambient temperature, but figure is from onset of internal browning (IB). Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season.

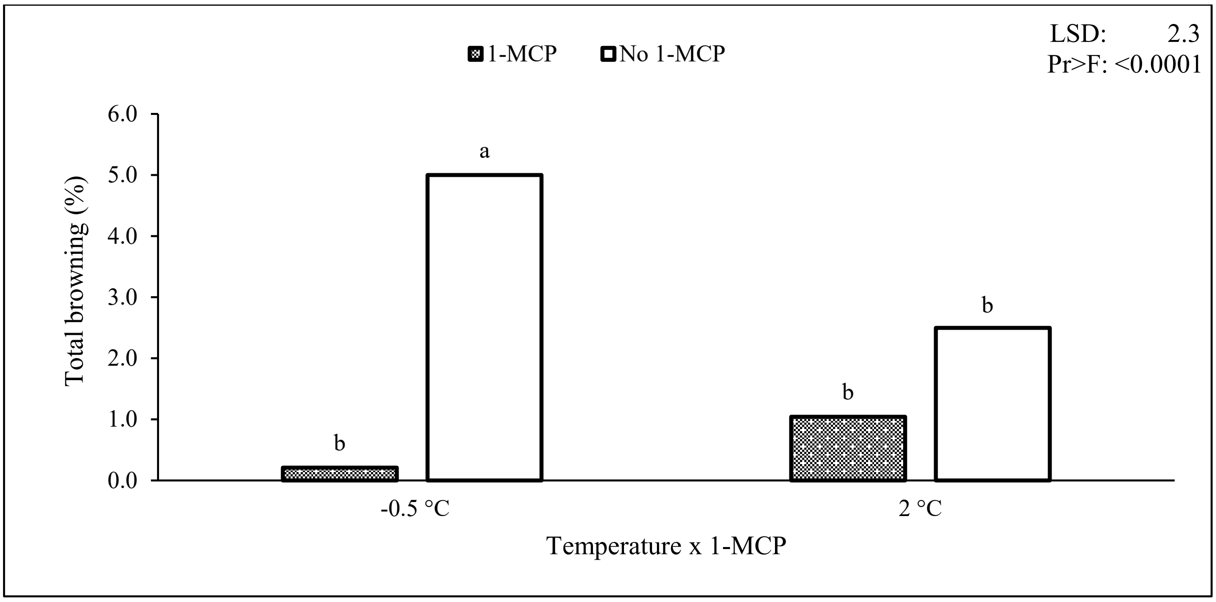

Figure 5: Percentage total browning by interaction between temperature and 1-MCP treatment for 'Rosy Glow' apples presented as mean of fruit that were stored for 3 months, 5 months and 7 months in controlled atmosphere (CA), each followed by 6 weeks in regular atmosphere (RA) and a 7-day shelf life period at ambient temperature. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season.

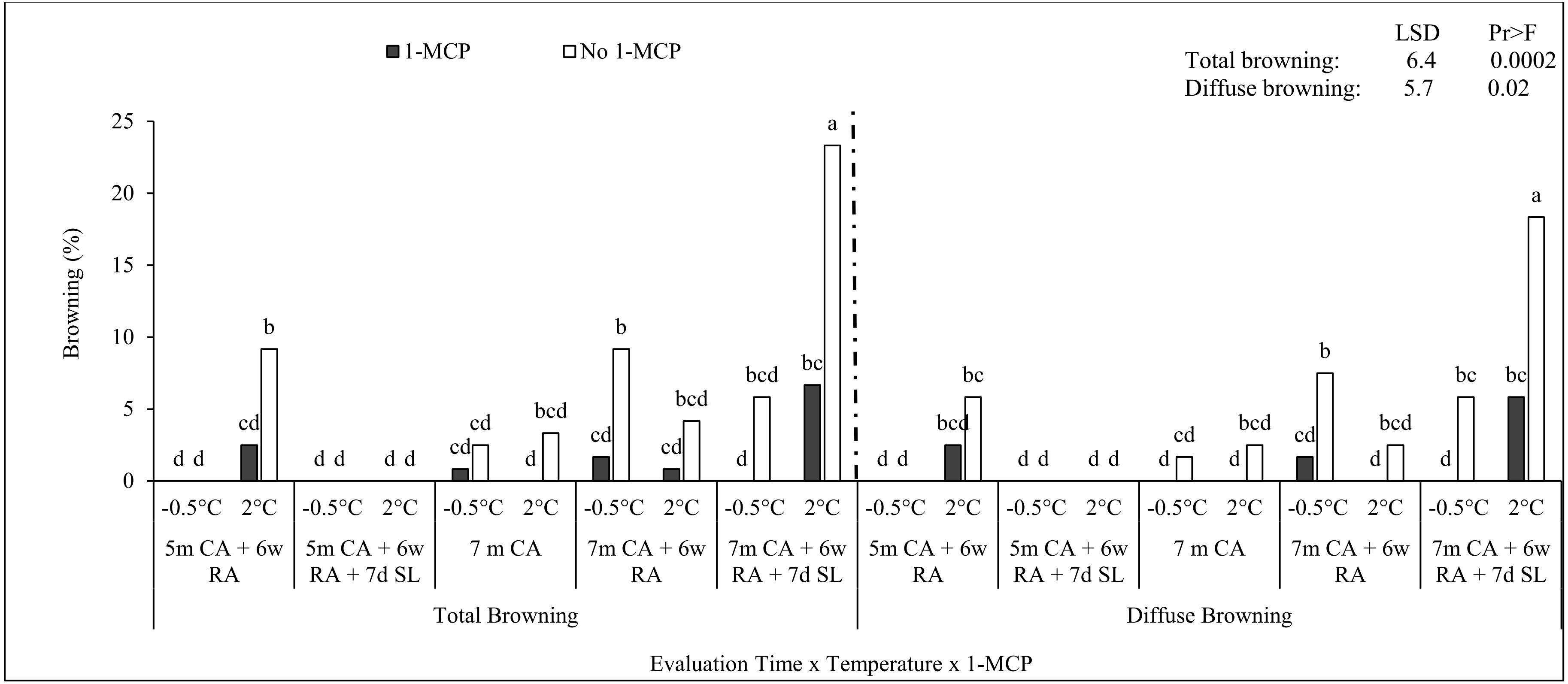

Figure 6: Percentage total and diffuse browning evaluated by the interaction between evaluation time (5 months (5m) and 7 months (7m) in controlled atmosphere (CA), each followed by 6 weeks in regular atmosphere (6w RA) and a 7-day shelf life (7d SL) period at ambient temperature), temperature (-0.5 °C and 2 °C) and 1-MCP treatment (1-MCP or No 1-MCP) for ‘Rosy Glow’ apples. Fruit were harvested from orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

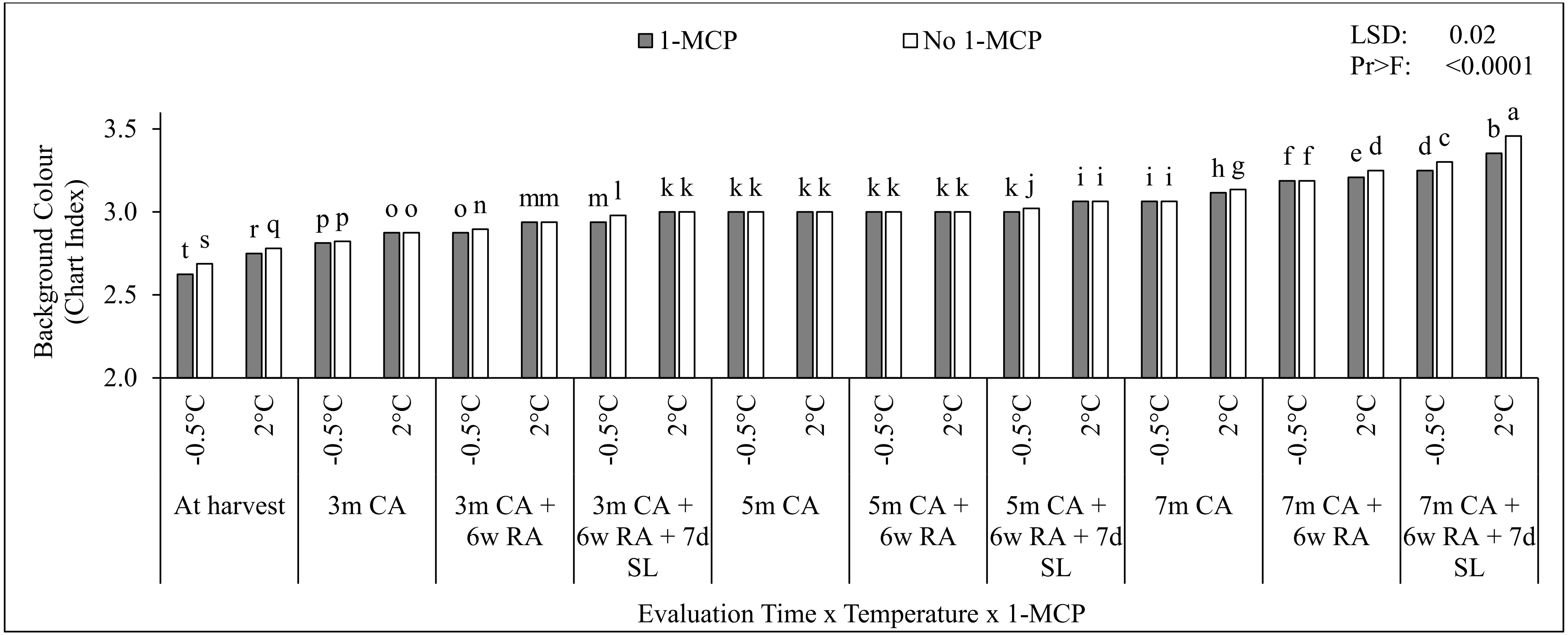

Figure 7: Background colour for the interaction between evaluation time, temperature and 1-MCP treatment in 'Rosy Glow' apples. Fruit were harvested in 2014 at optimum maturity from orchard 20 on Glen Fruin farm in the Elgin region of the Western Cape in 2014. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA), 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life (7d SL) period at ambient temperature).

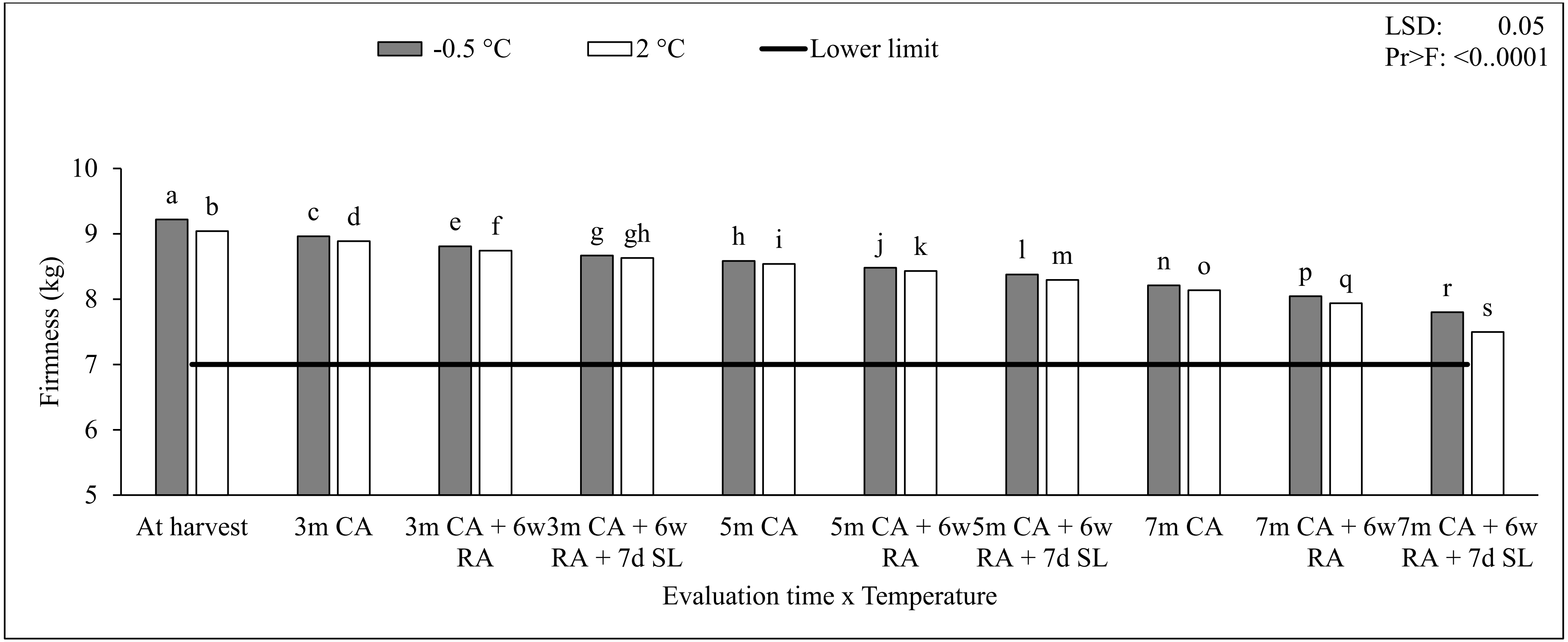

Figure 8: Firmness for the interaction between evaluation time and temperature in ‘Rosy Glow’ apples. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season. Measurements were made on fruit that were stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

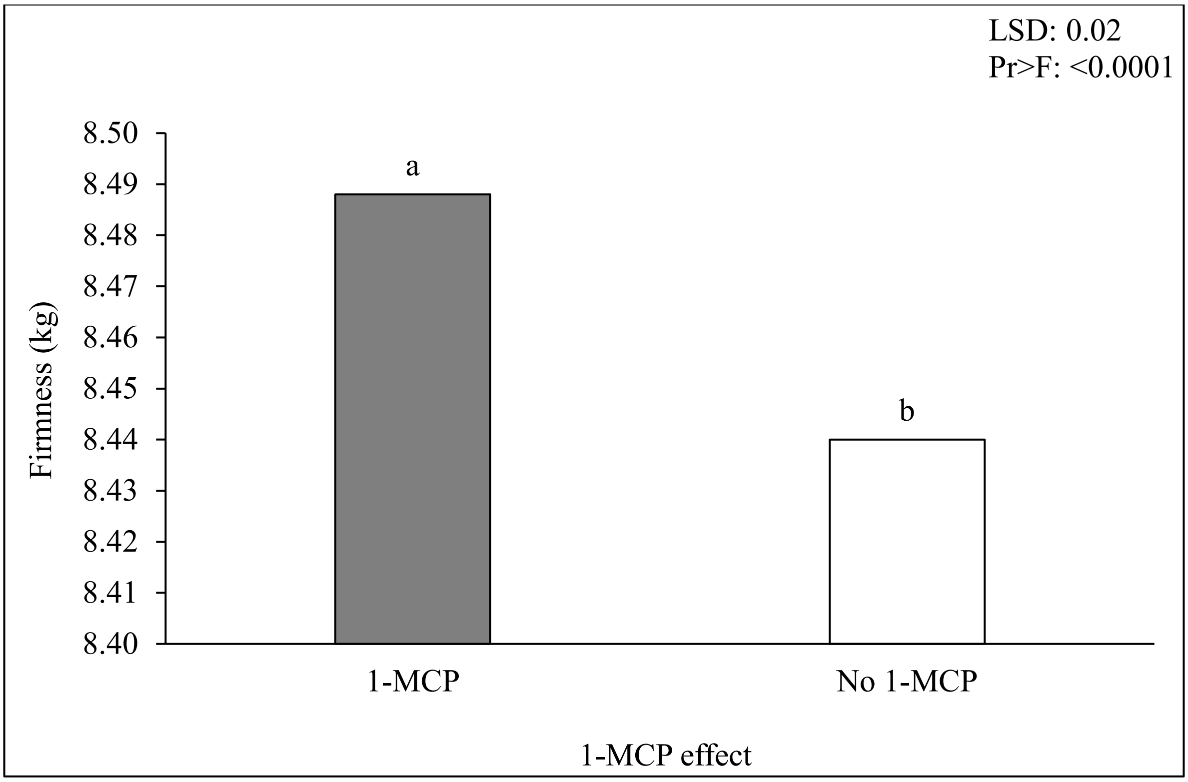

Figure 9: Firmness for the effect of 1-MCP in 'Rosy Glow' apples. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season. Measurements were made on fruit that were treated with or without 1-MCP.

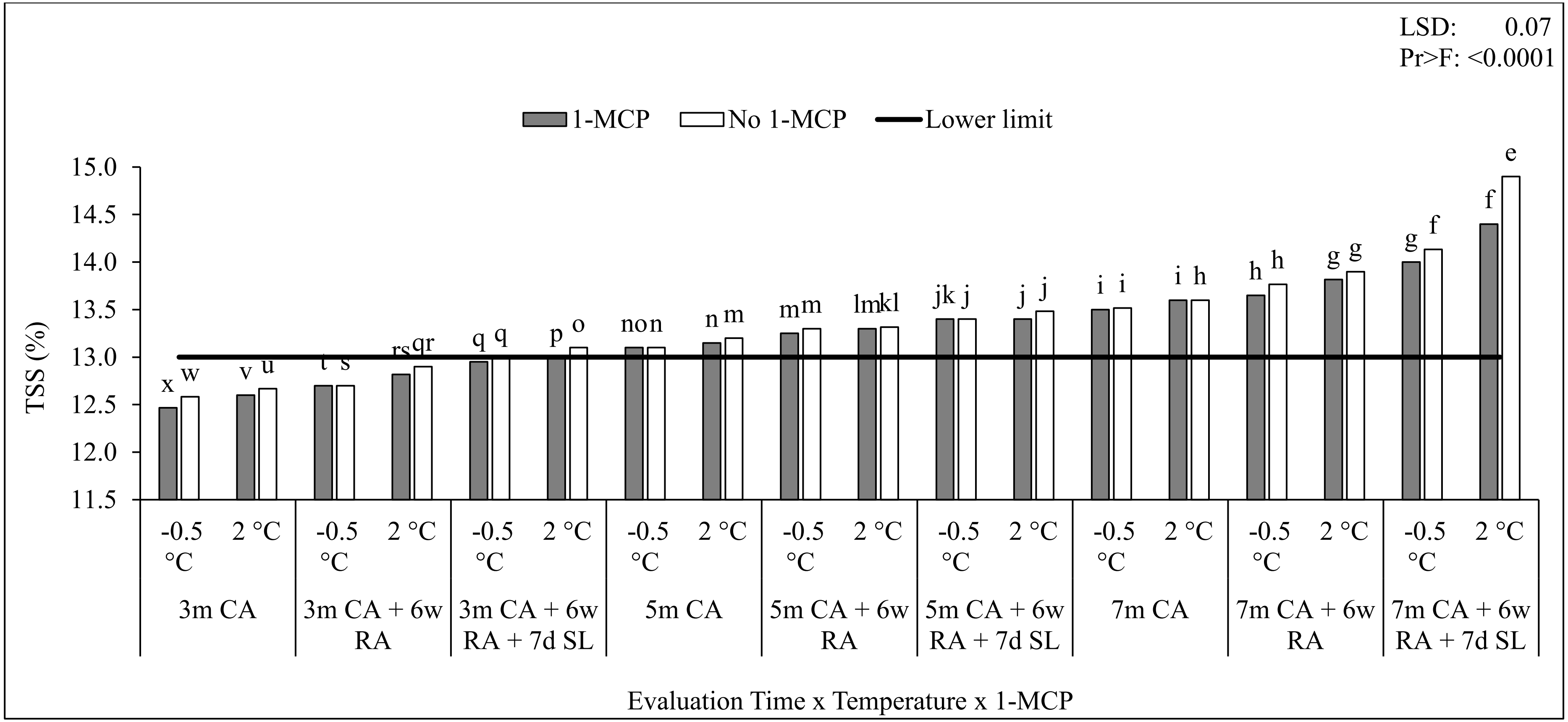

Figure 10: TSS for the interaction between evaluation time, temperature and 1-MCP treatment in 'Rosy Glow' apples. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C.) over specified times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

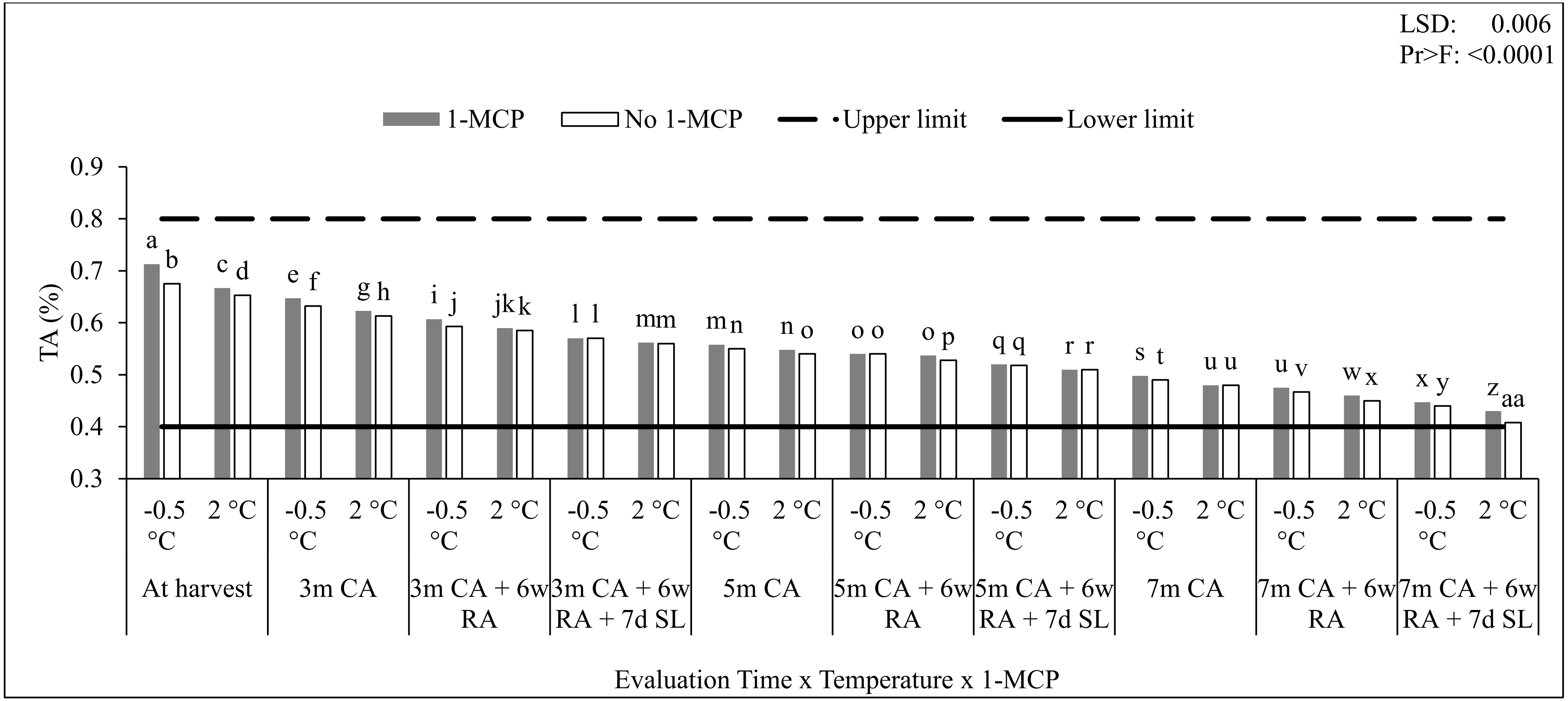

Figure 11: TA for the interaction between evaluation time, temperature and 1-MCP treatment in ‘Rosy Glow’ apples. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

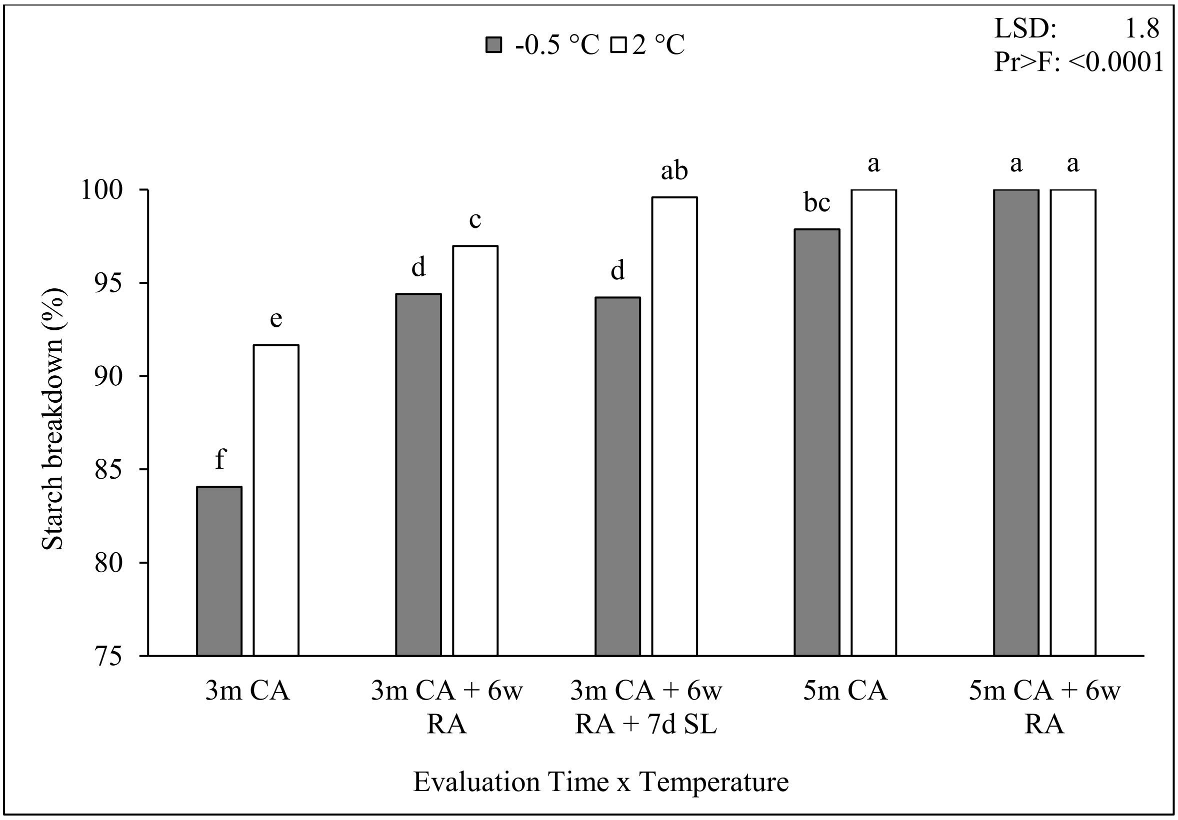

Figure 12: Starch breakdown for the interaction between evaluation time and temperature in ‘Rosy Glow’ apples. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season. Measurements were made on fruit that were stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

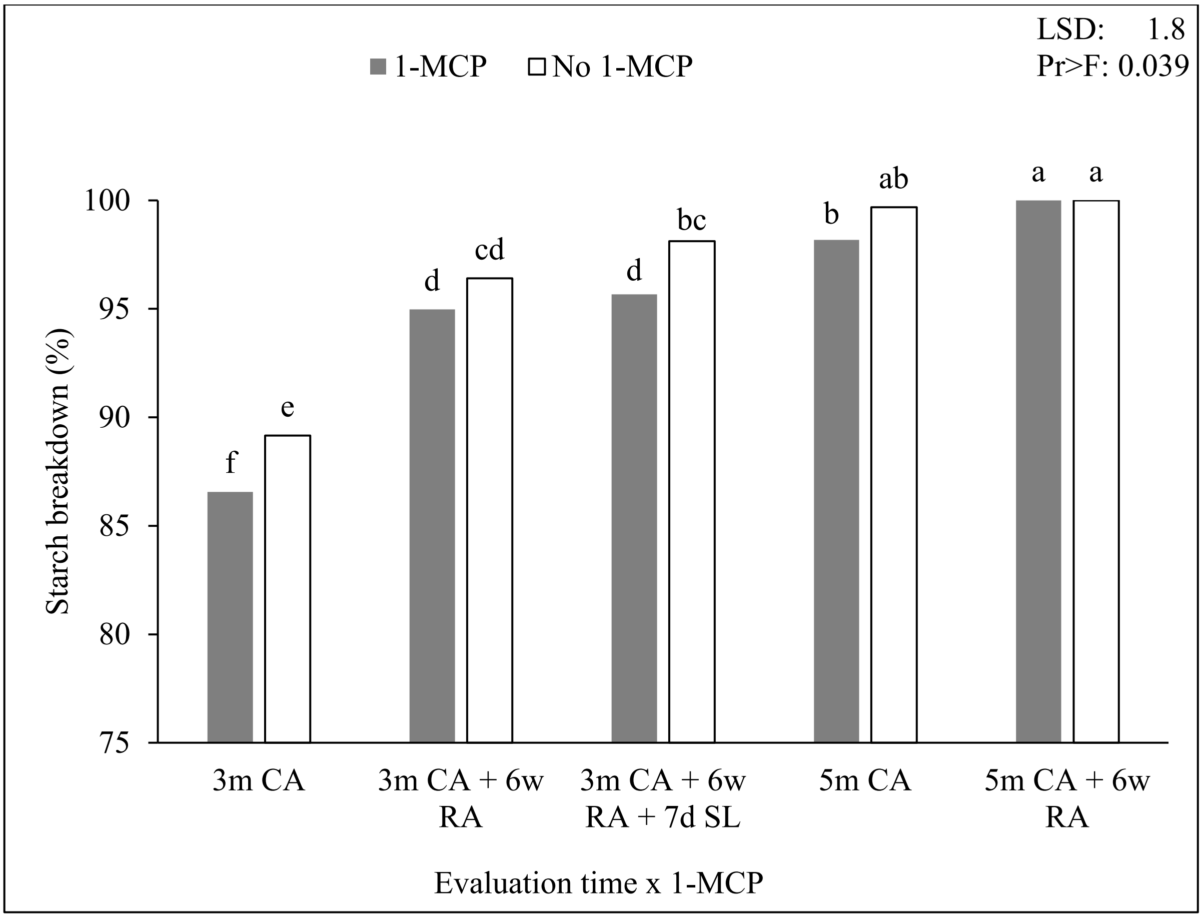

Figure 13: Starch breakdown for the interaction between evaluation time and 1-MCP treatment in 'Rosy Glow' apples. Fruit were harvested from orchard 20 on the Glen Fruin farm in the Elgin region of the Western Cape in the 2014 season. Measurements were made on fruit that were treated with or without 1-MCP and stored over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

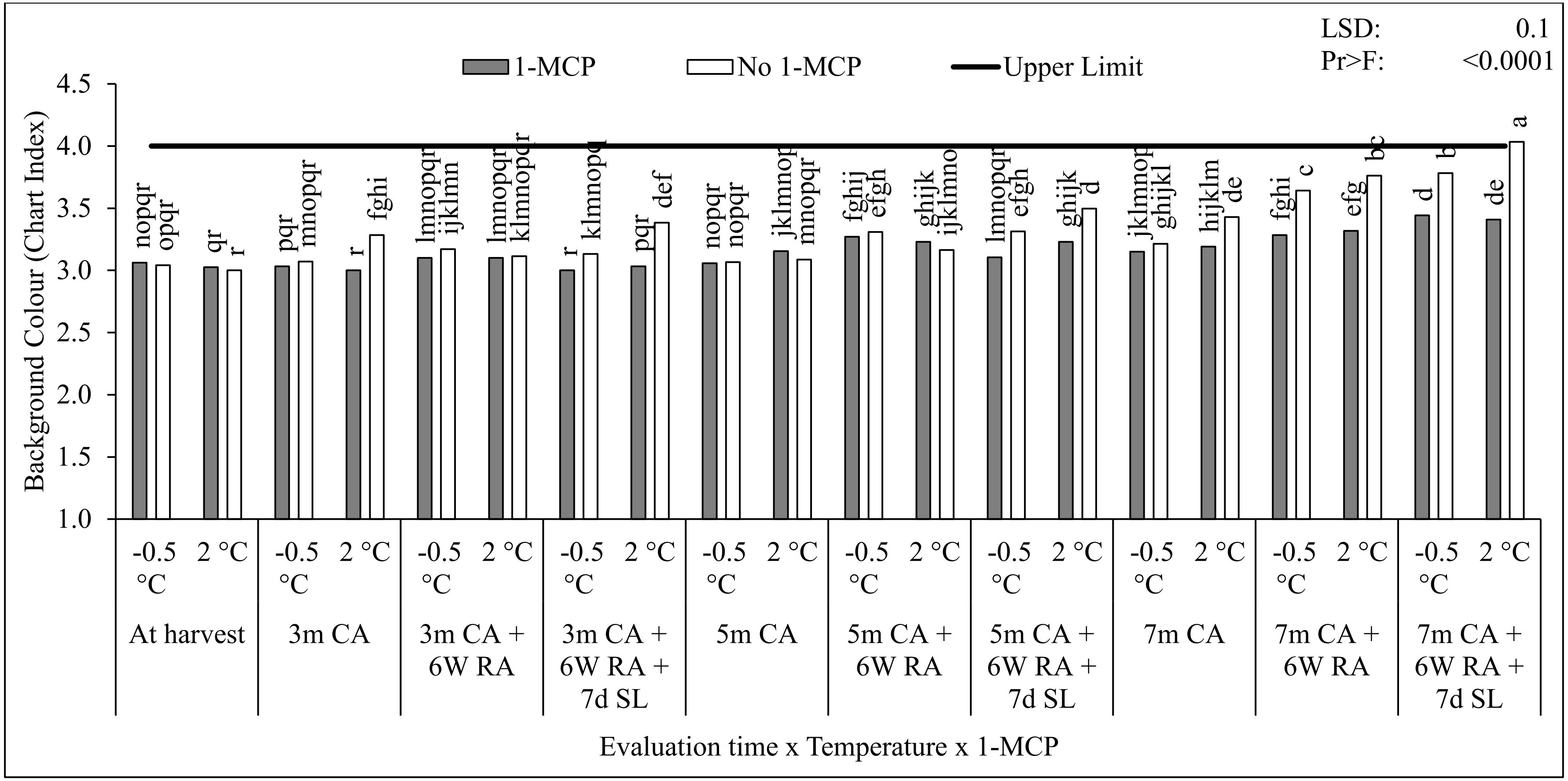

Figure 14: Background colour for the interaction between evaluation time, temperature and 1-MCP treatment in 'Rosy Glow' apples. Fruit were harvested at optimum maturity from orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

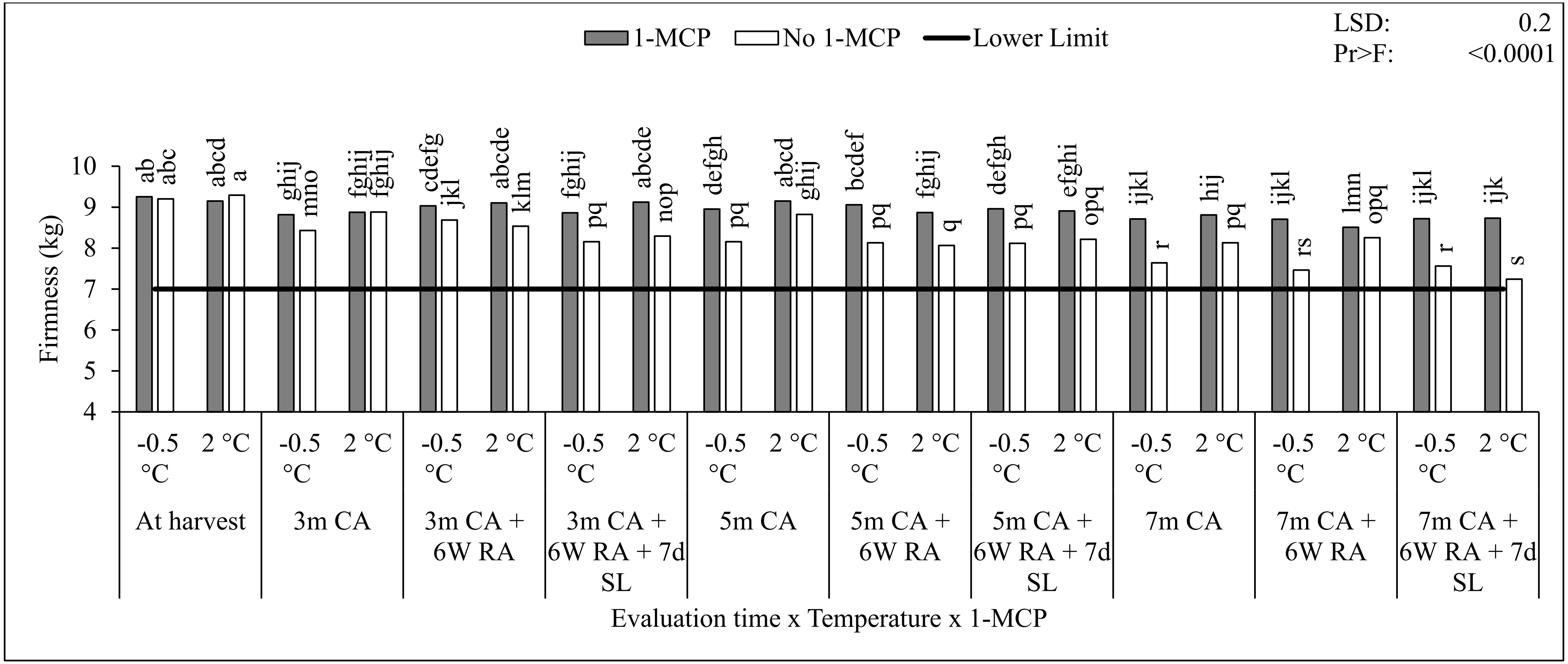

Figure 15: Firmness for the interaction between evaluation time, temperature and 1-MCP in 'Rosy Glow' apples. Fruit were harvested at optimum maturity from orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

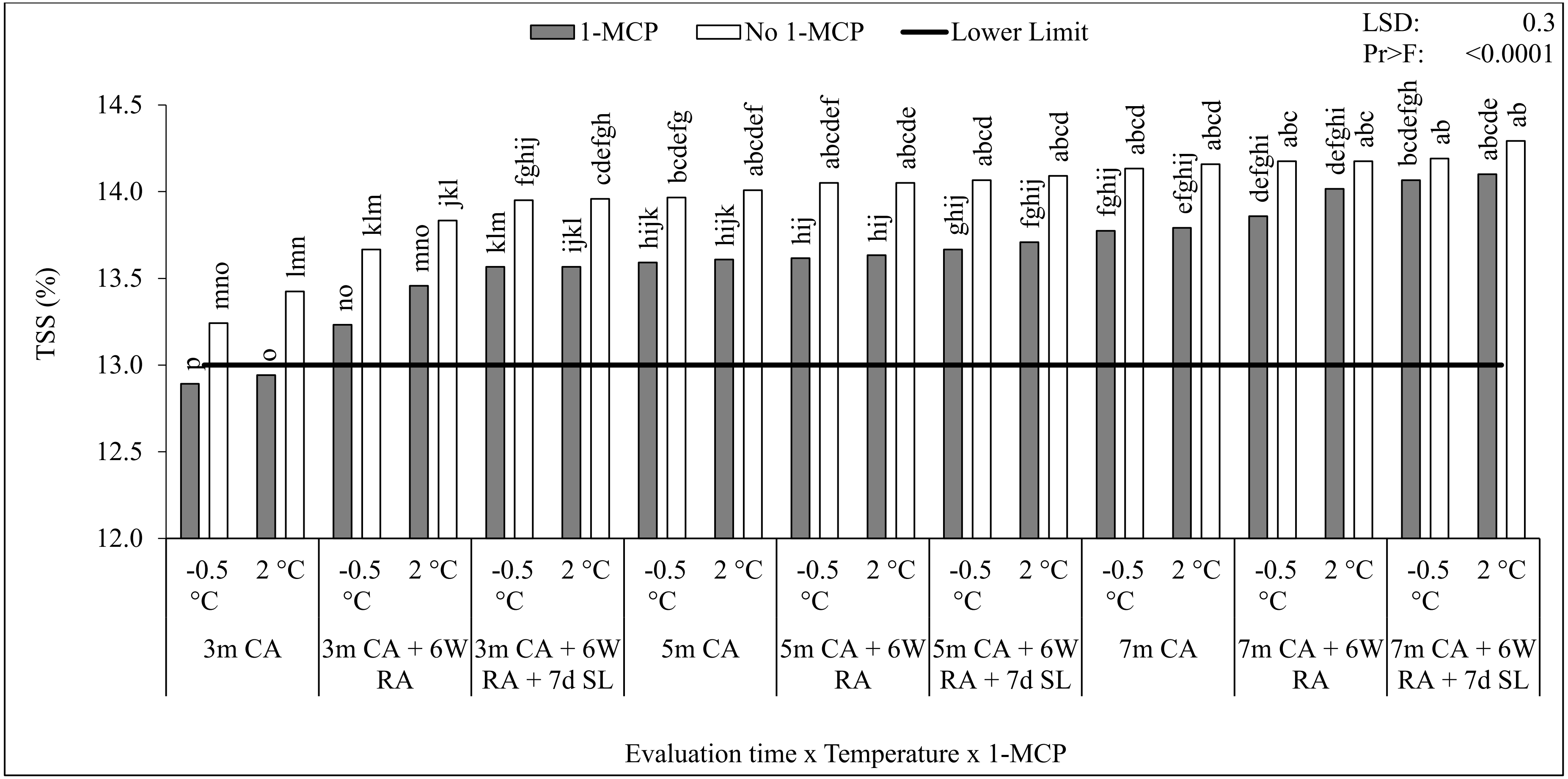

Figure 16: TSS for the interaction between evaluation time, temperature and 1-MCP in 'Rosy Glow' apples. Fruit were harvested at optimum maturity from orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

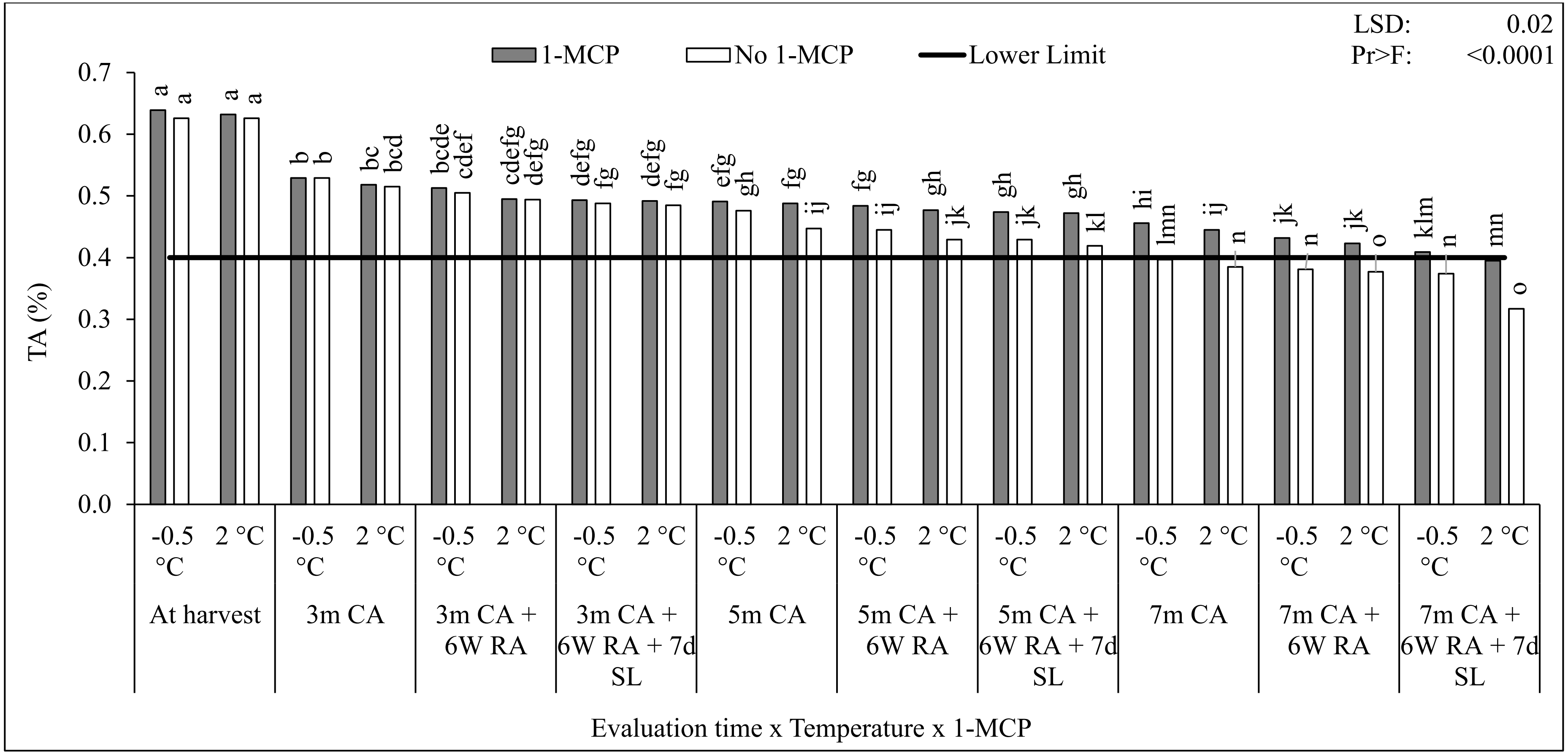

Figure 17: TA for the interaction between evaluation time, temperature and 1-MCP in 'Rosy Glow' apples. Fruit were harvested at optimum maturity from orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

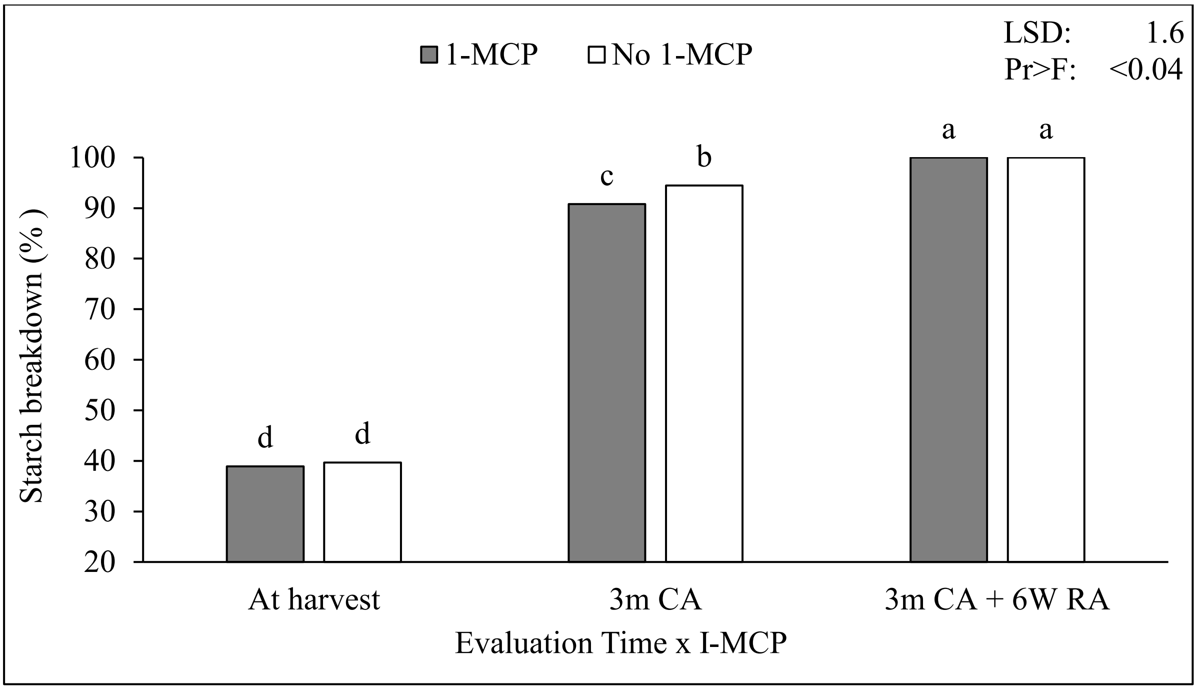

Figure 18: Starch breakdown for the interaction between evaluation time and 1-MCP treatment of 'Rosy Glow' apples. Fruit were harvested at optimum maturity from orchard 16 on the Damar farm in the Vyeboom region of the Western Cape in the 2015 season. Measurements were made on fruit that were treated with or without 1-MCP and stored over specified evaluation times (3, 5, 7 months (m) of controlled atmosphere (CA) + 6 weeks (6w) regular atmosphere (RA) and a subsequent 7-day shelf life period (7d SL) at ambient temperature).

Chapter 4: PAPER THREE

Susceptibility of 'Rosy Glow' apples to internal flesh browning in relation to tree age.

ABSTRACT

The effect of tree age on the development of the internal flesh browning (IB) in the 'Rosy Glow' apple cultivar after a long-term storage in controlled atmosphere (CA; 1.5% O_2 and 1% CO_2 at -0.5 °C) was investigated for two seasons. The objective was to determine whether the susceptibility of 'Rosy Glow' to IB after long term storage will decrease with tree age under South African conditions. Fruit were harvested at optimum maturity (<40% starch breakdown; SB) from 6 and 8 orchards in 2014 and 2015 season, respectively. Orchards in season one (2014) were in the fourth and sixth leaf, while those in season two (2015) were in their fourth and seventh leaf. Fruit were stored in CA conditions for 7 months and then moved into regular atmosphere (RA) for 6 weeks at -0.5 °C simulating shipping and stock rolling after which they were kept at ambient temperature (20 °C) for 7 days. Evaluations were done after each storage period to assess quality and susceptibility of apples to IB. Four types of IB (diffuse browning (DB), radial browning (RB), carbon dioxide browning (CO_2B) and combination browning (CB)) were observed in the two seasons with DB being the dominant IB type. Results indicated that, there was not enough evidence of a tree age effect on the development of IB. The interaction of evaluation time and orchard mostly affected browning development over the two seasons. Principal component analysis (PCA) of the data showed that total browning in the first season strongly positively associated with SB and TSS (75.5 and 86%, respectively) and positively but less strongly with background colour (50.2%). PCA analysis also indicated that total browning in the second season associated with background colour to a similar level as in the first season (55.9%). Although tree ages may not have been far apart enough, in order to create a clear contrast in the difference in fruit quality, another orchard factor namely harvest maturity may influence IB.

Keywords: controlled atmosphere, diffuse browning, evaluation time, long term storage, *Malus domestica* Borkh., orchard factors, radial browning

3.1 INTRODUCTION

The postharvest quality of fruit is affected by many factors including rootstock, tree age, mineral nutrition, soil management practices etc. (Bramlage, 1993; James, 2007; Butler, 2015). These factors can be divided into two main groups (pre and post-harvest) in conformity with the two main stages of fruit life. Bramlage (1993) further grouped the pre-harvest factors into two categories: cultural and climatic conditions.

Tree age has been reported to affect the quality of fruit (Khalid et al., 2012; Arshad et al., 2014; Butler, 2015). Butler (2015) observed significant variations in the incidence of browning (diffuse browning (DB) and combination browning (CB)), which was attributable to variations in tree age and said that, fruit from young 'Cripps' Pink' apple trees (7^{th} leaf) were more prone to the incidence internal browning (IB) compared to older trees (18^{th} leaf). Khalid et al. (2012) reported among others, a higher total soluble solid (TSS; °Brix) and percentage acidity in 18-year-old 'Kinnow' mandarins as compared to 3, 6, and 35-year-old ones. According to Bramlage (1993), young trees are most likely to be of higher vigour comparative to older trees, irrespective of the rootstock. Young apple trees are also more likely to bear bigger fruit (Bramlage, 1993), and may have a lower crop load in terms of fruit numbers (Treder et al., 2010). Fruit from young apple trees are more likely to contain less calcium and are thus, more prone to a variety of postharvest disorders (Bramlage, 1993). Tough et al. (1996) observed that, 'Braeburn' apple trees with low crop loads bore bigger fruits and the fruit contained low calcium but had a higher flesh firmness, soluble solids, background colour as well as starch pattern index (SPI). Low crop load 'Honeycrisp' apple fruit were also reported to contain low calcium levels, but were high in percentage blush colour, TA, soluble solids content, dry matter content and firmness (Serra et al., 2016). Ferguson and Watkins (1992) observed that fruit from low crop load trees were lower in calcium content, but higher in potassium concentrations and had more 'bitter pit' disorder as compared to high crop load tree fruit.

Apple trees, just like many other fruit trees, may bear more fruit as they age but consequently, the size of the fruit is most likely to reduce as the tree advances in age or as the crop load increases (Verheij, 1972; Wertheim, 1985; Crisosto et al., 1997). Wertheim (1985) reported that 'Karmijn' apple fruit used in their study had a reduced size as the tree aged. Butler (2015) observed that young (7^{th} leaf compared to older 18^{th} leaf) 'Cripps' Pink' orchard fruit were larger and more susceptible to browning. Crouch et al. (2015) indicated that younger trees (7^{th} leaf) were more susceptible to DB and CB, however tree age interacted with other factors like soil type to influence CB.

This study compared young 'Rosy Glow' trees (4th leaf) to slightly older ones (oldest available) in terms of their fruit quality and susceptibility to IB. The aim was to investigate whether 'Rosy Glow' fruit gets less susceptible to IB and has a better fruit quality (background colour (BC), starch breakdown (%SB), firmness (kg), total soluble solids (%TSS) and titratable acids (%TA) as the trees mature.

3.2. MATERIAL AND METHODS

3.2.1 Fruit material

'Rosy Glow' fruit were hand-picked from 6 and 8 commercial orchards in 2014 and 2015, respectively, in three locations: Vyeboom, Villiersdorp, and Elgin areas (Table 1). Fruit trees were grown and managed according to current commercial practices. Orchards were in their 4th and 6th leaf in the first season but 4th, 5th and 7th leaf in the second season (Table 1). Fruit were picked during the commercial harvest period, after a series of iodine tests, to check starch breakdown (SB percentage as a measure of maturity at harvest measured with 0.5 M potassium iodide (KI) on fruit cut surface).

3.2.2 Fruit number and harvest specifications

A total of 1920 (320 per orchard) and 2560 fruit were harvested at optimum maturity (30% - 40% SB) and evaluated in 2014 and 2015, respectively. Fruit were harvested at random from all parts of the tree canopy in same row per orchard and transported in boxes to the Department of Horticultural Science, Stellenbosch University and maturity evaluations were done immediately after harvest. Fruit were stored in regular atmosphere (RA) at -0.5 °C for one day to remove field heat before controlled atmosphere (CA) storage period commenced.

3.2.3 Fruit storage

Three fruit storage techniques were used in this trial namely CA, RA, and shelf-life (SL). Storage was done in CA at -0.5 °C for 7 months in Janny MT (France) CA plastic bins with sealed lids, hereafter called 'Janny bins', in the 2014 season. The Janny bins were 57 cm deep, 90.5 cm wide and 110 cm long (measuring from the inside) and contained 10 plastic lug boxes each containing fruit. Each Janny bin was connected to a pulsing inlet for nitrogen (N_2), oxygen (O_2) and CO_2 as well as a gas overflow. The CO_2 and O_2 concentrations were kept constant at 1 and 1.5%, respectively, using an automatic pulsing system (Janny MT Bins Pulse Gas Control System, Gas At Site (Pty) Ltd., South Africa). In the 2015 season, fruits were stored in the CA facility of the Agricultural Research Council (ARC-Infruitec) due to an increase in fruit numbers. In South Africa, Pink Lady® can be stored in CA (≥1.5% O_2 and ≤1% CO_2) conditions for 7 months at -0.5 °C (Hurndall and Fourie, 2003). Fruit were moved into RA at -0.5 °C for

6 weeks to simulate packing, shipping, and stock rolling after CA storage. Later, fruit were kept at room temperature (20 °C) for 7 days to simulate ripening or the SL period.

3.2.4 Fruit browning evaluation and description

At the end of each storage period, the number of fruit required for evaluation at that time was selected at random from the lot in storage. Individual fruit was cut equatorially but closer to the stem end for IB assessment. IB was assessed based on the pattern of brown colour observed in the flesh of fruit as well as the part of flesh affected as specified by James, (2007). IB type and presence of disorder were recorded for individual fruit after the evaluation was conducted.

3.2.5 Experimental design

The trial was a completely randomised design, analysed in a two-way analysis of variance (ANOVA) with tree age and evaluation time as factors. Assessment at each evaluation time consisted of ten replicates of eight fruit per orchard for both seasons. Fruit were evaluated at four evaluation times:

1. at harvest
2. after 7 months (m) of storage in CA (1.5% O_2, 1% CO_2) conditions at -0.5 °C (7m CA)
3. after 7m CA + 6 weeks (w) of RA storage at –0.5 °C (7m CA + 6w RA)
4. after 7m CA + 6w RA + 7 days (d) of SL at 20 °C (7m CA + 6w RA + 7d SL)

3.2.6 Physicochemical analyses

Maturity and quality measurements were conducted after CA, RA and SL. All assessments were done on each fruit for all replications, unless stated otherwise. IB (%), mass (g), diameter (mm), firmness (kg), background colour, greasiness, SB (%), TSS (%) and TA (%) measurements were performed as described in Chapter 2.

3.2.7 Statistical analysis

Physicochemical analyses data were subjected to ANOVA using SAS Enterprise guide version 5.1 (SAS Institute Inc., Cary, NC, USA) and XLSTAT version 2015.5 (Addinsoft Inc., NY, USA). Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means. Pearson's correlation coefficient was generated and used to establish correlations between tree age and/or quality parameters to IB development at harvest and after storage.

3.3 RESULTS

3.3.1 Browning incidence and browning types observed

3.3.1.1 Season one (2014)

From the 1920 fruit analysed, 376 (19.6%) were observed to have been affected with four types of IB (DB, RB, CO_2B and CB: Figs. 1A, B) over all storage periods. This percentage seems is commercially significant since it exceeds the maximum allowable incidence (<1%) that is stipulated by the International Pink Lady® Association (IPLA) standards. DB was the dominant browning type in this season, and it affected 339 fruit (90.2% of the total browning). RB affected 9 fruit, CO_2B affected 5 fruit and CB affected 22 fruit, contributing to 2.4, 1.3 and 6.1% of the total browning, respectively (Fig. 1B).

Six orchards were used in this season and they were each found susceptible to at least one type of browning disorder, albeit to different degrees (Fig. 2). Fruit from the Glen Fruin orchard exhibited only DB, 4th leaf Graymead orchard fruit exhibited only CO_2B, the Monteith fruit showed both DB and CO_2B, while fruit from the Damar orchard exhibited all the browning types except CO_2B (Table 2). Fruit from the Southfield and 6th leaf Graymead orchards exhibited all four IB types over all the storage periods (Table 2). Interestingly, two orchards, 6th leaf Graymead and 4th leaf Graymead, from the same farm and practices, exhibited the highest and the lowest incidence of IB, respectively. A new type of CB (RB+ CO_2B) was observed in fruit from Damar and Graymead (Table 2).

3.3.1.2 Season two (2015)

From the total of 2560 fruit harvested in 2015, 246 fruit (9.6%) were observed with four IB types over all the storage periods (DB, RB, CO_2B and CB; Fig. 3A, B). DB affected 148 fruit (60.2% of the total browning), also making it the dominant browning type in the 2015 season. RB affected 60 fruit, CO_2B affected 9 fruit and CB affected 29 fruit contributing to 24.4, 3.7 and 11.8% of the total browning, respectively (Fig. 3B).

Eight orchards were used in this season and the fruit from each orchard were susceptible to at least one type of browning (Fig 4). All browning types were observed in all orchards except that, CO_2B was not present in Chiltern 23 and De Rust (Table 3). Here again, it is worthy to note that two orchards, both 4th leaf, which lay side by side in the Chiltern farm, exhibited very different incidence levels of IB (Fig. 4). A new type of CB (RB+CO_2B) was observed this season also, in fruit from Chiltern 24 orchard (Table 3).

3.3.2 Effect of evaluation time and tree age on total browning and browning type

3.3.2.1 Season one (2014)

Results revealed that evaluation time, but not tree age, influenced total IB (Pr>F: <0.0001), DB (Pr>F: <0.0001) as well as CB (Pr>F = 0.002; Table 4). Even though RB only affected five fruit, it was influenced by the interaction of both evaluation time and tree age (Pr>F = 0.047; Table 4). Figure 5 shows the influence of evaluation time on total browning, DB and CB. Total browning and CB were the highest at 7m CA + 6w RA. Total browning and DB at evaluation time 7m CA + 6w RA were significantly higher than at 7m CA, but not at 7m CA + 6w RA + 7d SL even though total browning was highest at 7m CA + 6w RA + 7d SL (Fig. 5). CB was the highest at 7m CA + 6w RA and it was like CB at 7m CA, but significantly higher than that of evaluation time 7m CA + 6w RA + 7d SL (Fig. 5). According to Table 2, a new type of CB comprised of RB and CO_2B, was identified in three fruit. Further statistical analysis was not conducted on RB due to the low incidence. In general, tree age did not influence the incidence of browning. However, it is worthy to note that the two Graymead orchards which are situated side by side were used in this trial. These two orchards were not compared statistically, but data collected indicated that 1 and 129 fruit from the 4th and 6th leaf Graymead, were affected by IB, respectively (Table 2).

3.3.2.2 Season two (2015)

Results showed that evaluation time, but not tree age influenced total IB (Pr>F: <0.0001), DB (Pr>F: <0.0001), RB (Pr>F: <0.0001) and CB (Pr>F: <0.0001; Table 5). CO_2B was influenced by the interaction of evaluation time and tree age (Pr>F= 0.033; Table 5). Figure 6 shows how total IB and the four browning types varied with evaluation time. They all increased with time reaching their highest level after 7m CA + 6w RA + 7d SL, except for CB, which was significantly higher at 7m CA + 6w RA than after 7m CA + 6w RA + 7d SL (Fig. 6). RB at 7m CA was also not significantly different from RB at 7m CA + 6w RA. No further statistics was performed on CO_2B due low prevalence level.

3.3.3 Effect of evaluation time and orchard on total browning and browning types

3.3.3.1 Total browning: season one (2014)

According to Table 6, total IB and DB, RB and CO_2B types were influenced by the interaction of orchard and evaluation time. CB however, differed with evaluation time and orchard but not their interaction. Figure 7 shows how total browning varied between orchards over time. Graymead 6th had the highest percentage of flesh browning and did not differ significantly from Damar at 7m CA, but they both differed significantly from Monteith and Southfield. Glen Fruin and Graymead 4th had the lowest percentage browning at 7m CA but were statistically like Monteith. At 7m CA + 6w RA, total browning was significantly higher in the Damar orchard followed by Graymead 6th, which did not differ significantly from Southfield. Monteith, Graymead 4th and Glen Fruin exhibited significantly lower levels of the disorder and did not differ from one another at evaluation time 7m CA + 6w RA (Fig. 7). During the SL storage period (7m CA + 6w RA + 7d SL) fruit from Monteith showed a much higher browning level that was not seen in the previous evaluation times. Even though Graymead 6th recorded the highest incidence of browning at this evaluation time, it did not differ significantly from the percentages recorded by Monteith and Damar. Figure 7 indicates that fruit from Damar recorded the highest browning percentage per evaluation time, where 77% of fruit evaluated were affected by the disorder. However, Graymead 6th exhibited the highest overall incidence of browning.

3.3.3.2 Total browning: season two (2015)

According to Table 7, total browning and all the browning types, except CO_2B, were influenced by the interaction of orchard and evaluation time. All orchards except Glen Brae showed large variances in the browning percentages over the evaluation times (Fig. 8). Browning percentages generally increased with time in all the orchards, even though some increments were not significant for some orchards. The highest incidence of browning was seen for the Chiltern 24 orchard at 7m CA + 6w RA + 7d SL and it differed significantly from all other orchards and at all evaluation times, except from the Graymead orchard after 7m CA + 6w RA and 7m CA + 6w RA + 7d SL. De Rust, Glen Fruin and Southfield all exhibited significantly higher browning percentage at 7m CA + 6w RA + 7d SL as compared to 7m CA + 6w RA (Fig. 8). Chiltern 24 and Texel both exhibited significantly different browning percentages at the different evaluation times, while Graymead showed a different percentage after 7m CA but a similar value after 7m CA + 6w RA and 7m CA + 6w RA + 7d SL. The browning percentage of Chiltern 23 fruit at 7m CA was like that at 7m CA + 6w RA but significantly lower the one observed at 7m CA + 6w RA + 7d SL. Total browning percentage observed at 7m CA + 6w RA was similar to what was observed at 7m CA + 6w RA + 7d SL from the Chiltern 23 orchard (Fig. 8).

Chiltern 24 exhibited a significant increase in browning percentage with time and it was like Graymead after 7m CA and 7m CA + 6w RA + 7d SL, but significantly lower than Graymead after 7m CA + 6w RA (Fig. 8). Browning percentage observed in Chiltern 24 after 7m CA + 6w RA + 7d SL was the highest in the season and was significantly higher than the other orchards at all evaluation times but Graymead after 7m CA + 6w RA and 7m CA + 6w RA + 7d SL (Fig. 8). It is however worthy to note that Graymead exhibited the highest browning percentage after 7m CA + 6w RA. No browning incidence was recorded for the Texel orchard after 7m CA, but Texel exhibited browning percentages like Chiltern 24 and Graymead, after 7m CA + 6w RA and 7m CA + 6w RA + 7d SL respectively (Fig 8). The percentage browning for fruit from the Texel orchard was significantly higher than for Chiltern 23, De Rust, Glen Brae, Glen Fruin and Southfield after 7m CA + 6w RA and 7m CA + 6w RA + 7d SL (Fig. 8).

3.3.3.3 Diffuse browning: season one (2014)

Figure 1B indicates that approximately 90.2% of the browning incidence in this season was due to the DB type and according to Table 6, DB was influenced by the interaction of orchard and evaluation time. Figure 9 shows DB percentages for the season one orchards. Fruit from Damar, Graymead 6th and the Southfield orchards showed more susceptibility to DB after 7m CA and 7m CA + 6w RA, but after 7m CA + 6w RA + 7d SL, DB percentage was higher in the Damar, Graymead 6th and the Monteith orchards. Damar orchard exhibited the significantly highest DB percentage in the season (7m CA + 6w RA). However, similar DB percentage was observed in the Damar and Graymead 6th orchards after 7m CA, while after 7m CA + 6w RA + 7d SL, the Damar, Graymead 6th and Monteith orchards exhibited similar DB percentages (Fig. 9). The Glenfruin, Graymead 4th and Monteith orchards were least susceptible to DB throughout the season, but for the increase observed in the Monteith orchard after 7m CA + 6w RA + 7d SL. The Glenfruin and Graymead 4th orchards showed no diffuse browning after 7m CA + 6w RA and the Graymead 4th orchard again showed no DB after 7m CA + 6w RA + 7d SL.

Basically, it can be said that the effect observed for total IB from the interaction of orchard and evaluation time is as a result of DB.

3.3.3.4 Diffuse browning: season two (2015)

It can be observed from Figure 10 that Chiltern 23, Chiltern 24, De Rust, Glen Fruin, Southfield and Texel exhibited increasing DB percentages over time and their highest incidence was recorded after 7m CA + 6w RA + 7d SL. The highest incidence of DB recorded after 7m CA + 6w RA + 7d SL was for fruit from the Texel orchard and it differed significantly from all other orchards at all evaluation times. Graymead recorded a similar DB percentage after 7m CA + 6w RA + 7d SL as after 7m CA + 6w RA, however, a significantly lower percentage was recorded after 7m CA (Fig. 10). On the other hand, there were no significant differences between the DB percentages after 7m CA and 7m CA + 6w RA for Chiltern 23, Chiltern 24, Glen Fruin and Texel, whereas significantly higher percentages were recorded after 7m CA + 6w RA + 7d SL. Even though Texel exhibited the highest percentage DB at 7m CA + 6w RA + 7d SL, Chiltern 24 and Graymead also recorded high levels throughout the season (Fig. 10).

3.3.3.5 Radial, carbon dioxide and combination browning: season one (2014)

These three browning types formed up to about 9.8% of the total IB and from Figure 1B, CO_2B is the least prevalent of all the types. RB was highest in Damar at 7m CA (Fig. 11) and CO_2B was highest in Monteith at 7m CA (Fig. 12). Further statistical analysis was not conducted on RB and CO_2B due to low prevalence.

CB varied with orchard (Pr>F = 0.000) and evaluation time (Pr>F = 0.001), but not their interaction (Pr>F = 0.160; Table 6). Figure 13 indicates the prevalence of CB per orchard. Graymead 6th recorded the highest CB percentage (3.5%) and did not differ significantly from CB observed from the Damar orchard (2.2%) but differed significantly from Southfield (1.6%) and the rest of the orchards (0%). Damar, even though did not differ significantly from Southfield, was significantly different from Glenfruin, Graymead 4th and Montieth as well. Southfield did not differ significantly from Glenfruin, Graymead 4th and Montieth. Figure 14 shows that CB was the highest at evaluation time 7m CA + 6w RA and it differed significantly from evaluation time 7m CA + 6w RA + 7d SL but not significantly from 7m CA.

3.3.3.6 Radial, carbon dioxide and combination browning: season two (2015)

RB was first observed after 7m CA + 6w RA in this season in three orchards (Chiltern 24, Graymead and Texel) but after 7m CA + 6w RA + 7d SL it was observed in all the orchards (Fig.15). According to Table 7, RB differed with the interaction of orchard and evaluation time. The highest incidence of RB was observed in Chiltern 24 after 7m CA + 6w RA + 7d SL however, it did not differ significantly from RB incidence observed in the Graymead orchard at the same evaluation time. There was a general increase in the RB incidence in all the orchards from after 7m CA + 6w RA to after 7m CA + 6w RA + 7d SL (Fig. 15). Figure 15 also shows that RB incidence after 7m CA + 6w RA was similar in all the orchards except for Graymead, where RB incidence was significantly higher. At evaluation time 7m CA + 6w RA + 7d SL, RB was lowest in Texel and it was significantly different from RB incidence in the Chiltern 24, De Rust and Graymead orchards (Fig. 15). RB in De Rust was like Chiltern 23, Glen Brae, Glen Fruin and Southfield, significantly lower than Chiltern 24 and Graymead.

CB, like RB, was first observed after 7m CA + 6w RA, but in contrast to RB it was observed in all orchards except Southfield (Fig. 16). Texel exhibited the highest incidence of CB after

7m CA + 6w RA and it differed significantly from all orchards. Chiltern 23, Chiltern 24, De Rust and Graymead showed a similar level of CB incidence after 7m CA + 6w RA and they were higher than that of Glen Brae, Glen Fruin and Southfield at the same evaluation time (Fig. 16) even though, Glen Brae, Glen Fruin and Southfield were like De Rust. No CB was observed in Chiltern 23, Glen Brae, Graymead and Texel after 7m CA + 6w RA + 7d SL. A similar level of CB was observed in the remaining four orchards (Fig. 16). CO_2B observed in this season was neither influenced by evaluation time nor orchard according to Table 7. It can however be seen that the p-value of the interaction effect (0.079) was close to 0.05 (Table 7).

3.4 Effect of evaluation time and tree age on fruit maturity and quality

3.4.1 Firmness, titratable acidity and total soluble solids: season one (2014)

Table 8 indicates the effect of the interaction between tree age and evaluation time on fruit firmness and TA. Firmness and TA were significantly higher in 4th leaf fruit at harvest than it was throughout the season. The firmness remained similar for both ages at each of the subsequent evaluation times (Table 8). TA, however, was significantly higher in 4th leaf fruit, except at 7m CA + 6w RA + 7d SL when a similar TA was recorded for fruit from trees of both ages (Table 8). Firmness and TA were, however, maitained within the International Pink Lady® Association (IPLA) export acceptable levels (Firmness > 7 kg and TA = 0.4% – 0.8%; Hurndall and Fourie, 2003) throughout the season (Table 8). TSS was influenced by evaluation time alone and was lowest at harvest and highest after 7m CA + 6w RA + 7d SL but, was similar when measured after 7m CA and 7m CA + 6w RA (Table 11).

3.4.2 Firmness, titratable acidity and total soluble solids: season two (2015)

According to Table 9, firmness, TA and TSS significantly varied from one evaluation time to the other. Firmness and TA were the highest at harvest but, reduced significantly over time and were lowest at 7m CA + 6w RA + 7d SL. TSS on the contrary was the lowest at harvest and increased over time and was highest after 7m CA + 6w RA + 7d SL. Firmness and TSS varied also with tree age. According to Table 10, fruit from the 7th leaf trees had a significantly higher firmness, but lower TSS levels than 4th leaf tree fruit.

3.4.3 Background colour and starch breakdown: season one (2014)

According to Table 11, evaluation time significantly affected background colour and SB and they were all the highest at 7m CA + 6w RA + 7d SL. Background colour at 7m CA + 6w RA + 7d SL was significantly higher than at the other evaluation times but, the background colour at harvest and at 7m CA + 6w RA did not differ from one another. At 7m CA, background colour was significantly lower than all the other evaluation times (Table 11). On average, SB was 35% at harvest (Table 11).

3.4.4 Background colour and starch: season two (2015)

Table 12 indicates the effect of the interaction between tree age and evaluation time on fruit background colour and SB. Background colour was significantly higher in both 4th and 7th leaf fruit after 7m CA + 6w RA + 7d SL than it was throughout the season. However, after 7m CA + 6w RA + 7d SL, 7th leaf fruit exhibited a significantly higher or yellower background colour compared to 4th leaf fruit (Table 12). Background colour was similar for fruit from both ages of trees at harvest and after 7m CA + 6w RA. SB was complete (100%) for both tree ages after 7m CA. According to Table 12, 4th leaf fruit had a significantly higher level of SB than the 7th leaf fruit at harvest. SB was, however, within acceptable IPLA optimum harvest limits for both ages.

3.5 Effect of evaluation time and orchard on fruit maturity and quality

3.5.1 Background colour: season one (2014)

Background colour was influenced by the interaction of orchard and evaluation time (Table 13). According to Fig. 17, Graymead 6th and Damar ripened the most during 7m CA + 6w RA + 7d SL storage and while they did not differ significantly from one another, they did differ significantly from all the other orchards. Graymead 6th and Damar exhibited a high background colour at all the evaluation times throughout the season. At harvest, Damar was the most mature, followed by Glen Fruin and Graymead 6th. At 7m CA, Graymead 6th recorded the most advanced maturity and Glen Fruin the lowest (Fig. 17). At 7m CA + 6w RA, Graymead 6th maintained a maturity level like the level at 7m CA and this was again the significantly higher than all the other orchards. Ripening was similar in Monteith, Graymead 4th and Southfield, while Glen Fruin maintained the position for the significantly lowest ripening after 7m CA + 6w RA (Fig. 17). Glen Fruin exhibited the lowest ripening at SL but, did not differ significantly from Southfield. Graymead 4th and Monteith showed a fairly high level of ripening, but still lower than that of Damar and Graymead 6th after 7m CA + 6w RA + 7d SL (Fig. 17).

3.5.2 Background colour: season two (2015)

According to Table 14, at least one orchard recorded different background colours at different evaluation times. Figure 18 shows that all the orchards, except Glen Brae, ripened most when stored at ambient temperature (20 °C) on the shelf. Chiltern 24 and Texel recorded a similar background colour after 7m CA + 6w RA and after 7m CA. Texel and Graymead recorded the highest ripening levels after 7m CA + 6w RA + 7d SL and they were significantly higher than fruit from all other orchards. De Rust had the lowest background colour at harvest and exhibited a similar background colour after 7m CA. Fruit from the De Rust orchard ripened steadily when stored in 7m CA + 6w RA and subsequently reached its highest level at 7m CA + 6w RA + 7d SL, where it was like Chiltern 24 but, significantly higher than Southfield, Glen Brae and Chiltern 23 (Fig. 18). Generally, fruit lost background colour during 7m CA and ripened more at 7m CA + 6w RA + 7d SL.

3.5.3 Starch breakdown: season one (2014)

At harvest, Southfield exhibited the highest SB (34%), but did not differ significantly from Damar (Fig. 19). Glen Fruin had the least SB differing significantly from the other orchards (Fig. 19). Regardless of the SB level at harvest, all orchards exhibited complete SB after 7m CA storage (Fig. 19).

3.5.4 Starch breakdown: season two (2015)

From Figure 20, Chiltern 23 exhibited the highest SB (53%) which differed significantly from all the other orchards at harvest. Graymead, Southfield and Texel had a similar level of starch at harvest and Glen Fruin and De Rust. Chiltern 24 and Glen Fruin did not differ significantly from one another, just as Graymead did not differ from De Rust. Glen Brae had the least SB at harvest, and it differed significantly from the rest of the orchards. Regardless of the SB level at harvest, all orchards exhibited complete SB after 7m CA storage (Fig. 20).

3.5.5 Fruit firmness: season one (2014)

Fruit firmness for all the orchards was high at harvest and according to Table 13, fruit firmness varied with the interaction of orchard and evaluation time. Figure 21 illustrates the fruit firmness from the different orchards at various evaluation times. Graymead 4th recorded the most firm fruit at harvest, which differed significantly from other orchards except for Damar. Monteith and Graymead 6th had a lower fruit firmness than Damar and Graymead 4th but, a significantly higher firmness compared to Southfield and Glen Fruin fruit at harvest (Fig. 21). Graymead 4th fruit exhibited a consistently higher firmness, which was significantly different from the other orchards at all the evaluation times. Besides at harvest, Monteith showed fruit with the lowest firmness throughout the storage for this season but, it neither differed significantly from Damar and Glen Fruin at 7m CA, nor from Graymead 6th at 7m CA + 6w RA + 7d SL (Fig. 21). Overall firmness remained within acceptable IPLA limits (≥7 kg) in all the orchards except, for fruit from the Monteith orchard, which scored a firmness of 6.7 kg at 7m CA + 6w RA + 7d SL.

3.5.6 Fruit firmness: season two (2015)

Figure 22 demonstrates how fruit firmness differed over time in the different orchards. At harvest fruit from all the orchards exhibited their highest firmness levels. Even though some orchards maintained a high level of firmness until after 7m CA, there is enough evidence to state that most orchards lost firmness over time, since the lowest firmness levels were recorded after 7m CA + 6w RA + 7d SL (Fig. 22). Chiltern 24 and Texel fell below the IPLA standard after 7m CA and continued to decrease after 7m CA + 6w RA and 7m CA + 6w RA + 7d SL. Chiltern 23, Graymead, Glen Brae and Southfield maintained a high level of firmness throughout the 2015 season. Graymead recorded a significantly higher firmness level that differed from all the orchards at all the evaluation times, except after 7m CA + 6w RA where it was similar to Glen Brae. Besides Chiltern 24, Texel and De Rust, all the other orchards recorded IPLA acceptable firmness levels (≥7 kg) throughout the season (Fig. 22).

3.5.7 Total soluble solids: season one (2014)

At harvest Graymead 6th, Monteith and Damar had TSS levels acceptable by IPLA standards (13%), but Glen Fruin, Southfield and Graymead 4th were below the criteria (Fig. 23). Graymead 6th recorded the highest TSS, which was significantly different from all the other orchards at harvest and Glen Fruin was lowest at harvest. Damar and Monteith had a similar TSS and so did Southfield and Graymead 4th at harvest. The TSS level at evaluation time 7m CA was significantly higher in Graymead 6th, followed by Damar and Monteith which again recorded a similar level (Fig. 23). Graymead 4th and Southfield also had a similar TSS level, which was significantly higher than Glen Fruin but lower than Graymead 6th, Monteith and Damar at 7m CA. Glen Fruin again recorded the lowest TSS after 7m CA + 6w RA storage. However, Southfield and Monteith had levels similar to Graymead 6th, which again was the highest, while Damar and Graymead 4th followed after 7m CA + 6w RA (Fig. 23). After 7m CA + 6w RA + 7d SL storage Graymead 6th had a significantly higher TSS than all the other orchards, while Glen Fruin maintained the lowest TSS. Graymead 4th and Southfield had similar TSS levels and Damar and Monteith after 7m CA + 6w RA + 7d SL storage (Fig 23). It can be observed that the orchards show a similar pattern in their TSS levels over the evaluation times, except at 7m CA + 6w RA where Monteith and Southfield had TSS levels like Graymead 6th.

3.5.8 Total soluble solids: season two (2015)

Figure 24 indicated that the TSS level generally increased with time, as most of the orchards exhibited their highest TSS levels at 7m CA + 6w RA + 7d SL. Glen Brae recorded the highest

TSS at harvest and was significantly different from that of other orchards, while Southfield recorded the lowest TSS (Fig. 24). Chiltern 23 and 24 as well as Glen Fruin and De Rust exhibited a similar TSS at harvest but were all together significantly different from the other orchards (Fig. 24). Texel had the 2nd highest TSS significantly different from all the other orchards at harvest and TSS levels measured in fruit from Graymead at harvest was significantly lower than that of all the other orchards but not Southfield (Fig. 24). At 7m CA, Glen Brae again recorded the highest TSS, followed by Texel, while Southfield records the lowest TSS just like it did at harvest and all the other orchards exhibited similar levels of TSS (Fig. 24). TSS recorded at 7m CA + 6w RA, indicated Glen Brae fruit as maintaining the highest level, again followed by Texel, while Southfield still maintained the lowest TSS, but this time was like De Rust (Fig. 24). Graymead exhibited a TSS level like Chiltern 23 and 24 while Glen Fruin also recorded TSS levels like Graymead and Chiltern 23 after 7m CA + 6w RA, however, TSS recorded from Glen Fruin differed significantly from Chiltern 24 TSS after 7m CA + 6w RA (Fig. 24). Glen Brae maintained the highest TSS still followed by Texel, and Southfield recorded the lowest TSS but was again like De Rust at 7m CA + 6w RA + 7d SL (Fig. 24). Chiltern 23 and 24 had TSS like Glen Fruin and Graymead also exhibited TSS levels like Glen Fruin and Chiltern 24, but TSS from Chiltern 23 was significantly higher than TSS from Graymead at 7m CA + 6w RA + 7d SL (Fig. 24). Apart from Graymead and Southfield (at harvest), acceptable IPLA TSS levels were attained by all the orchards throughout the 2015 season including Graymead and Southfield for other storage and evaluation times.

3.5.9 Titratable acidity: season one (2014)

Generally, TA decreased over time in all the orchards and according to Table 13, TA varied from one orchard to the other at different evaluation times. Figure 25 illustrates that, at harvest, Monteith exhibited a significantly higher level of TA and Glen Fruin the lowest. Damar, together with the two Graymead orchards (4th and 6th leafs), recorded a similar TA, which was significantly lower than Monteith, but significantly higher than Southfield and Glen Fruin (Fig. 25). At 7m CA, Damar had the lowest TA but did not differ significantly from Southfield and Glen Fruin. The two Graymead orchards were similar at harvest and at 7m CA, but they differed significantly after 7m CA + 6w RA and 7m CA + 6w RA + 7d SL with the 4th leaf recording a significantly higher TA than the 6th leaf orchard fruit (Fig. 25). According to Figure 25, Graymead 4th remained similar to Monteith at all the evaluation times, except at harvest and it was significantly higher than Southfield, Glen Fruin and Damar after 7m CA and 7m CA + 6w RA. Graymead 4th was similar to Southfield, but significantly higher than Damar and Glen

Fruin after 7m CA + 6w RA + 7d SL. All the orchards maintained an export acceptable TA over all the evaluation times, except after SL where Damar and Glen Fruin fell below the minimum acceptable level (0.4%).

3.5.10 Titratable acidity: season two (2015)

General indications from Figure 26 are that there were decreases in TA levels from all the orchards throughout the season, even though some of the decreases were insignificant. All the orchards recorded their lowest TA levels after 7m CA + 6w RA + 7d SL. Besides Glen Brae and Texel, all the other orchards exhibited IPLA unacceptable TA levels at 7m CA + 6w RA + 7d SL (Fig. 26). Texel recorded the highest TA levels at all the evaluation times, but this did not differ significantly from Glen Brae at harvest, 7m CA and 7m CA + 6w RA + 7d SL, nor from De Rust after 7m CA and 7m CA + 6w RA. All other orchards, except, Glen Fruin and Chiltern 23, maintained IPLA acceptable TA levels (≥0.4%) after 7m CA + 6w RA but not after 7m CA + 6w RA + 7d SL (Fig. 26).

3.6 Relationship between orchards, browning and maturity/quality parameters

3.6.1 Season one (2014)

Table 15 represents the correlation analysis of the relationship between orchard, total browning and the browning types, as well as the quality parameters measured. From Table 15, total browning is positively correlated to TSS (0.707) and background colour (0.859). DB correlated highly positively with background colour (0.871) and TSS (0.70), but moderately negatively with firmness (-0.585; Table 15). CB correlated moderately positively with background colour (0.628) and TSS (0.666; Table 15). TA correlated highly negatively with SB (-0.853) and moderately negatively with background colour (-0.595), but highly positively with firmness (0.760; Table 15). TSS is highly positively associated with background colour (0.731; Table 15). Firmness correlated highly negatively with SB (-0.726), while SB correlated moderately positively with background colour (0.616; Table 15).

3.6.2 Season two (2015)

According to Table 16, total browning and all the browning types (except CB), are correlated to background colour. Total IB, DB and RB scored correlation values of 0.715, 0.736 and 0.647, respectively (Table 16). CB and SB both associated moderately negatively with fruit firmness with correlation values of -0.608 and -0.635, respectively (Table 16).

3.4 DISCUSSION

Four types of IB (Fig. 1B & 3B) were observed in the two seasons (2014 and 2015) of this study. DB was the dominant IB type in both seasons, which indicate that 'Rosy Glow' in South Africa may be prone to the DB type of IB (Bergman et al., 2012; Butler, 2015). However, contrary to Crouch et al. (2015) the tree age effect was not significant on DB in both seasons. Even though there was an evaluation time effect, DB was only progressive in the second season but not in the first possibly due to harvest maturity differences between seasons.

First season RB was low (Fig. 1B) and more RB was observed when more fruit were evaluated (Fig. 3B) in season two. RB was affected by the interaction of tree age and evaluation time in the first season but, not in the second (Tables 4 and 5). RB is known to be as a result of the interaction of many pre- and post-harvest factors which include harvest maturity, growing-degree-days (GDD), fruit nutrition, crop load and storage condition (Bergman et al., 2012; Butler, 2015; Moggia et al., 2015). The inconsistency in the results with regards to factors influencing RB suggests that there are many other factors that may influence RB in 'Rosy Glow', just like in 'Cripps Pink' or that higher levels of browning are required for bigger contrasts.

CO_2B observed in the study was low in both seasons and neither tree age nor evaluation time influenced CO_2B in season one (Table 4), but there was a tree age by evaluation time interaction effect on CO_2B in season two (Table 5). CO_2B is induced by a combination of low O_2 and high CO_2 levels during CA storage (Lau, 1998; James, 2007; de Castro et al., 2008). It is possible that CA storage used in the study may have induced CO_2B in 'Rosy Glow', or that fruit had not sufficiently cooled down when CA storage was induced.

CB incidence was similar in both seasons (Figs. 1 and 3) and the results indicated that CB was influenced by evaluation time in both seasons (Tables 4 and 5). The results showed that CB in both two seasons was the highest after 7m CA + 6w RA and not progressive in SL, which contradicts the findings of Crouch et al., (2015), that CB is progressive in SL.

Crouch et al., (2015) reported that young orchards suffered more DB and CB than older ones and factors (not evaluated in this study) such as harvest maturity and soil type were reported to have played a role. Also, Butler (2015), experimented on orchards that were 8 years for the young ones and 17 to 18 years for the old trees. These were much older than the orchards in this study. The difference between young and old orchards used in this study may not have been large enough to show an age effect on the browning observed due to trees at the time all being too recently planted to be classified as old.

The orchard factor effect on the incidence of IB and many other postharvest disorders have been studied (Crisosto et al., 1997). Bramlage (1992), indicated that there are many factors that may influence the development of disorders in harvested fruit. The results from this study indicated that orchard factors may play a greater role in influencing IB in 'Rosy Glow'. Tables 6 and 7 shows evaluation time by orchard interaction effect on total IB, DB and RB in both seasons. Figures 7 and 8 shows orchard similarity in IB incidence susceptibility as well as IB levels at different evaluation times, regardless of age.

Tables 13 and 14 illustrates that all the quality and maturity parameters measured were influenced by the interaction of evaluation time and orchard. Figures 17 to 26 indicate how the individual orchards were influenced at the evaluation times. It can be observed that, each orchard behaved differently with regards to certain quality and maturity parameters over time and that the age of the orchard was of less importance in this study. For example, Monteith and Southfield exhibited a similar background colour at harvest until after 7m CA + 6w RA, but not after SL in the first season, even though they are orchards with two different tree ages (Fig. 17). In the second season however, orchards of similar tree ages exhibited similar background colour at similar evaluation times. Nonetheless, there were also many similarities between orchards with different tree ages which may indicate many factors playing a role.

Tree age and crop load together with soil type and harvest maturity are all orchard factors that have been reported to affect the development of IB in apples (Bramlage, 1992; James, 2007; Crouch et al., 2014, 2015; Moggia et al., 2015). From this study there is enough evidence indicating that tree age was not part of the orchard factors that lead to the development of IB, or at least not between the tree ages studied. The association between various orchards, total browning and the quality/maturity parameters was tested. Total IB associated positively with TSS and SB (0.707 and 0.534, respectively) in season one, while total IB associated strongly positively with background colour (0.715) in season two (Table 16). There was a strong negative association between SB and TA (-0.777) as well (Table 16). The strong correlation of Total IB with DB, in both seasons is indicative of DB being the main type of IB in this trial. DB was positively correlated to BC in both seasons and in the second season also negatively correlated with firmness and positively correlated with TSS. These correlations indicate the role of harvest maturity in the development of the IB disorder in particular DB, as mentioned in paper one. It is thus possible that harvest maturity was the main factor influencing the IB observed in this study.

3.5 CONCLUSION

Four browning types (DB, RB, CO_2B and CO_2) were observed in this study, with the dominant type being DB. According to this study 'Rosy Glow' is more susceptible to DB than other browning types, and it may be progressive in SL. Due to the inconsistencies of the incidence of RB and CO_2B, further investigation is required to ascertain the susceptibility of 'Rosy Glow' to these types of IB. 'Rosy Glow' is susceptible to the CB type of IB, as is 'Cripps Pink' according to Butler (2015). However, in this study a new kind of CB (RB + CO_2B) was observed and needs to be further explored to determine the possibility of it being observed again and to further describe the physiology thereof. Tree age did not play a role in the development of IB observed in 'Rosy Glow' in the two seasons and might therefore not play a role in 'Rosy Glow' susceptibility to IB. Nevertheless, there is the need to conduct investigations using wider contrasts of tree ages to be able to confirm this finding. Orchard factors, especially fruit maturity, interacting with evaluation time was seen to influence the development of IB in 'Rosy Glow'. It is recommended that further investigations are conducted into determining the specific orchard factors that influence IB development in 'Rosy Glow' apples.

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3.7 TABLES AND FIGURES

Table 1: Area, orchard, farm, coordinates and rootstock of 'Rosy Glow' apples that were harvested in 2014 and 2015 from different tree ages

Area	Orchard	Farm	Coordinates	Rootstock	Season	Tree age (years)
Vyeboom	09A	Greymead	34°02'07.2"S 19°07'28.1"E	M793	One	4
Elgin	5B	Monteith	35°15'40"S 19°02'46"E	M793	One	4
Vyeboom	16	Damar	34°03'22.2"S 19°06'54.0"E	MM109	One and Two	4 & 5
Villiersdorp	Huttonsquire (D2)	Southfield	33°58'04.9"S 19°18'36.5"E	M793	One and Two	6 & 7
Elgin	20	Glen Fruin	34°11'23.64"S 19°3'10.38"E	MM109	One and Two	6 & 7
Vyeboom	B6B 6E	Greymead	34°02'07.2"S 19°07'28.1"E	M793	One and Two	6 & 7
Vyeboom	24E and 23E	Chiltern	34°03'03.9"S 19°09'29.2"E	M793	Two	4
Elgin	G2	De Rust	34°09'47.4"S 19°04'57.3"E	M7	Two	4
Elgin	-	Texel	34°14'48.7"S 19°04'48.7"E	MM109	Two	7
Elgin	22	Glen Brae	34°13'08.0"S 19°04'57.1"E	M793	Two	4

Table 2: Number of brown fruit and browning type (diffuse browning (DB), radial browning (RB), CO_2 browning (CO_2B) or combination browning (RB+CO_2B or DB+RB)) in 'Rosy Glow' apples that were harvested in 2014 at optimum maturity from two tree age groups (4th and 6th leaf) and from six farms (three for each age group). Measurements were made on fruit that were stored for 7 months in controlled atmosphere (CA) at -0.5°C, followed by 6 weeks in regular atmosphere (RA) at -0.5 °C and a subsequent 7-day shelf life period at 20 °C.

Age (years)	**Farm**	**Diffuse browning (DB)**	**Combination browning**		**Radial browning (RB)**	**Carbon Dioxide browning (CO_2B)**	**Brown fruit No.**	**Non-brown fruit No.**	**Total fruit No.**
			(RB+CO_2B)	**(DB+RB)**					
4	Damar	117	1	6	5	0	129	191	320
4	Graymead	0	0	0	0	1	1	319	320
4	Monteith	48	0	0	0	3	51	269	320
Total		**165**	**1**	**6**	**5**	**4**	**181**	**779**	**960**
6	Southfield	50	0	4	2	1	57	263	320
6	Graymead	115	2	9	2	1	129	191	320
6	Glen Fruin	9	0	0	0	0	9	311	320
Total		**174**	**2**	**13**	**4**	**2**	**195**	**765**	**960**

Table 3: Number of brown fruit and browning type (diffuse browning (DB), radial browning (RB), CO_2 browning (CO_2B) or combination browning (RB+CO_2B or DB+RB)) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity from two tree age groups (4th and 7th leaf) and from eight farms (four for each age group). Measurements were made on fruit that were stored for 7 months in controlled atmosphere (CA), followed by 6 weeks in regular atmosphere (RA) at -0.5 °C and a subsequent 7 – day shelf life period at 20 °C temperature.

Age (years)	Farm	Diffuse browning (DB)	Combination browning		Radial browning (RB)	Carbon Dioxide browning (CO_2B)	Brown fruit number	Non-brown fruit number	Total fruit number
			(RB+CO_2B)	(DB+RB)					
4	Chiltern 23	8	0	3	2	0	13	307	320
4	Chiltern 24	38	2	4	17	1	62	258	320
4	De Rust	8	0	5	5	0	18	302	320
4	Glen Brae	7	0	1	4	1	13	307	320
Total		**61**	**2**	**13**	**28**	**2**	**106**	**1174**	**1280**
7	Southfield	4	0	2	3	1	10	310	320
7	Graymead	43	0	3	22	4	72	248	320
7	Glen Fruin	8	0	2	4	1	15	305	320
7	Texel	32	0	7	3	1	43	277	320
Total		**87**	**0**	**14**	**32**	**7**	**140**	**1140**	**1280**

Table 4: P-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for evaluation time and tree age, as well as their interaction. Fruit were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2014 season.

Source of variation	Total browning	Diffuse browning	Radial browning	Carbon dioxide browning	Combination browning
			Pr>F		
Evaluation time	< 0.0001	< 0.0001	0.047	0.329	0.002
Tree Age	0.832	0.763	0.775	0.407	0.124
Evaluation time*Tree Age	0.310	0.148	0.047	0.105	0.827

Table 5: P-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for evaluation time and tree age, as well as their interaction. Fruit were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2015 season.

Source of variation	Total browning	Diffuse browning	Radial browning	Carbon dioxide browning	Combination browning
			Pr>F		
Evaluation time	< 0.0001	< 0.0001	< 0.0001	0.114	< 0.0001
Tree Age	0.076	0.081	0.643	0.087	0.854
Evaluation time*Tree Age	0.244	0.119	0.254	0.033	0.885

Table 6: P-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for evaluation time and orchard, as well as their interaction. Fruit were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2014 season.

Source of variation	Total browning	Diffuse browning	Radial browning	Carbon dioxide browning	Combination browning
			Pr>F		
Orchard	< 0.0001	< 0.0001	0.063	0.245	0.000
Evaluation time	< 0.0001	< 0.0001	0.032	0.293	0.001
Orchard*Evaluation time	< 0.0001	< 0.0001	0.001	0.003	0.160

Table 7: P-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for evaluation time and orchard as well as their interaction. Fruit were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2015 season.

Source of variation	Total browning	Diffuse browning	Radial browning	Carbon dioxide browning	Combination browning
			Pr>F		
Orchard	< 0.0001	< 0.0001	< 0.0001	0.170	0.227
Evaluation time	< 0.0001	< 0.0001	< 0.0001	0.108	< 0.0001
Orchard*Evaluation time	< 0.0001	< 0.0001	< 0.0001	0.079	0.006

Table 8: Maturity and quality indices measured for 'Rosy Glow' apples and the main and interactive effect of evaluation time and tree age. Fruit were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2014 season.

Evaluation time	**Tree age (leaf/ year)**	**Firmness (kg)**	**TA (%)**
At Harvest	4	9.1 a	0.70 a
At Harvest	6	8.4 b	0.62 b
7m CA	4	7.9 c	0.51 c
7m CA	6	7.9 c	0.47 de
7m CA + 6w RA	4	7.7 c	0.48 cd
7m CA + 6w RA	6	7.9 c	0.46 e
7m CA + 6w RA + 7d SL	4	7.3 d	0.42 f
7m CA + 6w RA + 7d SL	6	7.3 d	0.40 f
Source of variation:		**Pr>F**	
Evaluation time		< 0.0001	< 0.0001
Tree Age		0.022	< 0.0001
Evaluation time*Tree Age		< 0.0001	0.005

Table 9: Maturity and quality indices measured for 'Rosy Glow' apples and the main effect of evaluation time. Fruit were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2015 season.

Evaluation time	**Firmness (kg)**	**TSS (%)**	**TA (%)**
At Harvest	8.77 a	13.3 c	0.61 a
7months CA	7.65 b	14.7 ab	0.46 b
7months CA + 6w RA	7.42 c	14.6 b	0.44 c
7months CA + 6w RA + 7d SL	7.17 d	14.9 a	0.37 d
Source of variation:		**Pr>F**	
Evaluation time	< 0.0001	< 0.0001	< 0.0001
Tree Age	0.017	< 0.0001	0.748
Evaluation time*Tree Age	0.302	0.667	0.638

Table 10: Significance of firmness and TSS measured for 'Rosy Glow' apples by main effect of tree age. Fruit were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2015 season.

Tree Age	**Firmness (kg)**	**TSS (%)**
7^{th} leaf	7.8 a	14.2 b
4^{th} leaf	7.7 b	14.6 a
Source of Variation	**Pr>F**	
Tree Age	0.000	0.000

Table 11: Maturity and quality indices measured for 'Rosy Glow' apples and the main effect of evaluation time. Fruit were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2014 season.

Evaluation time	**Background colour (chart index)**	**Starch Breakdown (%)**	**TSS (%)**
At Harvest	3.4 b	35 b	12.8 c
7months CA	3.2 c	100 a	13.5 b
7months CA + 6w RA	3.4 b	100 a	13.4 b
7months CA + 6w RA + 7d SL	3.9 a	100 a	13.8 a
Source of variation		**Pr>F**	
Evaluation time	< 0.0001	< 0.0001	< 0.0001
Tree Age	0.678	0.674	0.883
Evaluation time*Tree Age	0.114	0.912	0.738

Table 12. Maturity and quality indices measured for 'Rosy Glow' apples and the main and interactive effect of tree age and evaluation time. Fruit were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2015 season.

Evaluation time	**Tree age (leaf/ year)**	**Background colour (chart index)**	**Starch breakdown (%)**
At Harvest	4	3.3 cd	38 b
At Harvest	7	3.4c	32 c
7months CA	4	3.0 e	100. a
7months CA	7	3.23 d	100 a
7months CA + 6w RA	4	3.3 cd	100 a
7months CA + 6w RA	7	3.4 c	100 a
7months CA + 6w RA + 7d SL	4	3.7 b	100 a
7months CA + 6w RA + 7d SL	7	4.2 a	100 a
Source of variation		**Pr>F**	
Evaluation time		< 0.0001	< 0.0001
Tree Age		< 0.0001	0.014
Evaluation time*Tree Age		< 0.0001	0.000

Table 13: The interaction of orchard and evaluation time on the maturity and quality indices of 'Rosy Glow' apples. Fruit were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin of the Western Cape in the 2014 season.

Source	**Background colour (chart index)**	**Starch Breakdown (%)**	**Firmness (kg)**	**TSS (%)**	**TA (%)**
			Pr>F		
Orchard	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Evaluation time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Orchard*Evaluation time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 14: The interaction of orchard and evaluation time on the maturity and quality indices of 'Rosy Glow' apples. Fruit were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin of the Western Cape in the 2015 season.

Source	**Background colour (chart index)**	**Starch Breakdown (%)**	**Firmness (kg)**	**TSS (%)**	**TA (%)**
			Pr>F		
Orchard	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Evaluation time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Orchard*Evaluation time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 15: Correlation matrix (top) and corresponding p-value (bottom) of the correlation between total browning percentage and quality parameters at the 0.05% significance level. 'Rosy Glow' apples were harvested at optimum harvest maturity from six different orchards in the Vyeboom, Villiersdorp and Elgin of the Western Cape in the 2014 season.

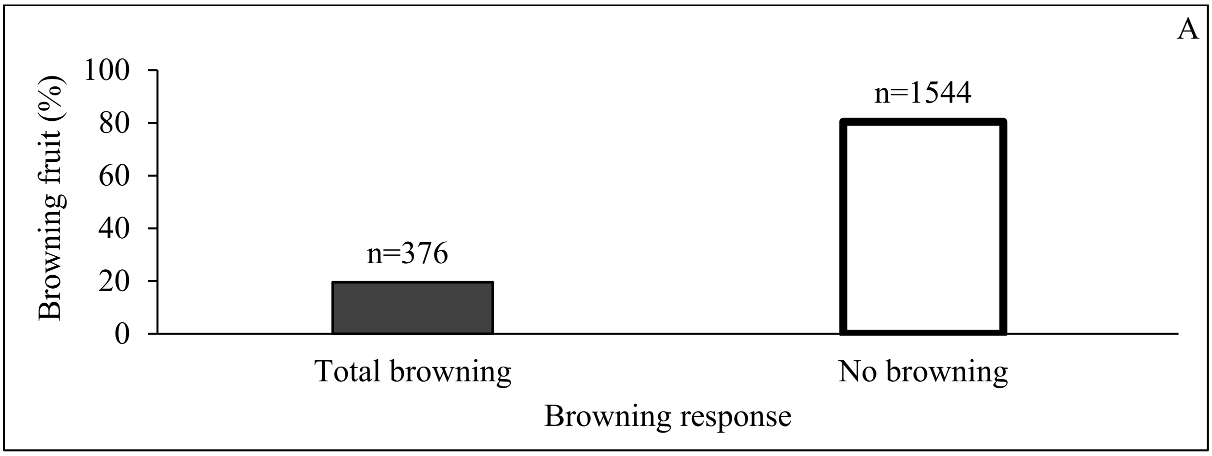
Variables:	Background colour (Chart Index)	Starch breakdown (%)	Firmness (kg)	TSS (%)	TA (%)	Total browning (%)	Diffuse browning (%)	Radial browning (%)	Carbon Dioxide browning (%)	Combination browning (%)
Background colour (Chart Index)	**1**	**0.616**	-0.513	**0.731**	**-0.595**	**0.859**	**0.871**	0.437	0.010	**0.628**
Starch breakdown (%)		**1**	**-0.726**	0.441	**-0.853**	0.534	0.526	0.358	0.394	0.384
Firmness (kg)			**1**	-0.357	**0.760**	-0.562	**-0.585**	-0.082	-0.307	-0.185
TSS (%)				**1**	-0.166	**0.707**	**0.700**	0.425	0.500	**0.666**
TA (%)					**1**	-0.503	-0.513	-0.255	0.079	-0.311
Total Browning (%)						**1**	**0.998**	**0.595**	0.040	**0.799**
Diffuse browning (%)							**1**	0.546	0.026	**0.774**
Radial browning (%)								**1**	-0.014	**0.665**
Carbon Dioxide browning (%)									**1**	-0.022
Combination browning (%)										**1**
Background colour (Chart Index)	**0**	**0.033**	0.088	**0.007**	**0.041**	**0.000**	**0.000**	0.156	0.974	**0.029**
Starch breakdown (%)		**0**	**0.008**	0.151	**0.000**	0.074	0.079	0.253	0.205	0.218
Firmness (kg)			**0**	0.255	**0.004**	0.057	**0.046**	0.799	0.332	0.565
TSS (%)				**0**	0.607	**0.010**	**0.011**	0.168	0.098	**0.018**
TA (%)					**0**	0.095	0.088	0.424	0.806	0.325
Total Browning (%)						**0**	**< 0.0001**	**0.041**	0.901	**0.002**
Diffuse browning (%)							**0**	0.066	0.936	**0.003**
Radial browning (%)								**0**	0.966	**0.018**
Carbon Dioxide browning (%)									**0**	0.947
Combination browning (%)										**0**

Values in bold are different from 0 with a significance level alpha=0.05

Table 16: Correlation matrix (top) and coefficient of correlation (down) of the correlation between total browning percentage and quality parameters at the 0.05% significance level. 'Rosy Glow' apples were harvested at optimum harvest maturity from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas of the Western Cape in the 2015 season.

Variables:	**Background Colour (Chart Index)**	**Starch breakdown (%)**	**Firmness (kg)**	**TSS (%)**	**TA (%)**	**Total browning (%)**	**Diffuse browning (%)**	**Radial browning (%)**	**Carbon Dioxide browning (%)**	**Combination browning (%)**
Background colour (Chart Index)	**1**	0.109	-0.231	0.194	-0.283	**0.715**	**0.736**	**0.647**	0.363	0.258
Starch breakdown (%)		**1**	**-0.635**	0.480	**-0.777**	0.393	0.379	0.296	0.300	0.412
Firmness (kg)			**1**	-0.448	0.442	-0.334	-0.360	-0.145	0.190	**-0.608**
TSS (%)				**1**	-0.155	0.212	0.257	0.080	0.065	0.195
TA (%)					**1**	-0.454	-0.407	-0.501	-0.239	-0.233
Total Browning (%)						**1**	**0.990**	**0.915**	**0.602**	0.513
Diffuse browning (%)							**1**	**0.872**	**0.581**	0.504
Radial browning (%)								**1**	0.504	0.217
Carbon Dioxide browning (%)									**1**	0.282
Combination browning (%)										**1**
Background colour (Chart Index)	**0**	0.711	0.427	0.506	0.327	**0.004**	**0.003**	**0.012**	0.203	0.374
Starch breakdown (%)		**0**	**0.015**	0.082	**0.001**	0.164	0.181	0.304	0.297	0.143
Firmness (kg)			**0**	0.108	0.113	0.243	0.206	0.622	0.515	**0.021**
TSS (%)				**0**	0.598	0.468	0.374	0.786	0.825	0.505
TA (%)					**0**	0.103	0.149	0.068	0.411	0.423
Total Browning (%)						**0**	**< 0.0001**	**< 0.0001**	**0.023**	0.061
Diffuse browning (%)							**0**	**< 0.0001**	**0.029**	0.066
Radial browning (%)								**0**	0.066	0.456
Carbon Dioxide browning (%)									**0**	0.329
Combination browning (%)										**0**

Values in bold are different from 0 with a significance level alpha=0.05



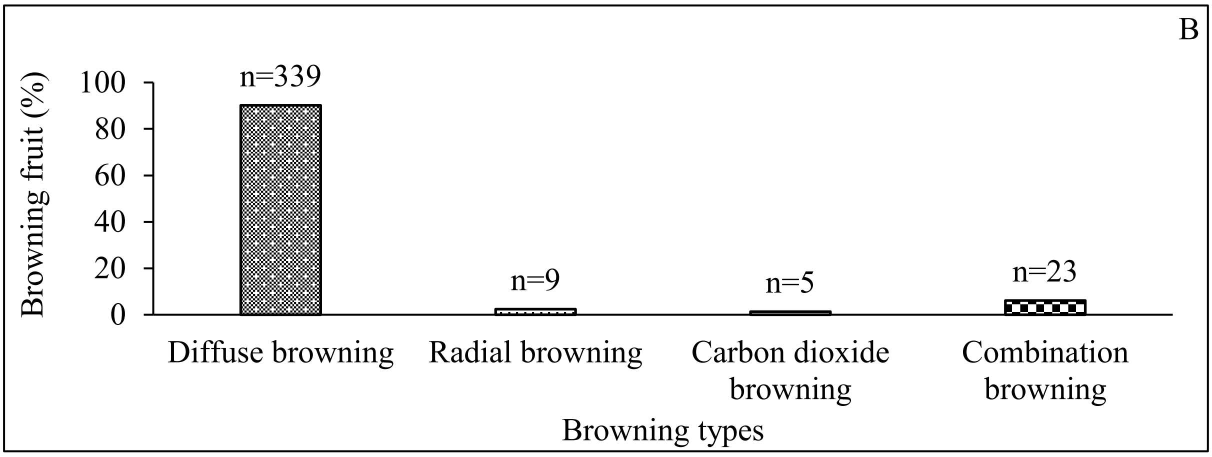


Figure 1: Total browning incidence in all the evaluated of fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples that were harvested on six orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (1.5% O_2; 1% CO_2), followed by 6 weeks in regular atmosphere at -0.5 °C and a 7-day shelf life period at 20 °C.

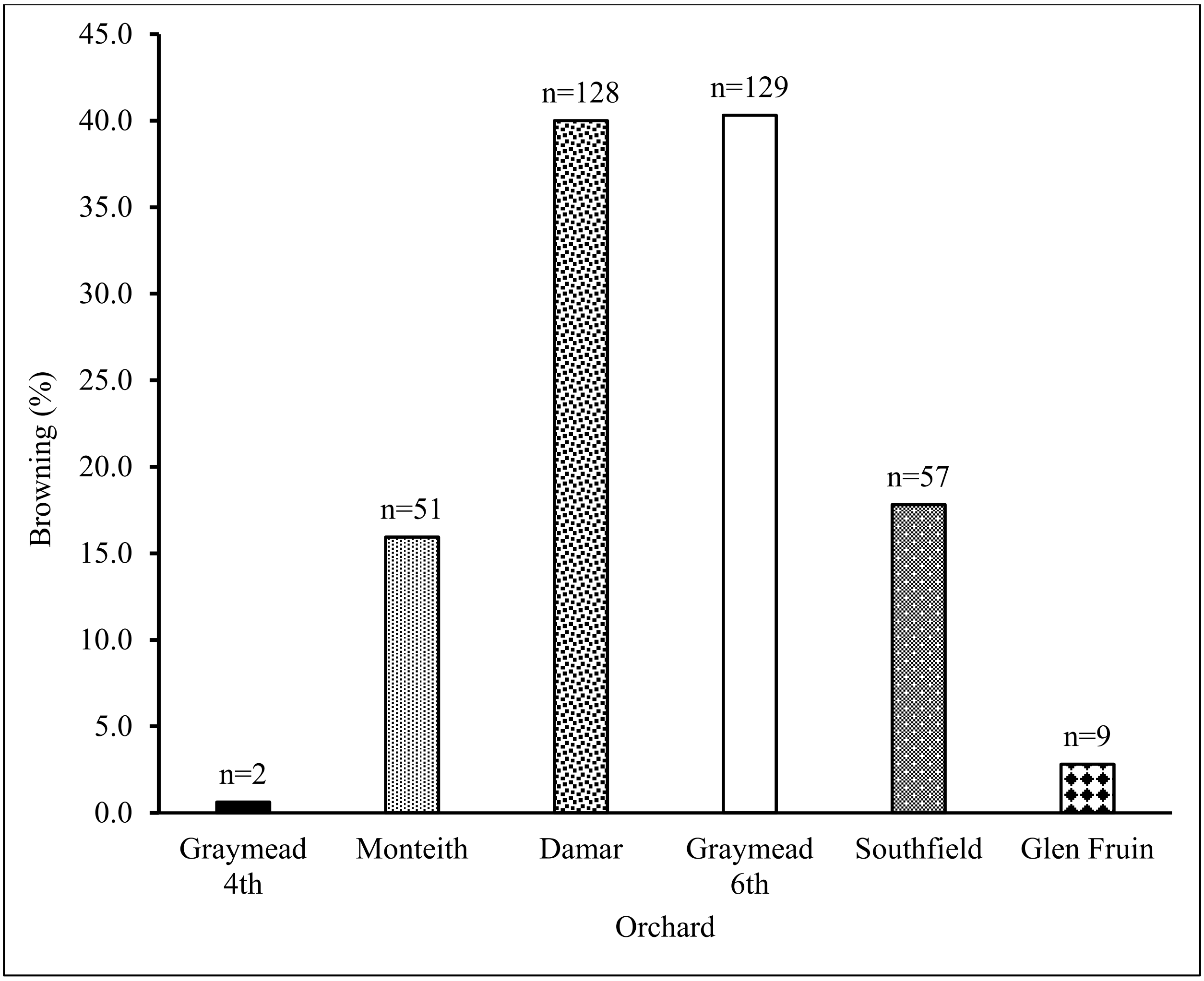


Figure 2: Browning incidence per orchard for 'Rosy Glow' apples harvested at optimum maturity from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (1.5% O_2; 1% CO_2), followed by 6 weeks in regular atmosphere at -0.5 °C and a subsequent 7-day shelf life period at 20 °C.

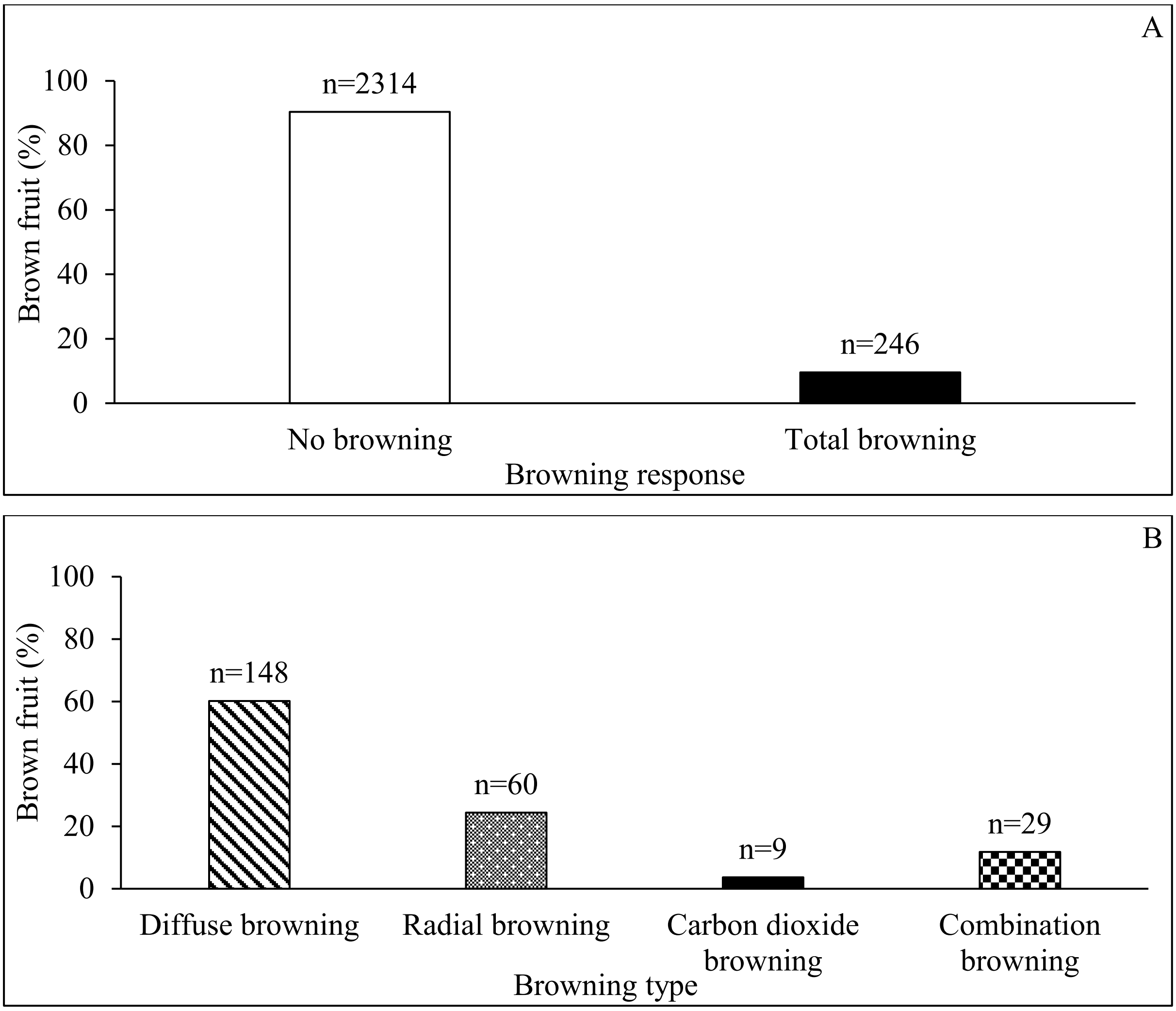


Figure 3: Total browning incidence in all the evaluated of fruit (A) and browning incidence per browning type (B) in 'Rosy Glow' apples that were harvested on eight orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (1.5 % O_2; 1 % CO_2), followed by 6 weeks in regular atmosphere at -0.5 °C and a 7-day shelf life period at 20 °C.

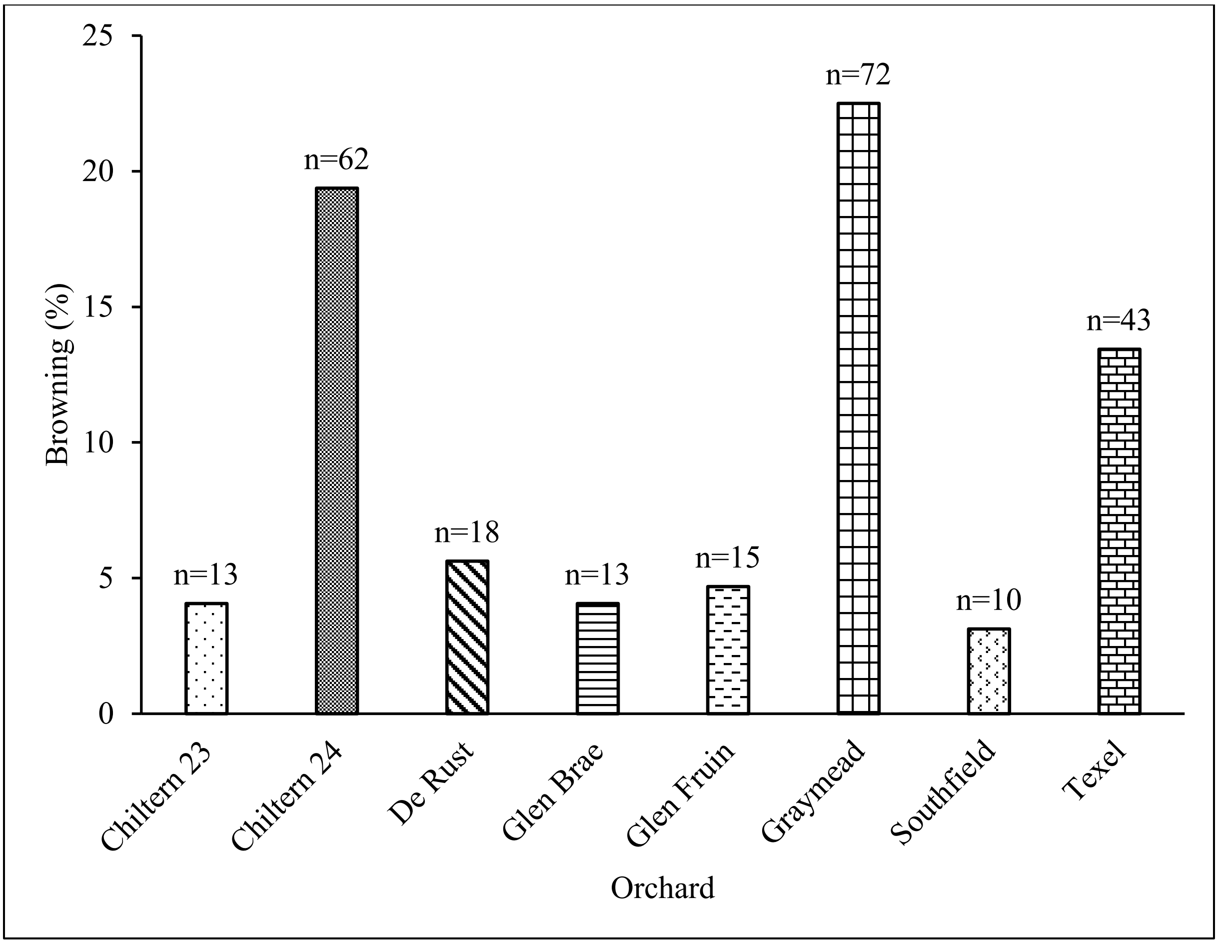


Figure 4: Browning incidence per orchard for 'Rosy Glow' apples harvested at optimum maturity from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (1.5% O_2; 1% CO_2), followed by 6 weeks in regular atmosphere at -0.5 °C and a subsequent 7-day shelf life period at 20 °C.

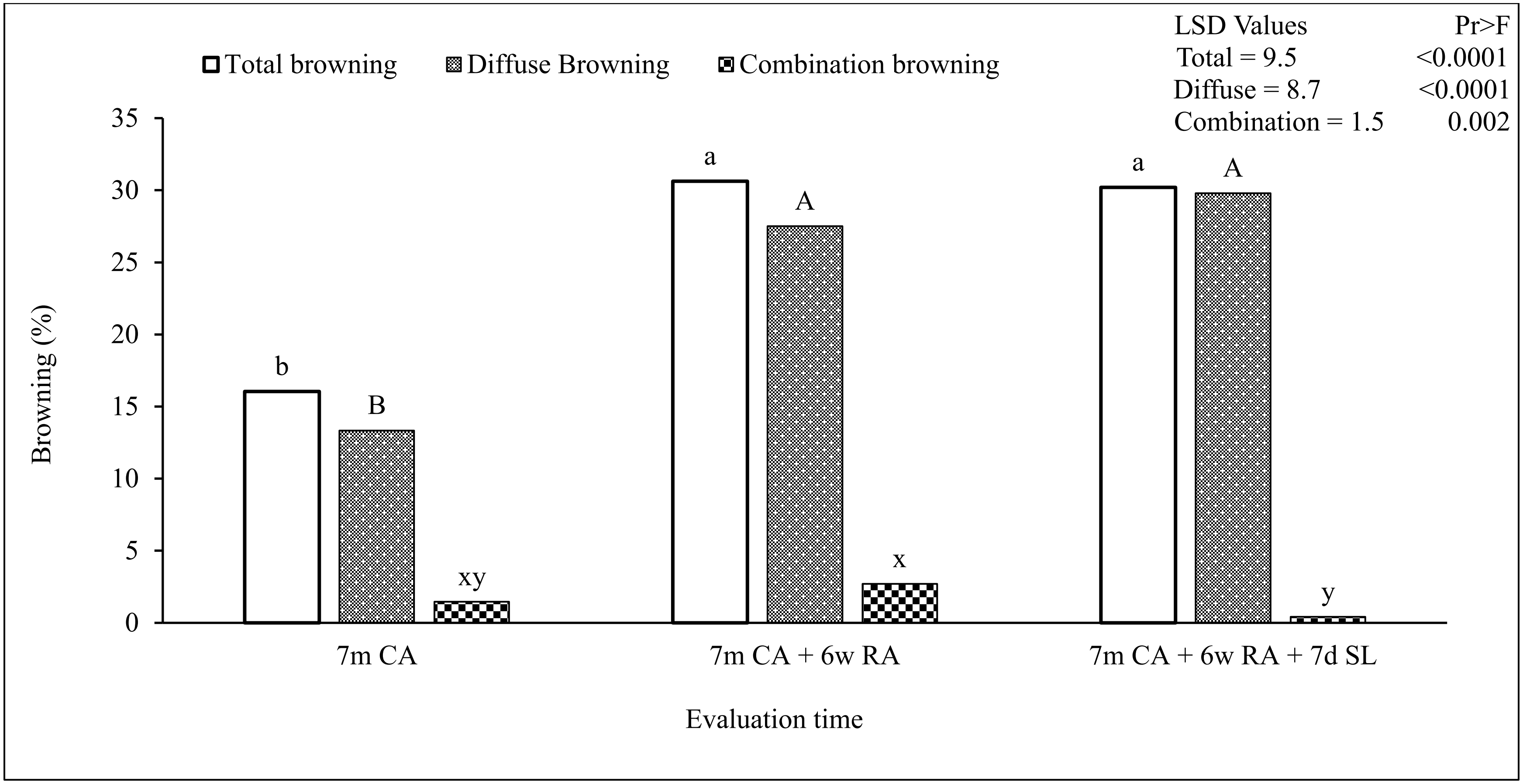


Figure 5: Percentage of total, diffuse and combination browning (statistically evaluated separately) per evaluation time for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season.

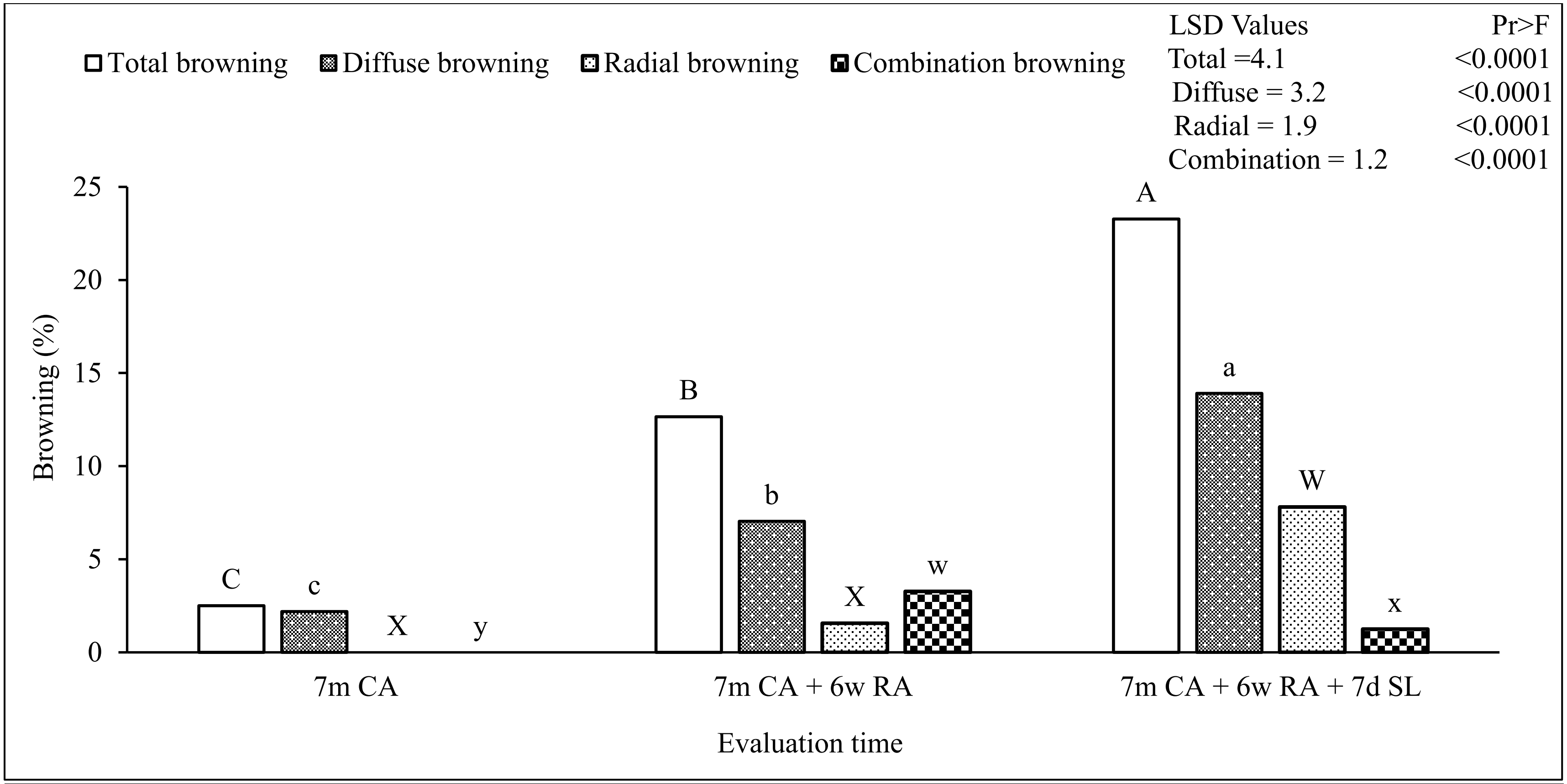


Figure 6: Percentage total, diffuse, radial and combination browning (statistically evaluated separately) per evaluation time for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season.

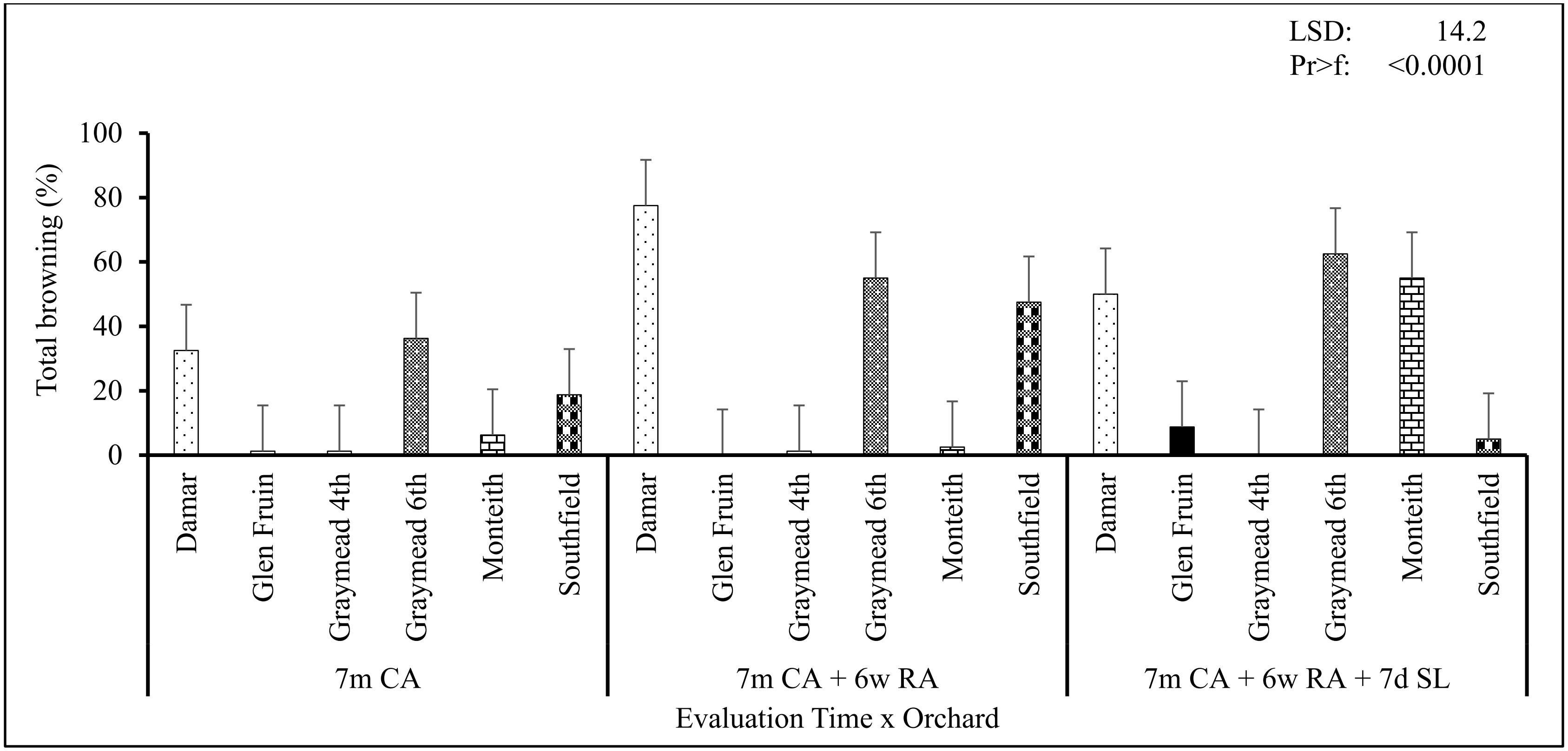


Figure 7: Percentage total browning per evaluation time and orchard interaction for 'Rosy Glow' apples that were harvested at optimum maturity from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C. The top error bar = the LSD value.

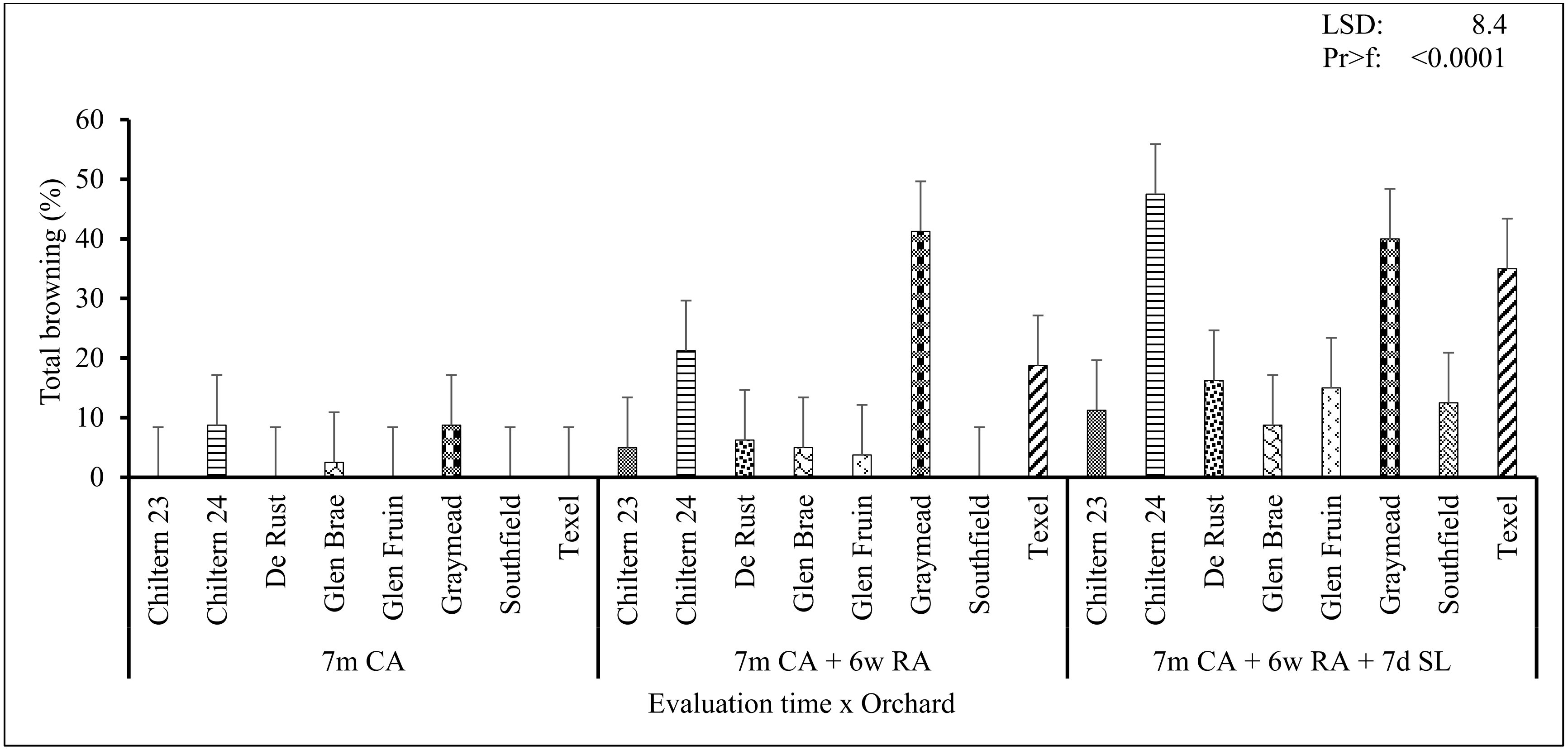


Figure 8: Percentage total browning per evaluation time and orchard interaction for 'Rosy Glow' apples that were harvested at optimum maturity from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C. The top error bar = the LSD value

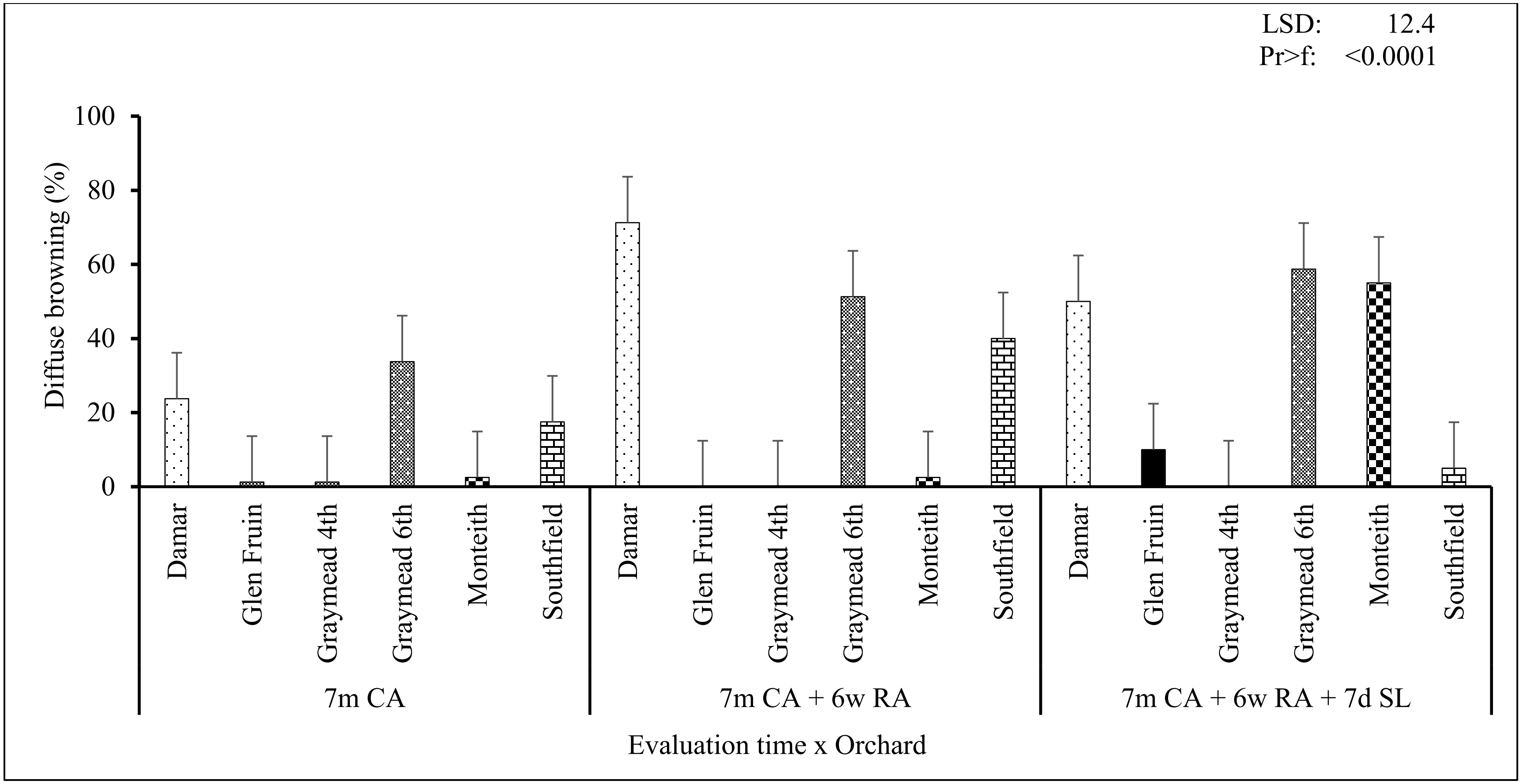


Figure 9: Percentage diffuse browning per evaluation time and orchard interaction for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C. The top error bar = the LSD value

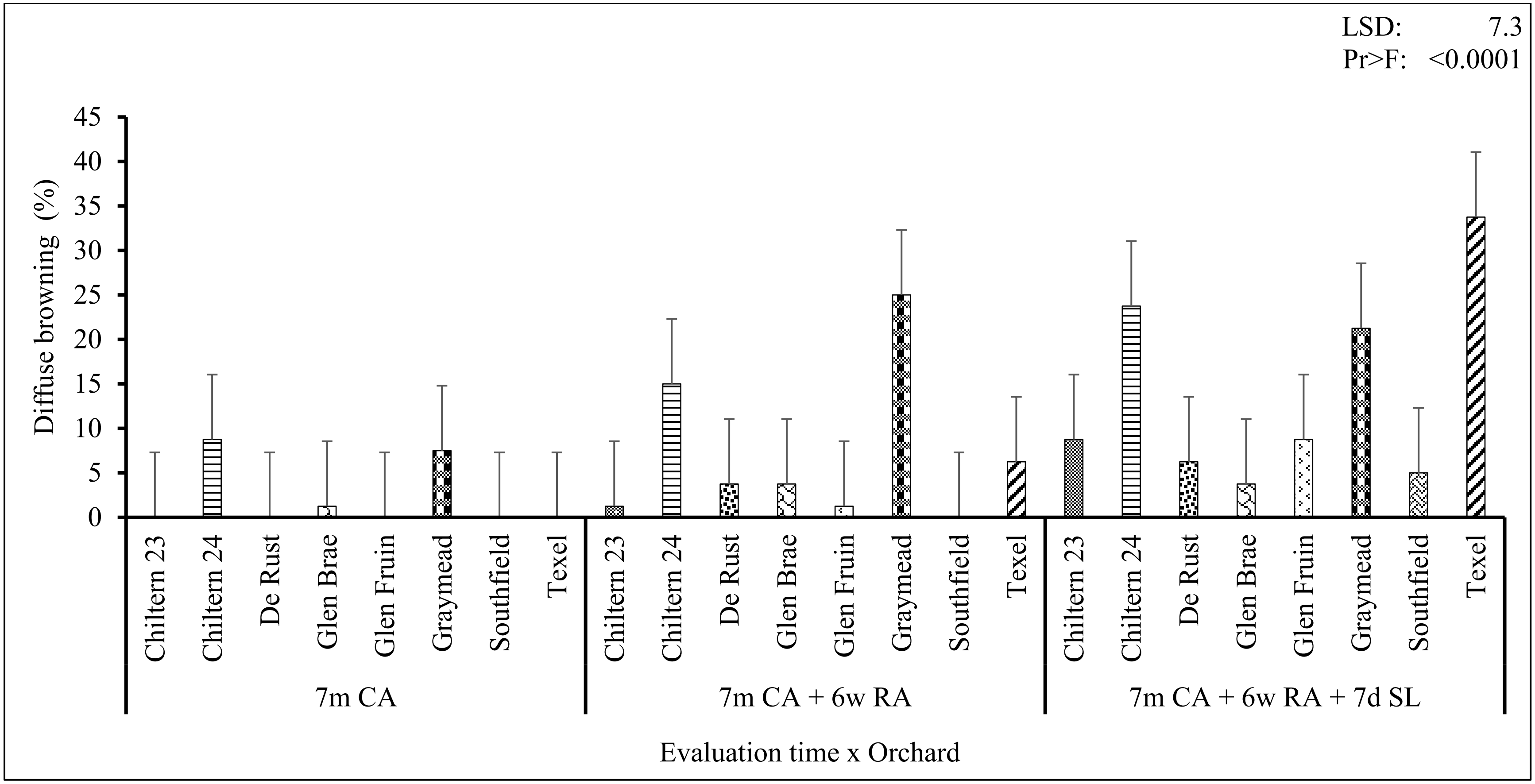


Figure 10: Percentage diffuse browning per evaluation time and orchard interaction for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

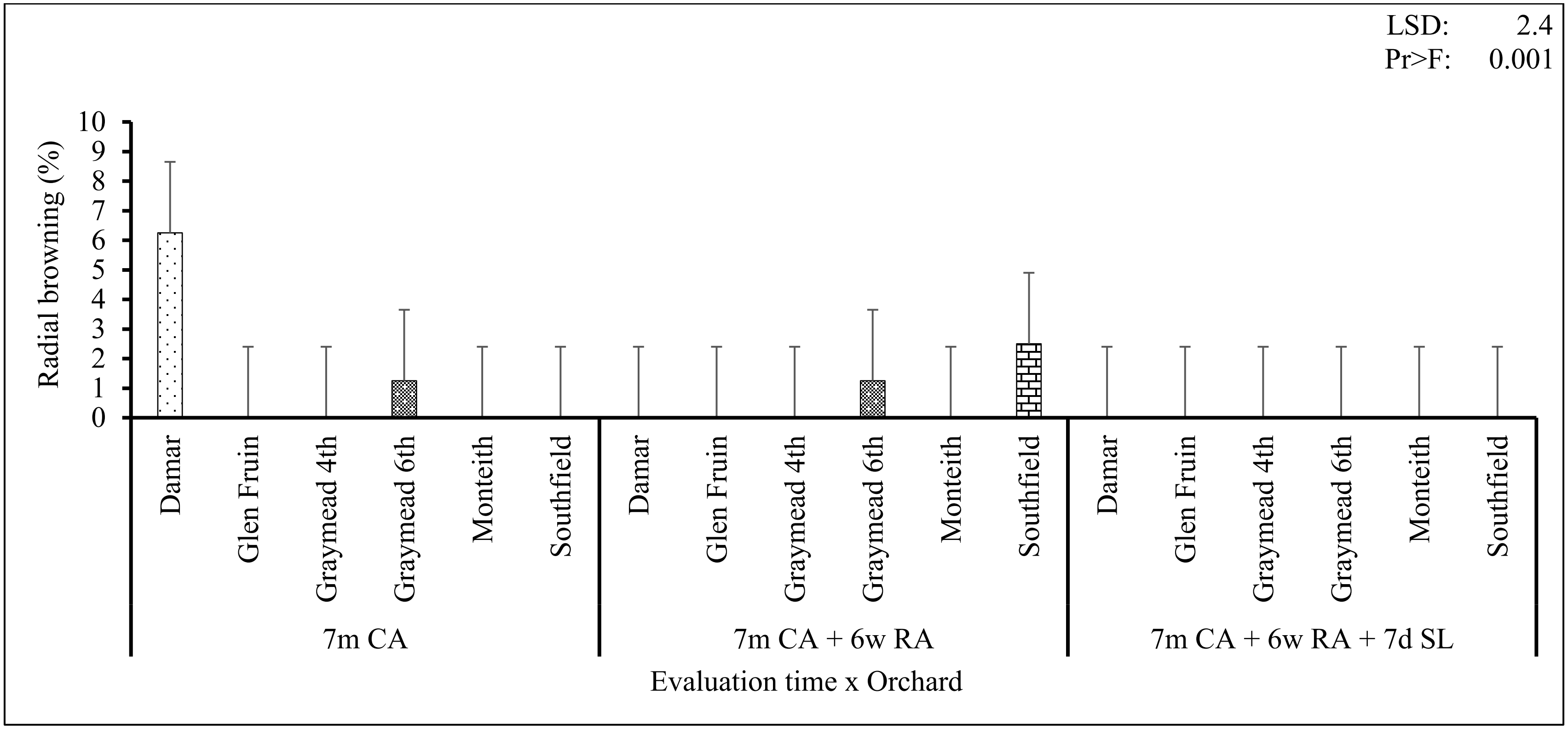


Figure 11: Percentage radial browning per evaluation time and orchard interaction for ‘Rosy Glow’ apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C. The top error bar = the LSD value

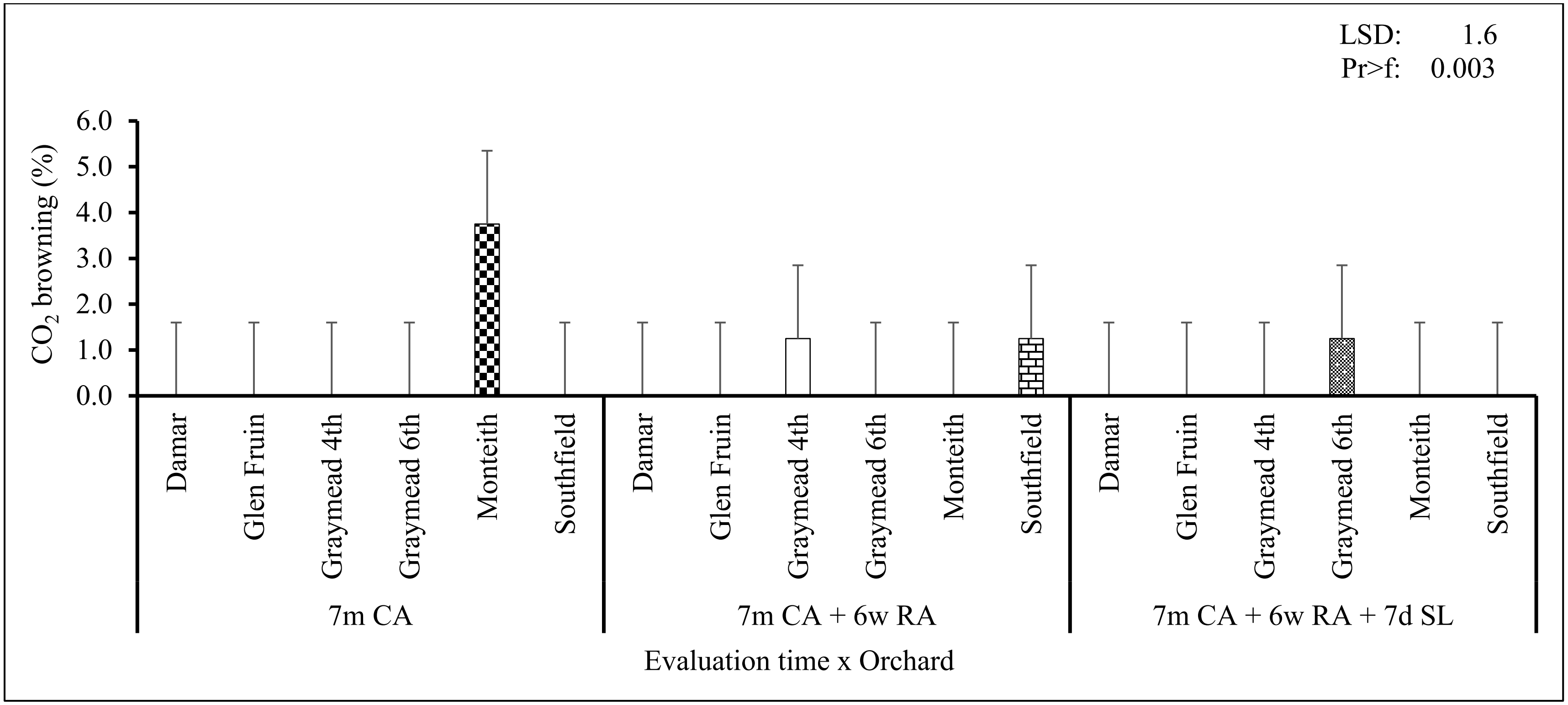


Figure 12: Percentage CO_2 browning per evaluation time and orchard interaction for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C. The top error bar = the LSD value.

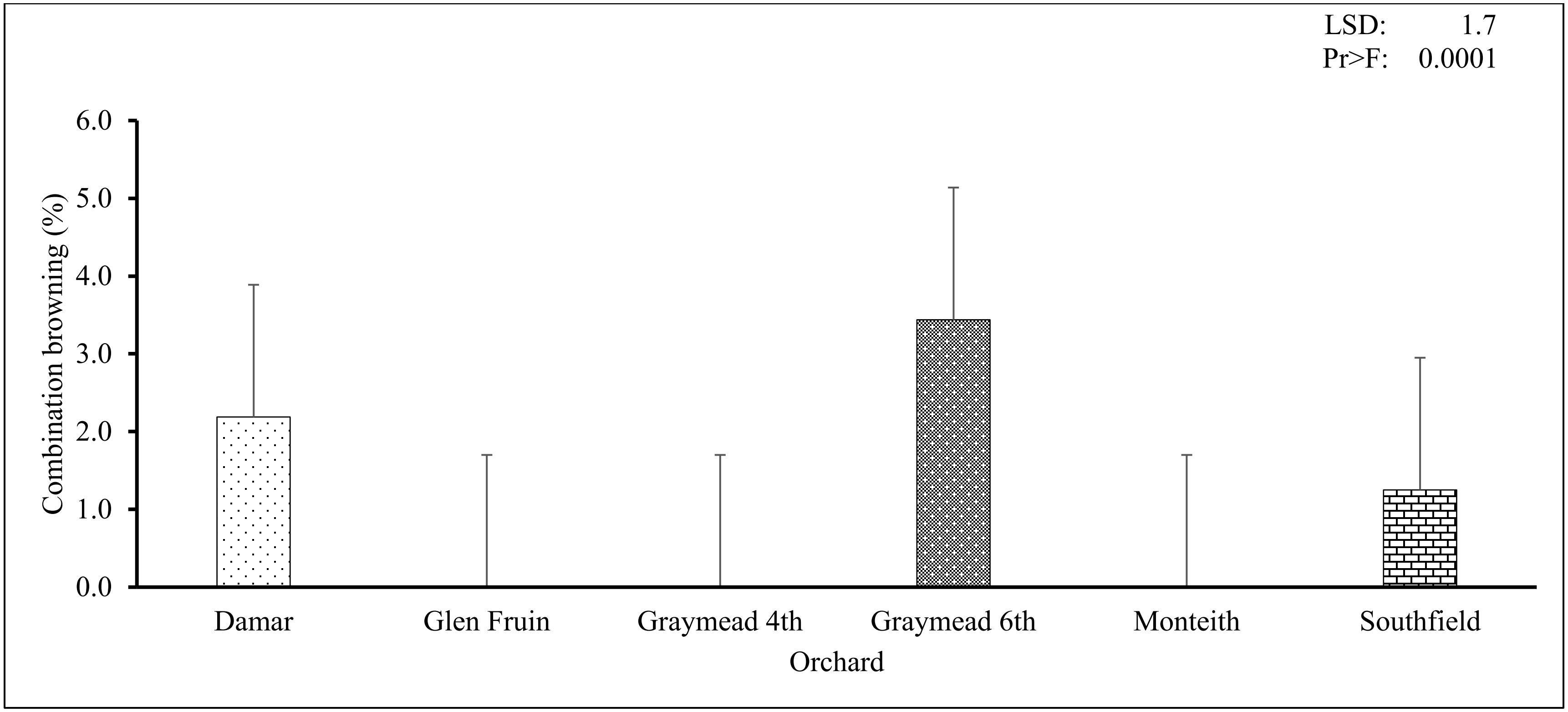


Figure 13: Percentage combination browning per orchard for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7–day shelf life period at 20 °C. The top error bar = the LSD value.

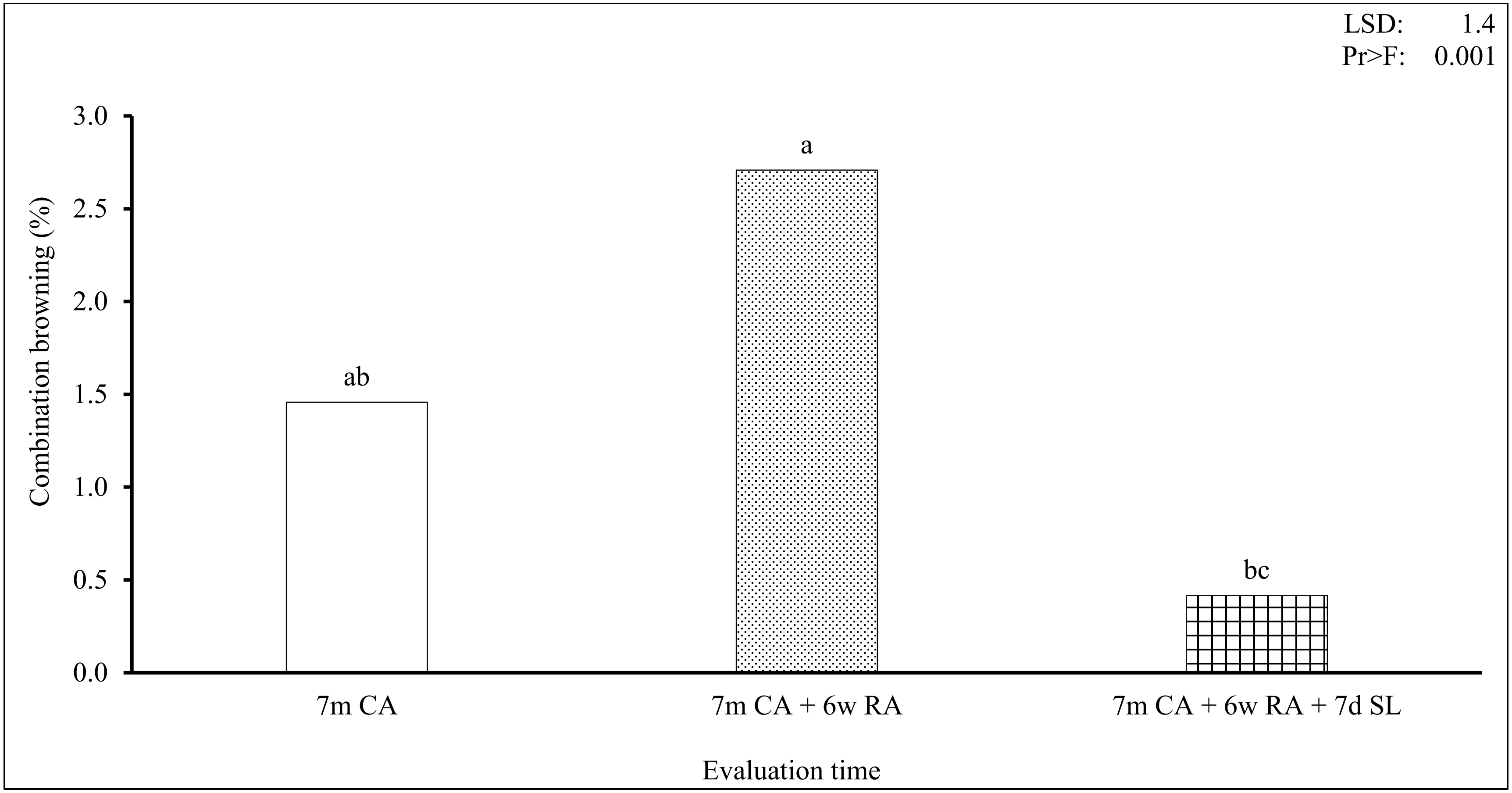


Figure 14: Percentage combination browning per evaluation time for ‘Rosy Glow’ apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

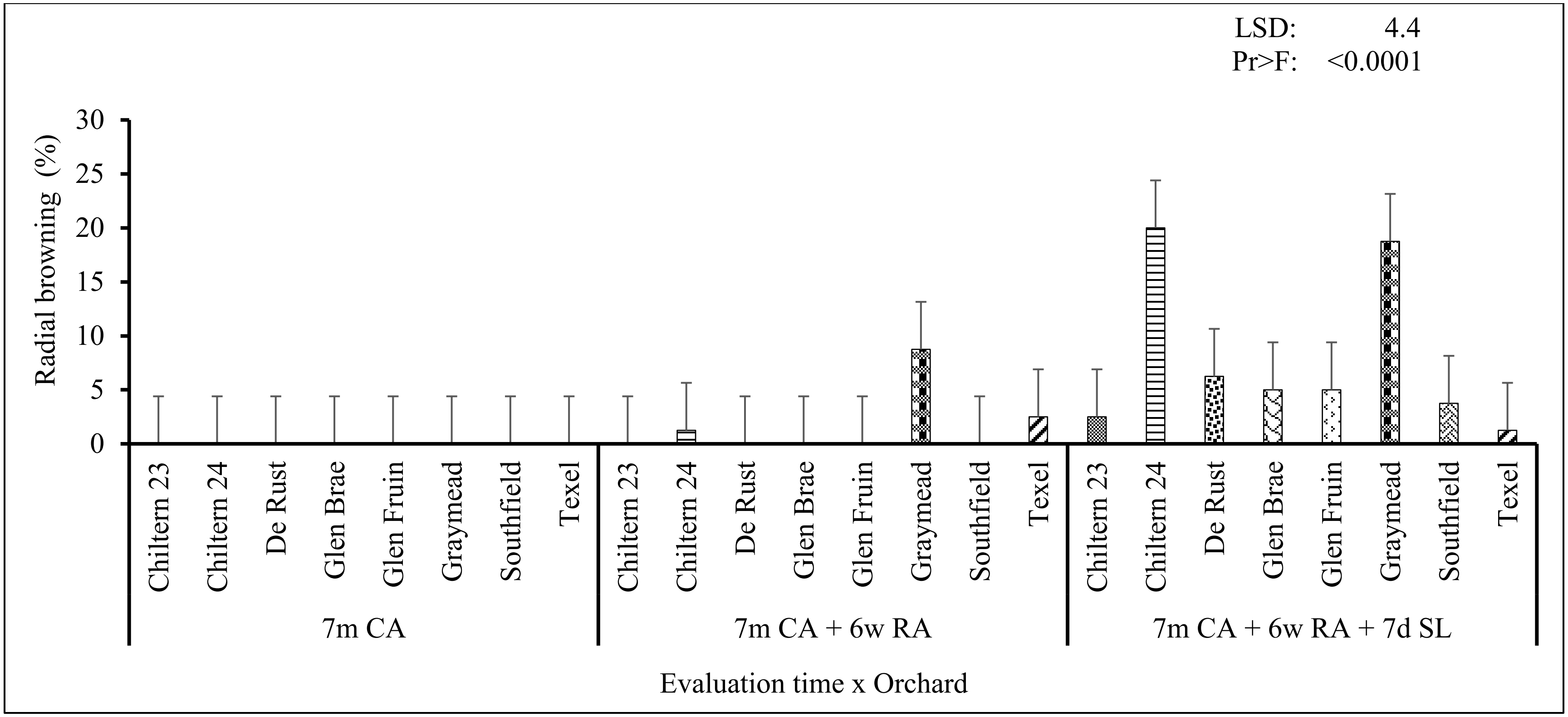


Figure 15: Percentage radial browning per evaluation time and orchard interaction for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C. The top error bar = the LSD value.

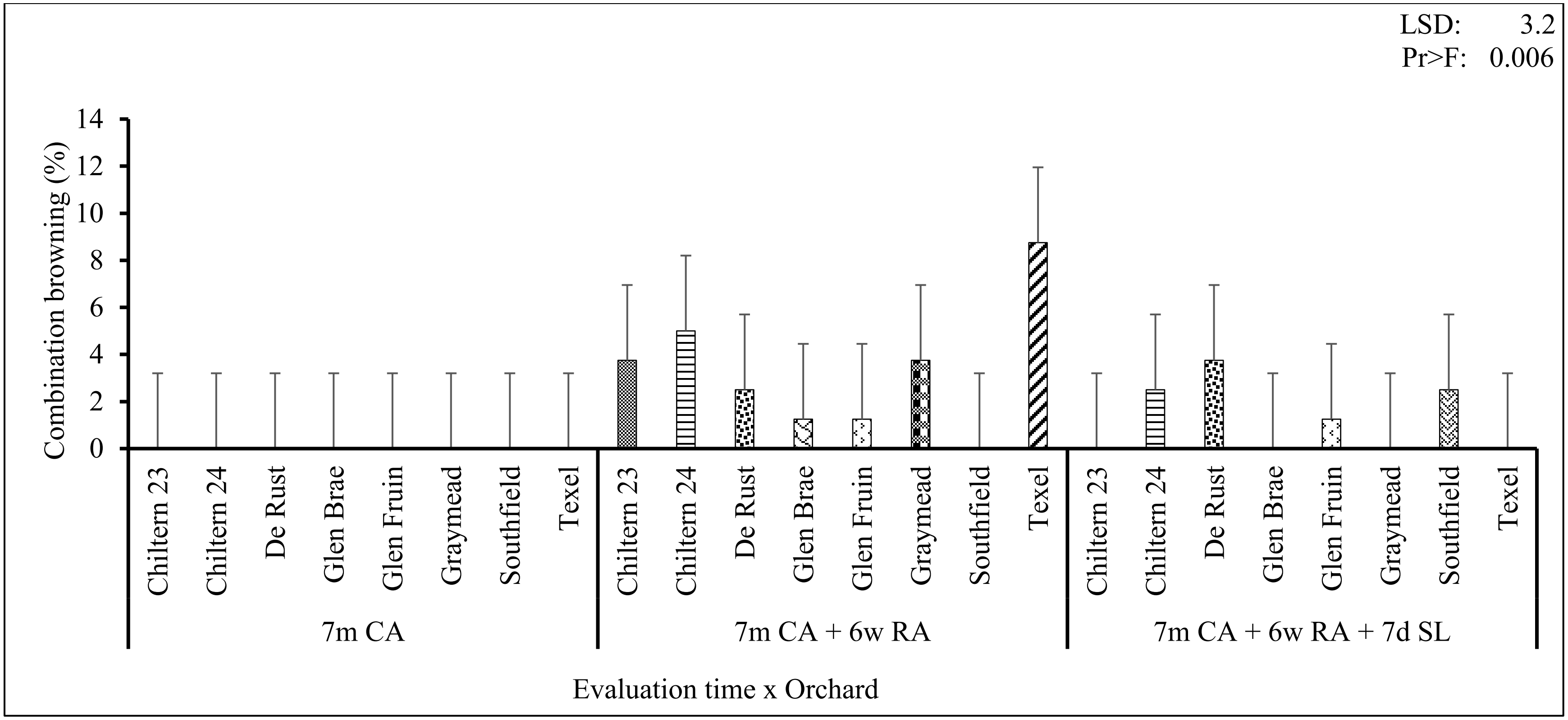


Figure 16: Percentage combination browning per evaluation time and orchard interaction for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C. The top error bar = the LSD value.

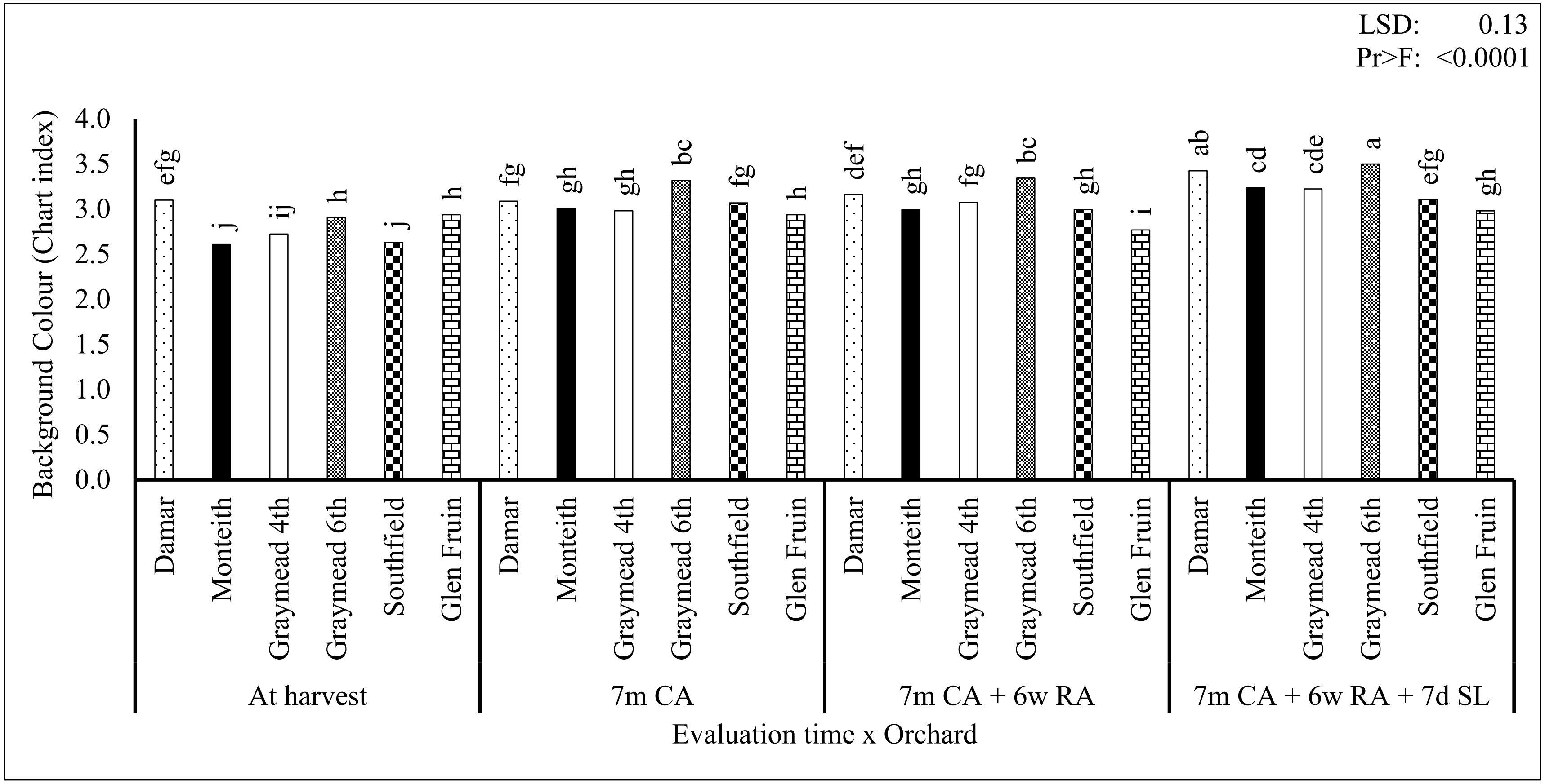


Figure 17: Multiple comparison test of the interaction between evaluation time and orchard on background colour for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

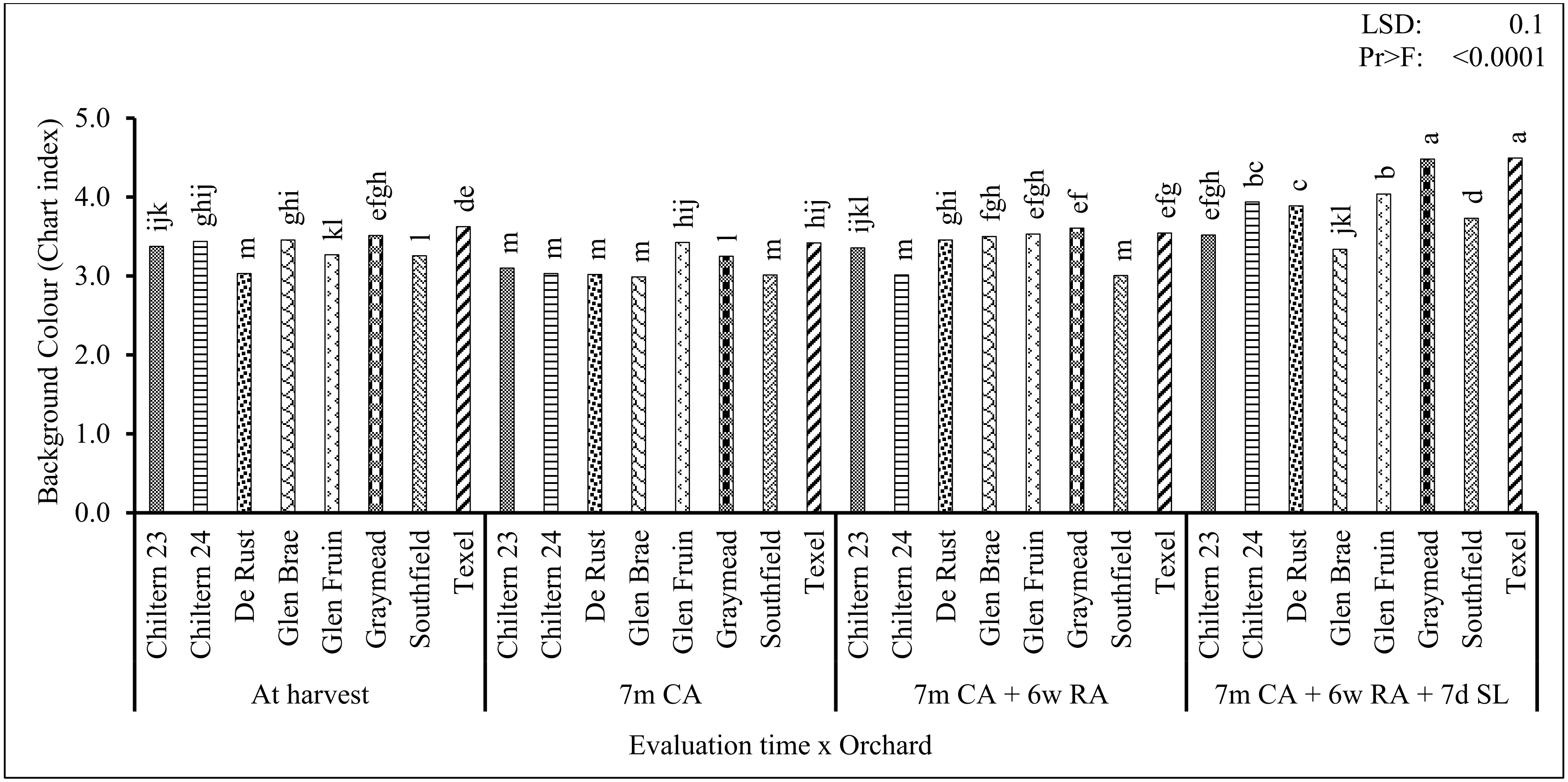


Figure 18: Multiple comparison test of the interaction between evaluation time and orchard on background colour for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

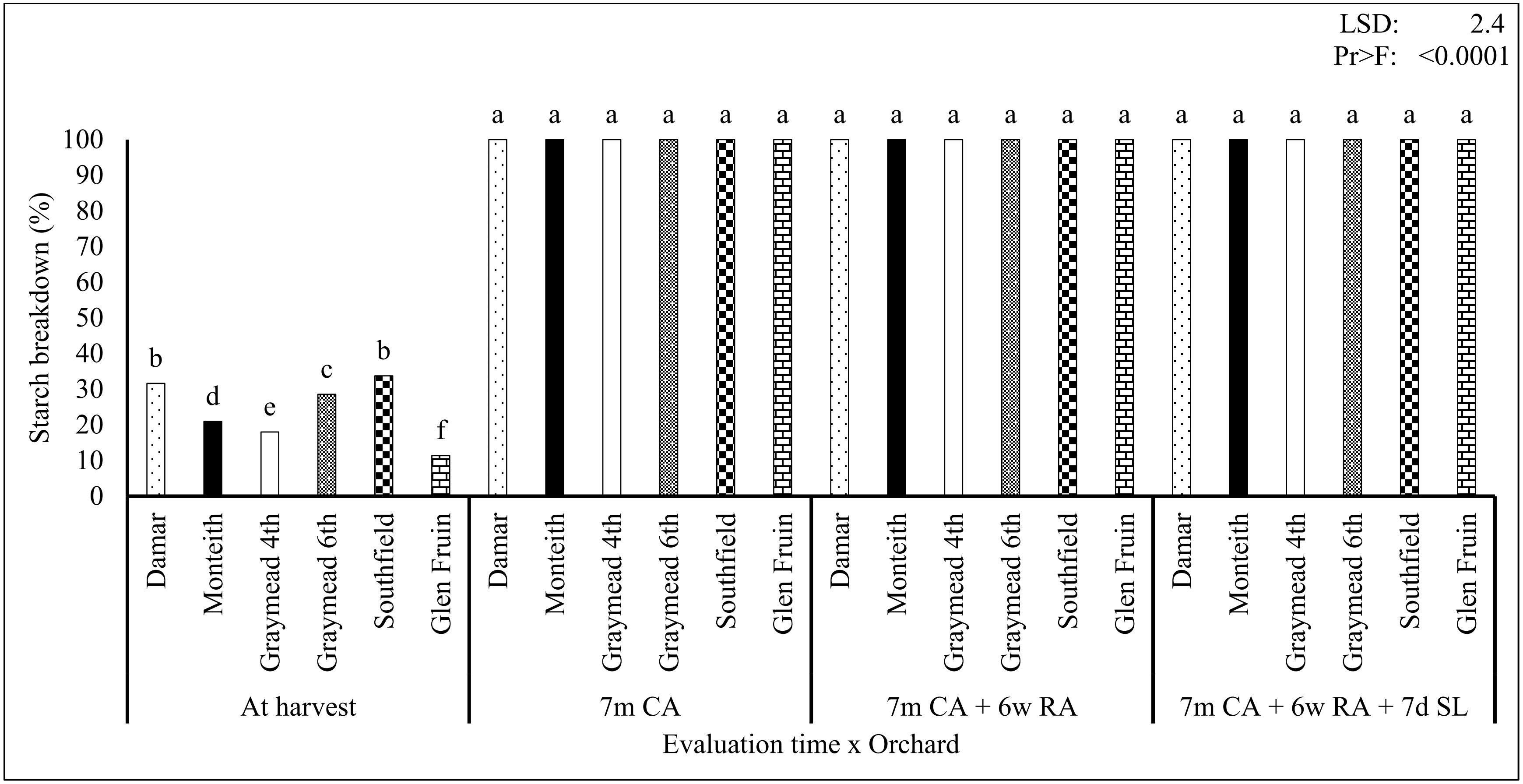


Figure 19: Multiple comparison test of the interaction between evaluation time and orchard on starch breakdown (SB) for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

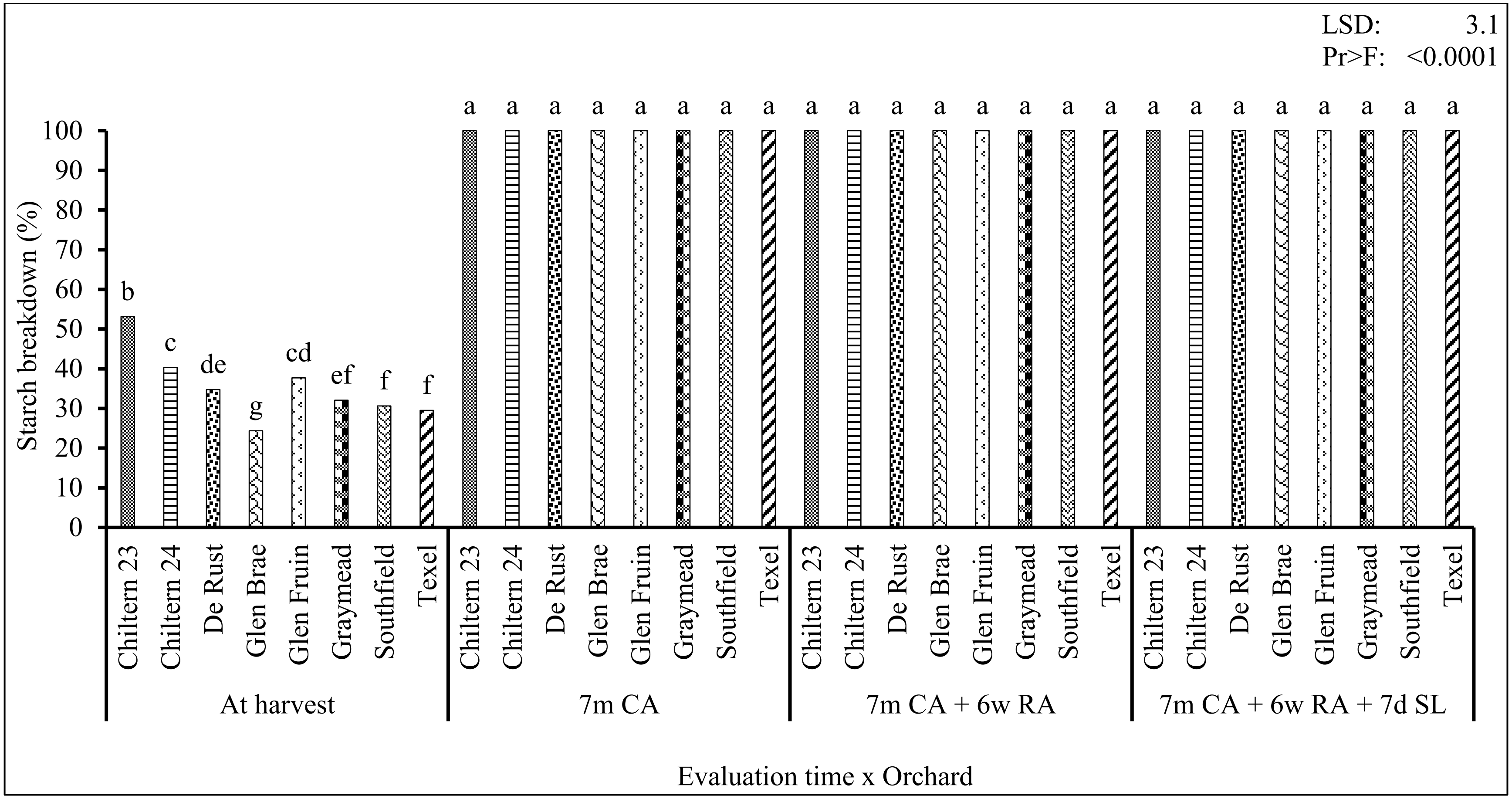


Figure 20: Multiple comparison test of the interaction between evaluation time and orchard on starch breakdown (SB) for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

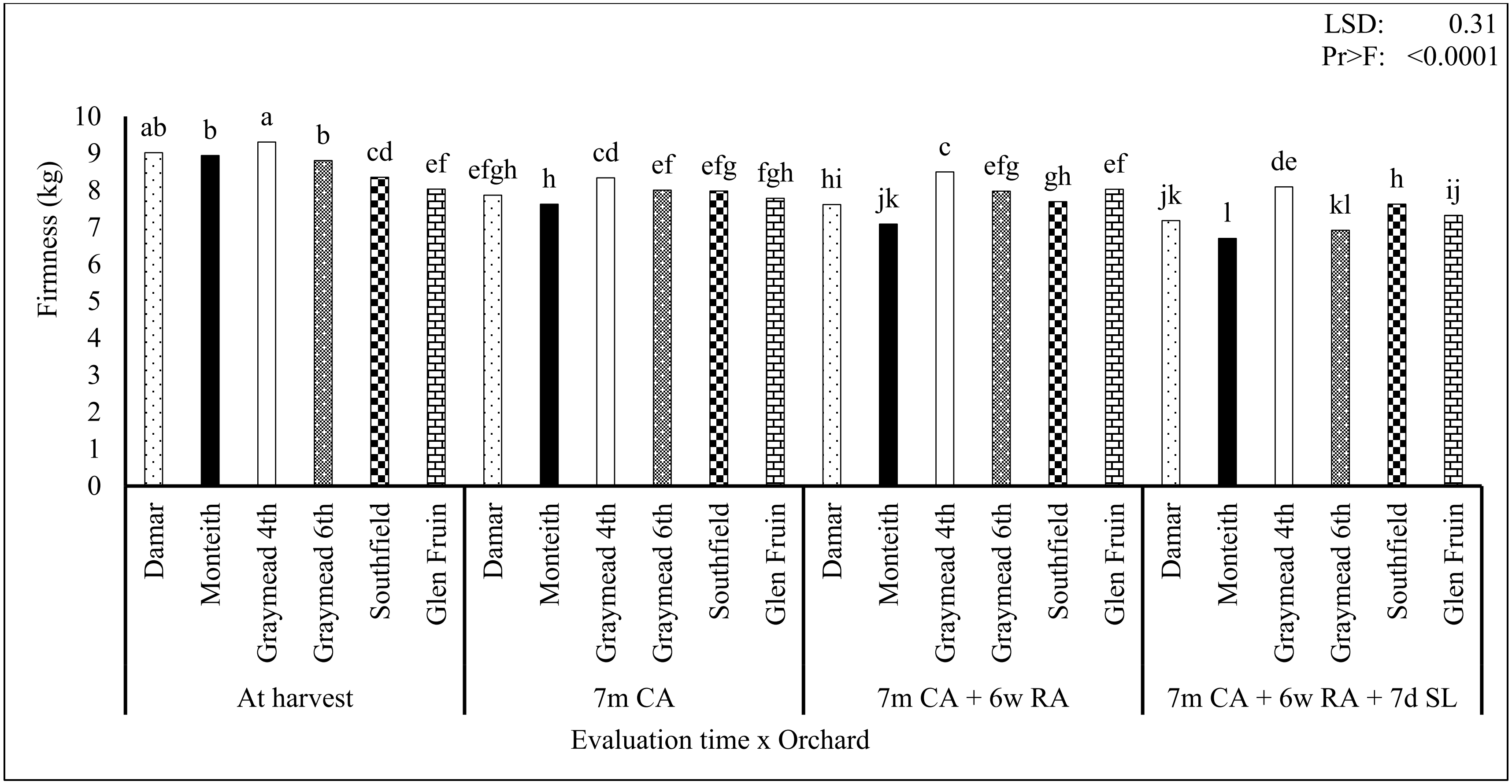


Figure 21: Multiple comparison test of the interaction between evaluation time and orchard on firmness for ‘Rosy Glow’ apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

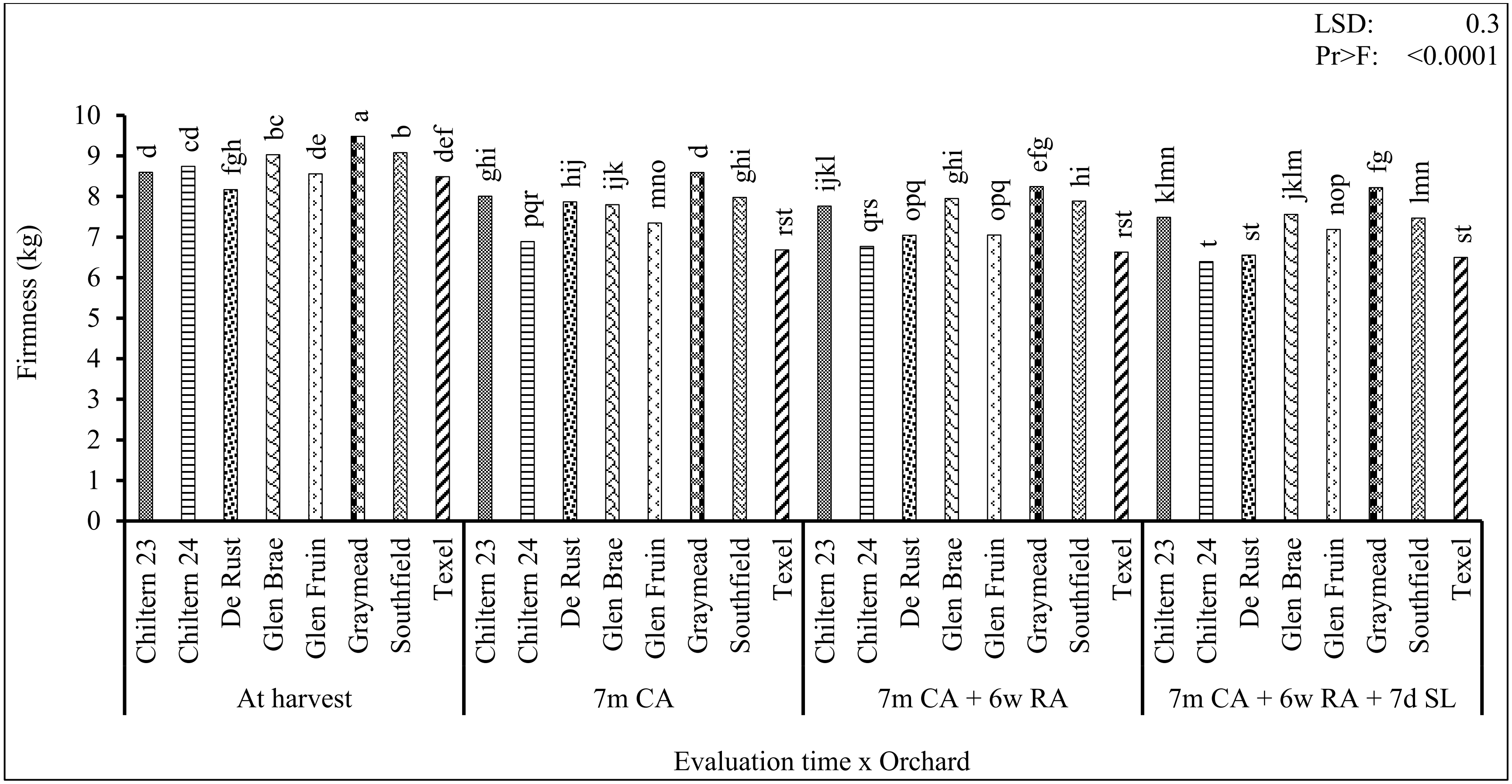


Figure 22: Multiple comparison test of the interaction between evaluation time and orchard on firmness for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

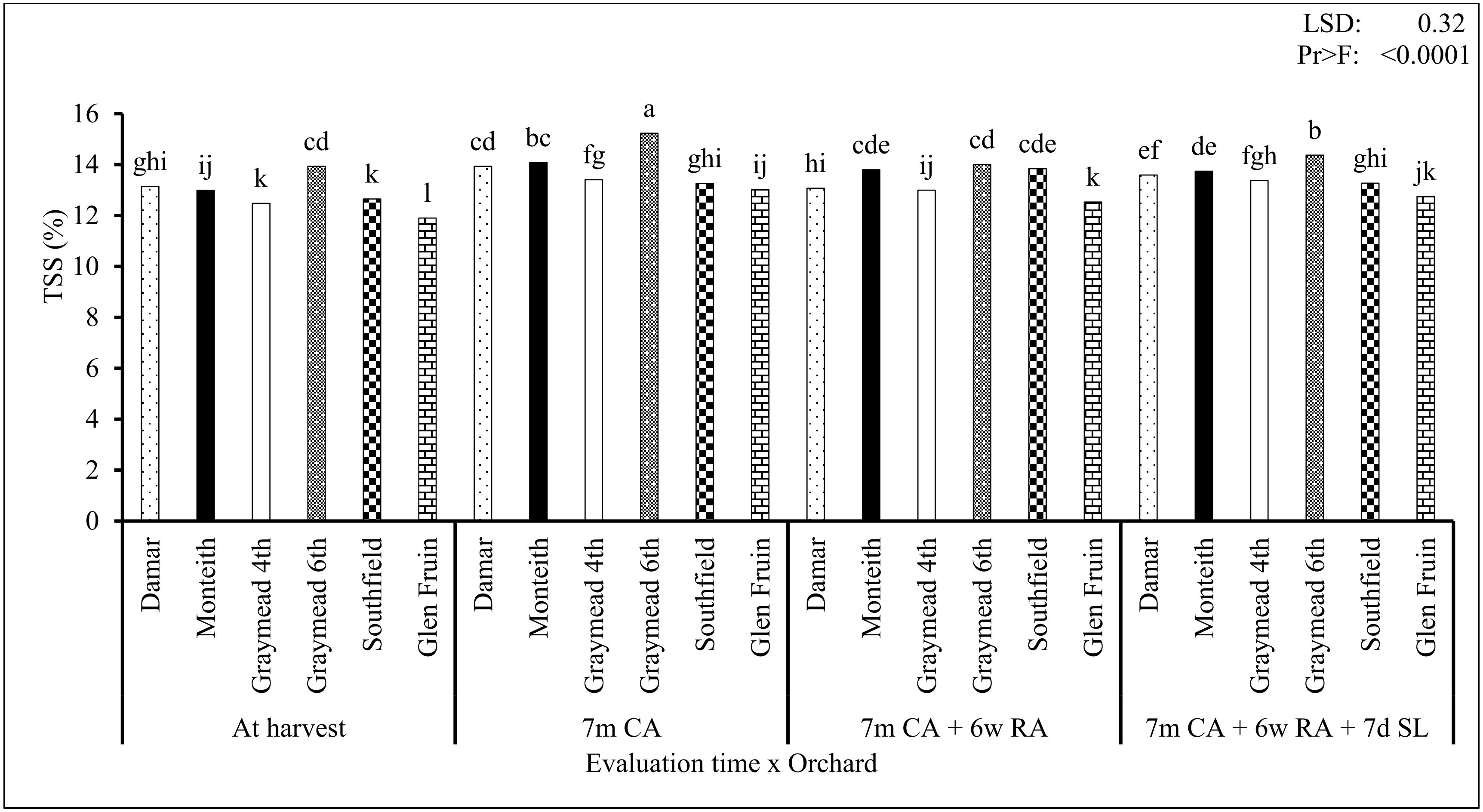


Figure 23: Multiple comparison test of the interaction between evaluation time and orchard on TSS for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

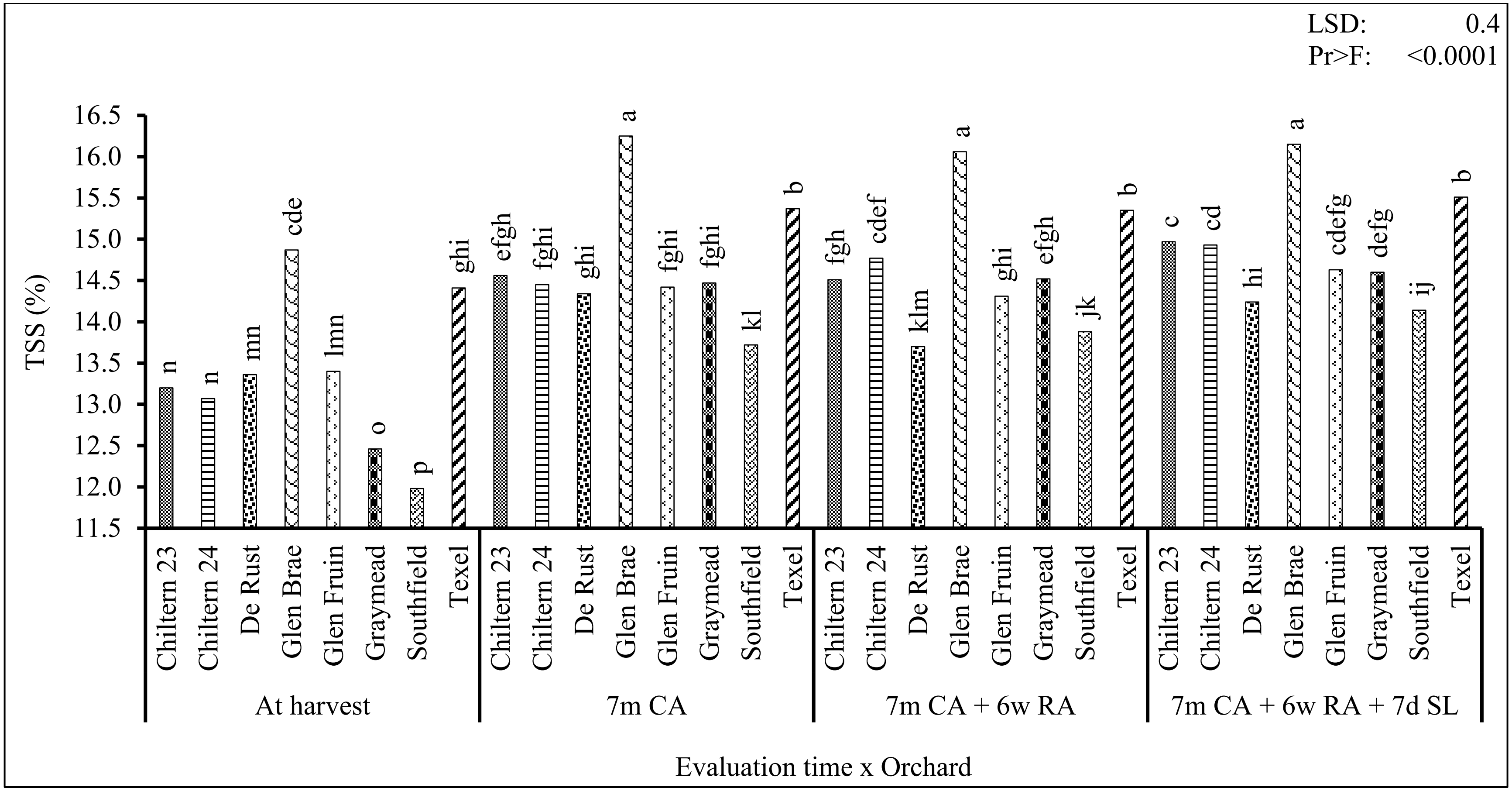


Figure 24: Multiple comparison test of the interaction between evaluation time and orchard on TSS for 'Rosy Glow' apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

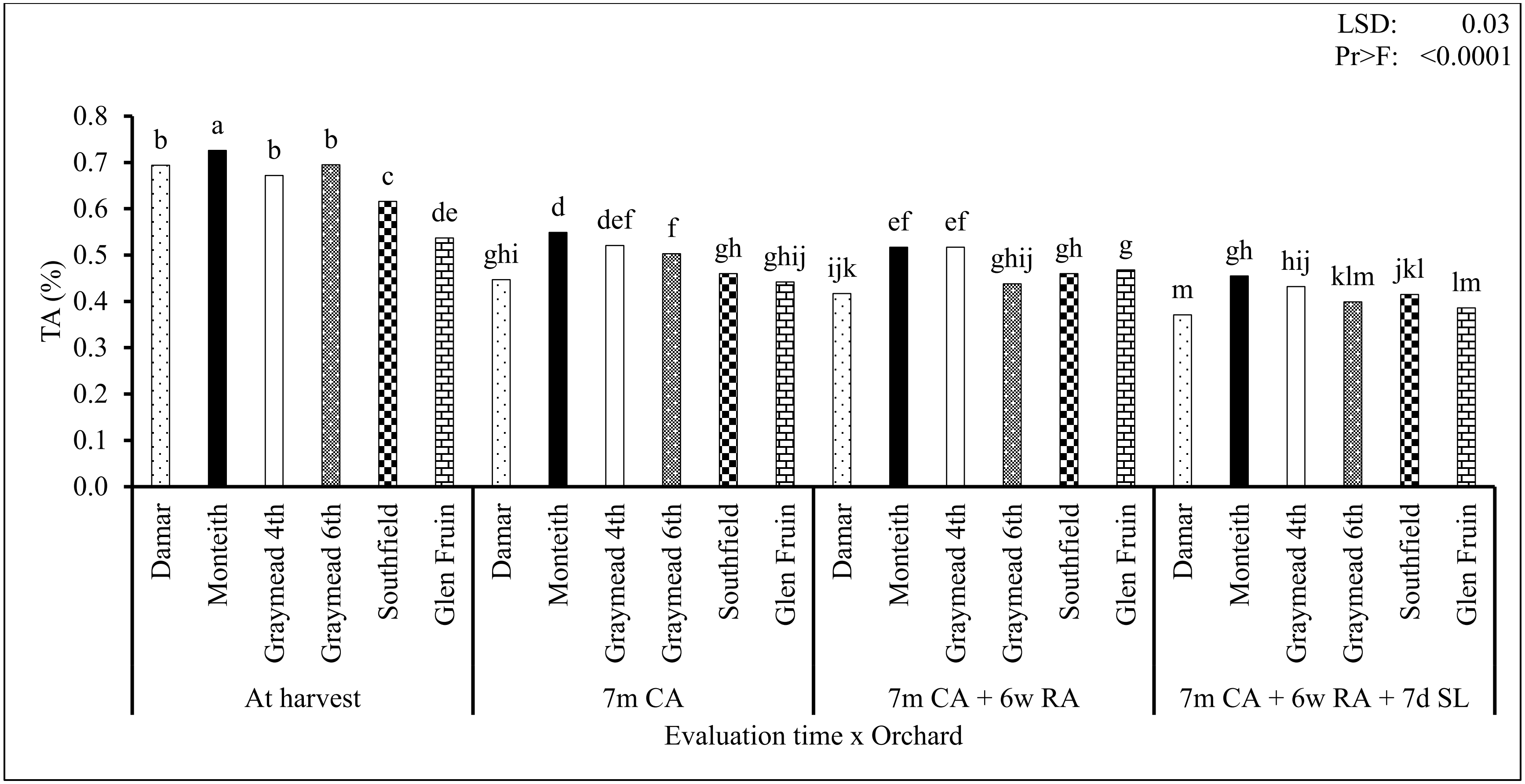


Figure 25: Multiple comparison test of the interaction between evaluation time and orchard on TA for 'Rosy Glow' apples that were harvested from six different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2014 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

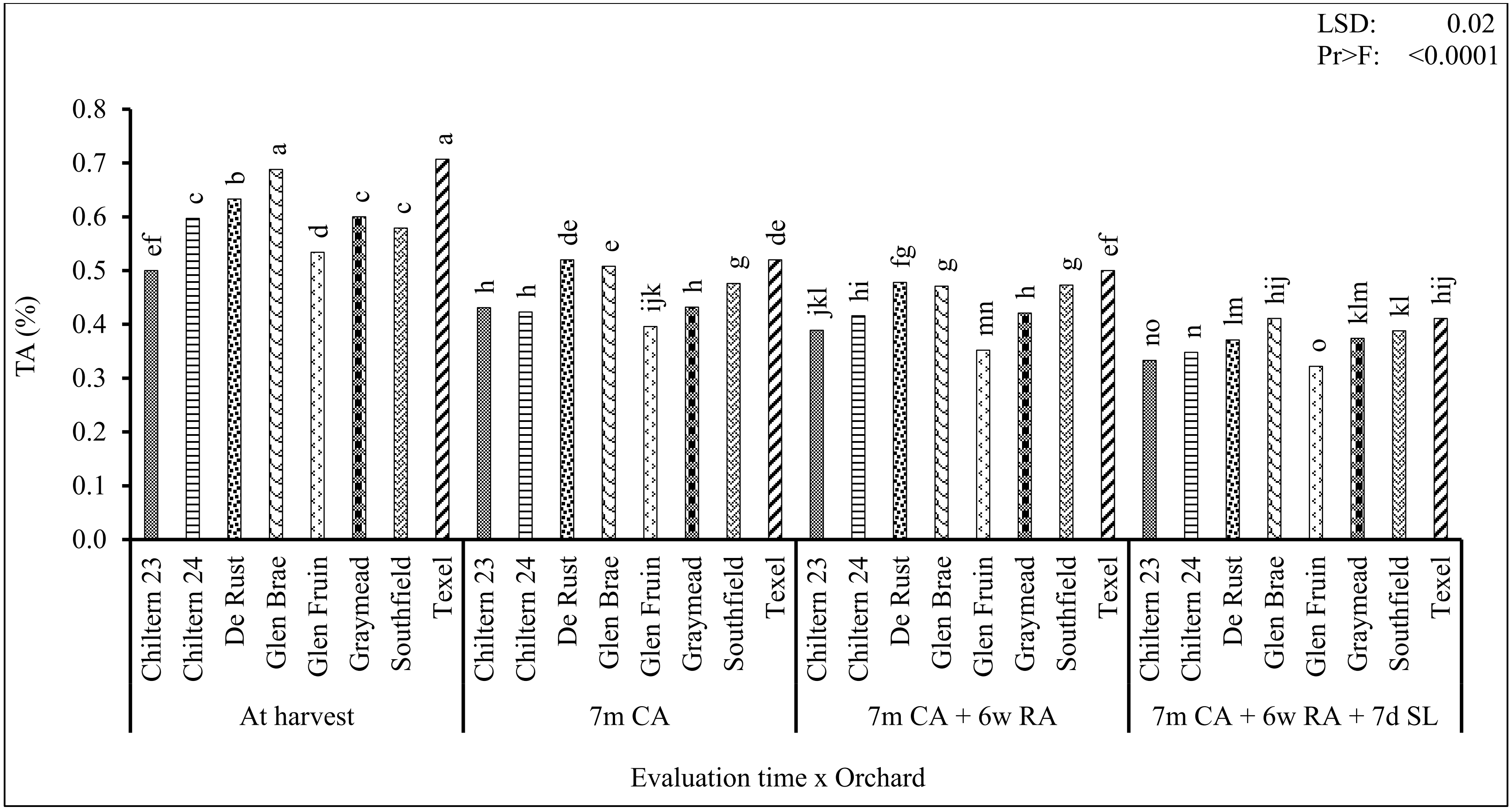


Figure 26: Multiple comparison test of the interaction between evaluation time and orchard on TA for ‘Rosy Glow’ apples that were harvested from eight different orchards in the Vyeboom, Villiersdorp and Elgin areas in the Western Cape in the 2015 season. Measurements were made on fruit that were stored at -0.5 °C for 7 months under controlled atmosphere + 6 weeks regular atmosphere and a subsequent 7-day shelf life period at 20 °C.

GENERAL DISCUSSION AND CONCLUSIONS

The first trial was aimed to assess the risk of internal browning development during storage as a result of post-optimum harvest maturity for the 'Rosy Glow' apple grown under South African conditions. There was enough evidence of harvest maturity influence on diffuse browning (DB) and radial browning (RB) in both seasons (2015 and 2016). However, harvest maturity interacted with storage time to affect DB and RB in the second season. The results further showed that early harvested fruit (13% starch breakdown (SB)) exhibited more DB in the first season, which is contrary to the finding of Jobling (2002), James (2007), Majoni et al. (2013) and Crouch et al. (2015). Data from the second season showed that post-optimum fruit was more susceptible to DB. This was also reported by James et al. (2005), Bergman et al. (2012), Majoni et al. (2013), Crouch et al. (2014, 2015), and Butler (2015). The average SB of the fruit harvested in the first season was 13%, which may have caused a restricted gas diffusion in the flesh of the fruit as reported by Park et al. (1993) and caused them to be more susceptible to browning. This is also in line with the observation of Toivonen (2008). Alternatively a low level of antioxidant was present at this early time of harvest as antioxidants also influence browning development in 'Cripps' Pink' as a result of harvest maturity (Crouch et al., 2014, 2015). The details of this finding needs to be studied further as no gas diffusion or antioxidant levels were measured in this trial to physiologically back up possible explanations.

There was a lower incidence of RB in the first season but the data from the second season showed that post-optimum harvested fruit were more susceptible to RB and it was progressive during the shelf-life. RB has been reported to be influenced by many factors including seasonal variations (Butler, 2015), low temperature during the cell expansion stage (Butler, 2015), growing–degree–hours (GDH; Moggia et al., 2015), as well as low storage temperature, and high CO_2 in the storage atmosphere (James and Jobling, 2008). The results clearly demonstrated the influence of harvest maturity and further confirmed that fruit harvested post-optimum exhibited a high susceptibility to RB. However, the progression of RB during shelf-life contradicts what was reported by Crouch et al. (2015). It is possible that one or more of the factors mentioned earlier may have contibuted to the development of RB in this study, more so, when fruits were stored in CA (1.0% CO_2, 1.5% O_2) at-0.5 °C or that incidence differences in some seasons aAquinore too low in order to make clear deductions. Nonetheless, fruit harvested at optimum maturity were less prone to the RB disorder, regardless of all other factors.

The incidence of CO_2 browning (CO_2B) and combination browning (CB) observed were too low in the two seasons, to draw sound conclusions from the data. The results however, showed that, CB in the first season was influenced by only storage time and in the second season, by an interaction of storage time and harvest maturity. CO_2B on the other hand was not influenced by any of the factors studied in both seasons. Butler (2015) and Moggia et al. (2015) both reported on factors influencing CB and stated that CB may be influenced by any of the factors that influences the individual browning types. CO_2B is reported to mainly be as a result of an increase in CO_2 gas in the storage atmosphere of fruit and this is mostly associated with CA storage (Jobling, 2002; Majoni et al., 2013). CO_2B observed in this study may have been a result of the CA technique employed or perhaps fruit temperature at the time of initiating CA storage.

The second trial sought to investigate the effect of 1-methylcyclopropene (1-MCP) application on the postharvest and post long term storage quality of ‘Rosy Glow’ apples and to determine the optimum storage temperature applicable to the realization of superior quality disorder-free post long term storage ‘Rosy Glow’ apples. The results revealed that the temperature effect was not very clear because the two storage temperatures used in the study gave contrasting results over the two seasons. There was a higher internal flesh browning (IFB) incidence in fruit stored at -0.5 °C in the first season, while the second season showed high browning incidence in fruit stored at temperature 2 °C. Reports from earlier studies are not strict on which temperature to use. Jobling (2002) suggested that storing ‘Cripps’ Pink’ fruit at temperatures higher than -0.5 °C (0 °C to 3 °C) will reduce IFB, while another study recommended -0.5 °C for long period storage of ‘Cripps’ Pink’ (Hurndall and Fourie, 2003). It therefore seems that storage temperature is influenced by other factors, such as the composition of storage atmosphere (Jobling, 2002) as well as perhaps harvest maturity and perhaps the higher storage temperature of 3 °C should be studied or alternatively step-wise cooling from these higher temperatures. Further investigation is necessary to draw a distinct conclusion on the optimum storage temperature for ‘Rosy Glow’ apples.

DB is undoubtedly the dominant type of browning observed, implying that ‘Rosy Glow’ apple may be more susceptible to this type of browning. All the other browning types observed (RA, CO_2B, and CB) require further investigation. The principal factor reported to influence DB development is chilling injury (Jobling, 2002; Bergman et al., 2012; Majoni et al., 2013; Butler, 2015). However, it is also reported that preharvest factors such as GDH, soil, climate, and maturity may take influence the susceptibility of 'Cripps' Pink' apples to browning (Jobling, 2002; Butler, 2015). The maturity factor was investigated in Paper I and it was found that harvesting fruit at optimum maturity is very likely to reduce the susceptibility of 'Rosy Glow' to DB. It is possible that some pre-harvest factors played a role in the susceptibility of 'Rosy Glow' to the IFB observed. It is therefore recommended that future investigations look into other pre-harvest factors that may influence the DB observed in this study in conjunction with antioxidant levels. 1-MCP has been established to maintain apple fruit and inhibit or even prevent disorders associated with long period storage, especially in CA where the level of O_2 is low and that of CO_2 is high (Sisler and Blankenship, 1996; Sisler and Serek, 2003; Watkins, 2006; Bergman et al., 2012; Majoni et al., 2013). In this study, 1-MCP treatment interacted with either temperature, time point and/or both to maintain 'Rosy Glow' quality. However, it was observed that in general 1-MCP treated fruit was better maintained in storage, mainly after 5 months CA, compared to 1-MCP untreated fruit.

The possibility of a reduced IFB incidence after storage as the 'Rosy Glow' apple trees grow older was investigated in the third trial of this study. After storage quality of fruit from 4 year old orchards, to that of 6 and 7 year old orchards were evaluated in the first and second seasons, respectively. The results indicated that total browning was influenced more by storage time, rather than tree age over the two seasons, possibly due to the small difference in tree ages available at the time of the study. DB was also influenced by storage time in both seasons, while RB was influenced by an interaction between storage time and tree age in the first season but by time point alone in the second season. CO_2B was affected by an interaction between storage time and tree age in the second season, but was not influenced by any of the two factors in the first season. CB was consistently influenced by storage time over the two seasons.

This study indicates that 'Rosy Glow' apples are susceptible to IFB types similar to those reported by Jobling (2002) and Butler (2015) for 'Cripps' Pink' apples. Even though specific experiments were not conducted into the causative physiology of the browning types in this study, it is believed that the work done by Jobling (2002), Majoni (2012) and Butler (2015) is very valid for the explanation of the IFB observed in this study. This study therefore concludes that 'Rosy Glow' IFB may be influenced by harvest maturity. Temperature at which fruit is stored and 1-MCP application may also mitigate the prevalence of the IFB disorder. The fruit storage duration in CA also plays a very important role with regards to IFB development and even the browning types that develop. Furthermore, storage duration affects harvest maturity, storage temperature or even 1-MCP application and in that way, influence IFB in ‘Rosy Glow’ apples.

It is recommended that ‘Rosy Glow’ apples in South Africa are harvested at optimum harvest (30−40%) and treated with a commercial concentration of 1-MCP for long-term CA storage. This study was not able to recommend a specific storage temperature, however, temperatures ranging from -0.5 °C to 2 °C may be used to store ‘Rosy Glow’ fruit in CA and RA for up to 7 months, but 5 months will be the optimum time period. Higher storage temperatures and stepdown cooling should be further studied. This study also acknowledges seasonal variations and preharvest factors/orchards, as well as possible climatic effects on the susceptibility of ‘Rosy Glow’ to IFB. It is therefore recommended that investigations should be conducted into the possible effects of these factors and a possible repeat of the tree age experiment using a wider age variation.

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