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**FINAL REPORT
(March 2018)**

1. PROGRAMME AND PROJECT LEADER INFORMATION

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2. PROJECT INFORMATION

Research Organisation	Stellenbosch University
Project number	A-13-USH-PH12
Project title	Harvest and storage condition plus duration influencing internal browning and fruit quality of 'Rosy Glow'
Short title	'Rosy Glow' fruit quality and internal browning after CA storage

Fruit kind(s)	Apple (Rosy Glow)		
Start date (mm/yyyy)	Nov 2013	End date (mm/yyyy)	Sept 2016
Key words	Temperature, Controlled Atmosphere, Harvest Maturity, 1-MCP Rosy Glow, Internal browning.		

Approved by Research Organisation Programme leader (tick box)



Objectives & Rationale

'Rosy Glow' is regarded prone to internal flesh browning. This study investigated tree age, harvest maturity, storage temperature, 1-methylcyclopropene (1-MCP) treatment and storage duration in CA as factors influencing 'Rosy Glow' internal browning.

Methods

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 3 fruit were stored for 7 months in CA (1% CO₂ and 1.5% O₂) plus 6 weeks in air 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 7 day shelf-life periods. Fruit numbers used in the trial were increased in the second season in order to decrease variability. The results therefore mainly focused mainly on the second season although both seasons are taken into consideration.

Key Results

Diffuse (DB), radial (RB), and combination (CB) as well as CO₂ browning were observed in both seasons. Optimum harvested fruit (<40%) were less susceptible to DB and RB. 1-MCP treated fruit had a lower internal browning incidence and no tree age effect was observed (4th and 7th leaf). DB and RB were first observed after 5 months in CA plus 6 weeks RA at -0.5 °C and increased with storage time and was highest after the shelf-life period. A weak (60%) correlation was observed with background colour and browning incidence after storage and shelf-life.

Conclusion/Discussion

Diffuse browning was the main type of browning present. Post-harvest maturity (>50% SB) played a significant role in 'Rosy Glow' browning development. Fruit quality was better retained at -0.5 °C than at 2 °C in the second season, while 1-MCP treated fruit quality was better maintained than control fruit over time. A large orchard influence was observed on 'Rosy Glow' browning. Ripening rates over time for various orchards and factors influencing ripening rate requires further investigation.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

The Rosy Glow apple (*Malus domestica* Borkh.) is a mutant variety of the Cripps' Pink cultivar and it was discovered in an orchard at Masons Road, Forest Range, South Australia in 1996. It is described as a highly and early colouring variety (Mason and Mason, 2003) in warmer winters and is currently therefore the main type of 'Cripps' Pink' planted in South Africa and other southern Hemisphere countries. 'Rosy Glow' has been predicted to be more prone to the flesh browning disorder than its parent cultivar Cripps' Pink (Dall, 2008) and therefore the need to investigate browning development as well as its handling practices in order to reduce browning and maintain fruit quality. Kupferman (2002) outlined a number of factors, both preharvest and postharvest, that influence the susceptibility of apples to browning disorders. However, two recent studies on 'Cripps' Pink' browning in South Africa identified crucial pre- and postharvest factors that may be involved in browning development (Butler, 2015; Majoni, 2011).

This study therefore sought to determine whether pre-harvest factors tree age and harvest maturity will have an effect on the susceptibility of 'Rosy Glow' apples to develop flesh browning during long term CA storage at -0.5 °C.

Postharvest it is important to understand when flesh browning occurs and whether storage temperature (-0.5 °C and 2 °C) in combination with 1-methylcyclopropene (1-MCP) over time could have an influence on internal browning and fruit quality during CA storage and was therefore also evaluated. This study was done in the Western Cape, South Africa on 'Rosy Glow' apple fruits grown in the Grabouw Area and is being conducted over two seasons (2014-2015).

References

- Butler, L., 2015. Internal flesh browning of 'Cripps' Pink' apple (*Malus domestica* Borkh.) as influenced by pre-harvest factors and the evaluation of near infrared reflectance spectroscopy as a non-destructive method for detecting browning. MScAgric Thesis, Department of Horticultural Science, Stellenbosch University.
- Dall, P., 2008. 'Pink Lady'TM news. International 'Pink Lady' alliance secretariat, North Melbourne, Victoria, Australia.
- Kupferman, E. (2002). Minimizing internal browning in apples and pears. Tree Fruit Research and Extension Centre, Washington State University-Postharvest Information network. [<http://postharvest.tfrec.wsu.edu/EMK2002D.pdf>]. Accessed in June, 2014
- Majoni, T.J., 2011. Physiology and biochemistry of 'Cripps' Pink' apple (*Malus domestica* Borkh.) ripening and disorders with special reference to postharvest flesh browning. MSc (Agric) Thesis. Faculty of AgriSciences, Department of Horticultural Sciences, Stellenbosch University.
- Mason, H. C., Mason, A. G. (2003). Apple tree named 'Rosy Glow'. United States Plant Patent Application Publication. Pub. No.: US 2003/0226181 P1.

5. DETAILED REPORT

a. PERFORMANCE CHART (for the duration of the project)

Objectives	Milestones	Original Target Date as per application	Achieved
Background info	Literature review MSc	15 April 2014	yes
Harvest Evaluations	Optimum Trial 1: Starch breakdown 30-40% Post-optimum Trial 1,2,3 (T1, 2, 3): Starch breakdown >50%	>15 April 2015	yes
Storage Evaluations	3 (T2), 5 (T2), 7 (T1, 2, 3) months in CA	≈ > 15 Jul, >16 Sept, >13 Nov	yes
After RA Evaluations	3 (T2), 5 (T2), 7 (T1, 2, 3) months in CA + 6 weeks	≈ > 31 Jul, > 7 Oct, >4 Dec	yes
After shelf-life Evaluations	3 (T2), 5 (T2), 7 (T1, 2, 3) months in CA + 6 weeks + 1 week shelf-life	(Last evaluations occurred Jan. 2016).	yes
Final report	Final report	Aug 2016 Handed in Final report on research report template. Therefore handing in again on Final Report template.	Aug 2016 March 2018
Presentations	<ol style="list-style-type: none"> 1. Presentation at the Combined Congress at Bloemfontein. (Best presentation award.) 2. South African Pink Lady Association AGM Technical Presentations. Top Fruit, Simondium. 3. Australian Producer's Association Limited. Postharvest Seminar, Adelaide. 4. ISHS 7th International Conference in Managing Quality Chains. 	<ol style="list-style-type: none"> 1. Jan 2016. 2. Nov 2016 3. Jan 2017 4. Sept 2017 	Yes
Papers:	<ol style="list-style-type: none"> 1. Doe, J.M., Schoeman, L., Crouch, E.M., 2017. Effect of harvest maturity, storage condition and duration, as well as tree age, on internal flesh browning and quality of 'Rosy Glow' apples grown in South Africa. Acta Hort. 	<ol style="list-style-type: none"> 1. Accepted Published 2018 	Yes

b) WORKPLAN (MATERIALS AND METHODS)

Trial One: Harvest Maturity

Experimental Site:

All lab experiments, regular atmosphere (RA) as well as shelf-life (SL) storage were carried out in the Department of Horticulture, Faculty of AgriSciences of the University of Stellenbosch in the Western Cape Province, South Africa. Controlled atmosphere (CA) storage was conducted in the Department of Horticulture, Faculty of AgriSciences of the University of Stellenbosch in 2014 (season one) and the CA facility of the Agricultural Research Council (ARC-Infruitec), located on the Helshoogte Rd, Stellenbosch, 7599, South Africa (33°55'31.2"S 18°52'26.2"E) in 2015 (season two) due to increase in fruit numbers.

Plant material

The apple cultivar (*Malus domestica* Borkh.) on which experiments were performed was Rosy Glow, a single branch sport of Cripp's Pink. 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 the Grabouw Area (Elgin) and Damar (block 16, 34°03'22.2"S 19°06'54.0"E planted in 2010) Vyeboom area in the Western Cape Province of 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 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 harvesting period, after a series starch breakdown percentage measurements as an indication of maturity and was measured with 0.5 M potassium iodide painted on the fruit cut surface.

Fruit numbers and harvest specifications

Optimum maturity fruit were harvest at a starch breakdown of 30% - 40% and post-optimum maturity fruit at >50% starch breakdown for both seasons. A total of 640 (2014 season) and 1600 (2015 season) fruit were harvested at random from all parts of the tree canopy (four trees and nine trees, respectively in same row per harvest maturity). Fruit were transported in lug boxes to the Horticulture Department of Stellenbosch University and maturity evaluations were done immediately after harvest. Fruit were stored at -0.5 °C in regular atmosphere for one day before CA storage commenced.

Fruit Storage

Three fruit storage techniques were used in this trial namely controlled atmosphere (CA), regular atmosphere (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 cm wide and 110 cm long and contained 10 plastic lug boxes containing fruit. Each Janny bin was connected to a pulsing inlet for nitrogen (N), oxygen (O₂) and carbon dioxide (CO₂) as well as a gas overflow. The CO₂ concentrations were kept constant by an automatic pulsing system (CA Janny bin Pulse Gas control System, Gas at Site (PTY) LTD., South Africa) at 1% and O₂ at 1.5%. In the 2015 season, fruit were stored in the CA facility of the Agricultural Research Council (ARC-Infruitec), located on the Helshoogte Road, Stellenbosch, 7599, South Africa (33°55'31.2"S 18°52'26.2"E) due to increase in fruit numbers for the trial. In South Africa, it has been

reported that 'Pink Lady'TM can be stored for up to 7 months under CA conditions ($\geq 1.5\%$ O₂ and $\leq 1\%$ CO₂ 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 in the Department of Horticulture storage facility, located in the Horticulture Department of Stellenbosch University. They were later kept at room temperature (20 °C) for seven days to simulate ripening or the SL period.

Experimental Design

The trial was a completely randomised design, analysed in a two-way analysis of variance (ANOVA) with harvest maturity (Maturity) and evaluation time (Time) as factors. Evaluation at each treatment /evaluation time consists of 10 replicates of 8 fruit each in the 2014 season and 20 replicates of 10 fruit each in the 2015 season. Fruit were evaluated at four evaluation times namely:

1. at harvest
2. after 7 months of storage in CA (1% CO₂, 1.5% O₂) at -0.5 °C (7m CA)
3. after 6 weeks of RA storage at -0.5 °C (7m CA + 6w RA)
4. after 7 days of SL at 20 °C (7m CA + 6w RA + 7d SL)

Physicochemical Analyses

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

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 across the stem end – blossom end plane but closer to the stem end for internal flesh browning assessment. Internal flesh browning 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, (2002). Browning type, presence of disorder and browning intensity were recorded for every individual fruit after the evaluation was conducted.

Fruit mass (g), diameter (mm) and firmness (kg)

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 with a maximum load capacity of 3 kg for determining fruit mass, a DMF apparatus for determining diameter, as well as an electronic calliper, with automated reading capturing, was used to assess these parameters. Firmness of fruit (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 sites with a potato peeler.

Background colour

Colorimeter measurement

Visual acuity of fruit skin colour in humans is best emulated by the calculation of the hue angle and chroma (Thai and Shewfelt, 1990). For this reason, this parameter was assessed using the chromameter (Model CR-400; Minolta Co., Ltd., Tokyo, Japan) where lightness (L), chroma (C), and hue angle (h°) were documented. Two chromameter measurements were done (reddest and greenest side) on each fruit in all replications. The L

values range from black = 0 representing a darker colour to white = 100. The h° estimates colour based on trigonometric functions on a scale of 0° to 360° (red-purple = 0° , yellow = 90° , bluish-green = 180° and blue = 270°) reading anticlockwise (Hunter, 1942; McGuire, 1992). A measure of the C is a measure of how saturated a pure hue colour may be (deviation from gray or white toward hue colour).

Colour chart index

Background colour was also measured via using the Colour Chart for Apples and Pears (Unifruco Research Services [Pty] Ltd., 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 note the colour transition from a green to yellow ground colour change with ripening.

Greasiness

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

Starch Breakdown (SB)

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

Total Soluble Solids (TSS) and Titratable Acidity (TA)

Equatorial apple slices representing all sides from the fruit (seeds punched out with 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 refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). TA was measured using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland) by titrating 5 g of juice from each apple sample with 0.1 M NaOH to a pH of 8.2. The TA was expressed as % malic acid while TSS was expressed as %Brix. In 2014, ten pooled samples from a composite of eight fruit per sample were recorded for each replicate, while in 2015, 20 pooled samples from a composite of 10 fruit per sample were recorded for each replicate.

Statistical Analysis

Physicochemical analysis data, were subjected to analysis of variance (ANOVA) by SAS version 9.2 (SAS Institute Inc., 2008, Cary, North Carolina, USA). Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means. Further correlations were done determining maturity/quality parameter's relation to browning development at harvest and after storage.

Trial two: Storage temperature, duration and 1-MCP (SmartFresh™) treatment

All laboratory evaluations, CA and RA as well as SL storage were carried out in the Department of Horticulture, Faculty of AgriSciences of the University of Stellenbosch in the Western Cape Province, South Africa.

Fruit sources

The study was conducted on the Rosy Glow apple cultivar (*Malus domestica* Borkh.), a single branch sport of Cripp's Pink. Fruit were hand-picked from two commercial orchards: Glen Fruin Farm (Blok 20, 34°11'23.6"S 19°03'10.4"E planted in 2008) in the Grabouw Area (Elgin) and Damar (Block 16, 34°03'22.2"S 19°06'54.0"E) in the Vyeboom area in the Western Cape Province of 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 orchard was in its 6th leaf at the time of this study (2014) and the orchard at Damar was in its 5th leaf (2015). Fruit were picked during the commercial harvest period, after a series of iodine tests (starch breakdown percentage as a measure of maturity measured with 0.5 M potassium iodide on fruit cut surface).

Fruit numbers and harvest specifications

Fruit were harvested at optimum maturity (<40% starch breakdown) in both seasons. A total of 1920 fruits (6 reps of 8 fruit per treatment combination) were evaluated in the 2014 season and in the 2015 season, 4800 fruits (12 reps of 10 fruit per treatment combination) were evaluated. Fruit were harvested at random from all parts of the canopy of trees in the same row and transported in lug boxes to the Horticulture Department, Stellenbosch University. Maturity evaluations were done immediately after harvest and remaining fruit stored at -0.5 °C in RA before being placed in the CA containers after checking pulp temperatures.

Fruit treatment and storage

Fruit were divided into two equal parts (A and B) according to two storage temperatures (A = -0.5 °C and B = 2 °C). One-half of each (A/2 and B/2) were treated with 1-MCP (SmartFresh™; at the commercially recommended time and rate) while the second half were left untreated (control). CA (1% CO₂, 1.5% O₂) and RA storage techniques were used to store fruits of the various treatments. Fruit for the respective temperatures were simultaneously stored for 3, 5 or 7 months in CA in Janny bins at both temperatures (-0.5 °C and 2 °C). Fruit were taken out after each CA period, further stored under RA for 6 weeks, simulating packing, shipping and stock rolling, still at respective temperatures. 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 N₂, oxygen O₂ and CO₂ as well as a gas overflow. The CO₂ concentrations were kept constant by an automatic pulsing system (CA Janny bin Pulse Gas control System, Gas at Site (PTY) LTD., South Africa) at 1% and O₂ at 1.5%. Fruit were finally ripened for 7 days at 20 °C simulating the shelf-life and ripening period.

Experimental Design

The trial was a completely randomised design, analysed in a three-way analysis of variance (ANOVA) with 1-MCP, temperature and evaluation time (Time) as factors. Evaluation at each evaluation time consists of 6 replicates of 8 fruit each in the 2014 season and 12 replicates of 10 fruit each in the 2015 season. Fruit were evaluated at 10 evaluation times namely:

1. at harvest
2. after 3 months of storage in CA (1% CO₂, 1.5% O₂) at -0.5 °C and 2 °C (3m CA)

3. after 6 weeks of RA storage at -0.5 °C and 2 °C (3m CA + 6w RA)
4. after 7 days of SL at 20 °C (3m CA + 6w RA + 7d SL)
5. after 5 months of storage in CA (1% CO₂, 1.5% O₂) at -0.5 °C and 2 °C (5m CA)
6. after 6 weeks of RA storage at -0.5 °C and 2 °C (5m CA + 6w RA)
7. after 7 days of SL at 20 °C (5m CA + 6w RA + 7d SL)
8. after 7 months of storage in CA (1% CO₂, 1.5% O₂) at -0.5 °C and 2 °C (7m CA)
9. after 6 weeks of RA storage at -0.5 °C and 2 °C (7m CA + 6w RA)
10. after 7 days of SL at 20 °C (7m CA + 6w RA + 7d SL)

Physicochemical Analyses

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

Maturity and quality indices:

Internal browning (%), mass (g), diameter (mm) and firmness (kg), background colour, greasiness, SB, TSS and TA measurements were performed as described in Trial 1.

Statistical Analysis

Physicochemical analysis data were subjected to analysis of variance (ANOVA) by SAS version 9.2 (SAS Institute Inc., 2008, Cary, North Carolina, 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. Further correlations and Principal Component Analysis were done determining 1-MCP, temperature and or quality parameter's relation to browning development at harvest and after storage.

Trial three: Tree age

All evaluations and RA as well as SL storage were carried out in the Department of Horticulture, Faculty of AgriSciences of the University of Stellenbosch in the Western Cape Province, South Africa. CA storage was conducted in the Department of Horticulture, Faculty of AgriSciences of the University of Stellenbosch in 2014 (season one) and CA facility of the Agricultural Research Council (ARC-Infruitec), located on the Helshoogte Rd, Stellenbosch, 7599, South Africa (33°55'31.2"S 18°52'26.2"E) in 2015 (season two) due to increase in fruit numbers.

Fruit sources

The study was also conducted on the 'Rosy Glow' apple cultivar. Fruit were hand-picked at optimum harvest maturity (30% - 40% starch breakdown) from six and eight commercial orchards for 2014 and 2015 assessments, respectively in three different locations (Vyeboom, Villiersdorp and Elgin areas) (Table 1). Fruit trees were grown and managed with current commercial practices and were picked during the commercial harvest period, after a series SB percentage as a measure of maturity were measured with 0.5 M potassium iodide on fruit cut surface.

Fruit numbers and harvest specifications

A total of 1920 (320 per orchard) fruits were evaluated in the 2014 and 2560 fruits were evaluated in the 2015. Fruit were harvested at random from all parts of the trees' canopy in the same row per orchard and transported in plastic lug boxes to the Horticulture

Department, Stellenbosch University. Maturity evaluations were done immediately after harvest and remaining fruit stored at -0.5 °C, where after, fruit were placed in CA containers after checking core temperatures.

Fruit storage

As with trial 1 and 2 fruit were evaluated after CA storage as described in the previous trials (1% CO₂ and 1.5% O₂) at -0.5 °C for 7 months in Janny bins in the 2014 season. In the 2015 season, fruit were stored in the CA facility of the ARC-Infruited, under the same conditions. Fruit were moved into RA at -0.5 °C for 6 weeks to simulate packing, shipping and stock rolling after CA storage in the Department of Horticulture storage facility, located in the Horticulture Department of Stellenbosch University. They were later kept at ambient temperature (20 °C) for seven (7) days to simulate ripening or the SL period.

Experimental Design

The trial was a completely randomised design, analysed in a two-way analysis of variance (ANOVA) with tree age and evaluation time (time) as factors. Evaluation at each time point consists of 10 replicates of 8 fruit per orchard in the both seasons. The four evaluation times were as follows:

1. at harvest
2. after 7 months of storage in CA (1% CO₂, 1.5% O₂) at -0.5 °C (7m CA)
3. after 6 weeks of RA storage at -0.5 °C (7m CA + 6w RA)
4. after 7 days of SL at 20 °C (7m CA + 6w RA + 7d SL)

Physicochemical Analyses

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

Maturity and quality indices:

Fruit mass (g), diameter (mm) and firmness (kg), background colour, greasiness, SB, TSS and TA were performed as described in Trial 1.

Statistical Analysis

Physicochemical analysis data were subjected to analysis of variance (ANOVA) by SAS version 9.2 (SAS Institute Inc., 2008, Cary, North Carolina, 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.

c) RESULTS AND DISCUSSION

Trial 1: Harvest Maturity

Internal browning

After storage at the end of season two the incidence of internal browning was only 5% (85 of 1600 fruit evaluated) (Fig. 1A). Of these, four fruit exhibited internal flesh browning after 7m CA, six fruit after 7m CA + 6w in RA and 75 fruit after 7m CA + 6w RA + 7d SL. Four types of internal browning were observed namely diffuse browning, radial browning, CO₂ browning and combination browning, with the most common being diffuse browning (65%) (Fig. 1B).

Radial browning was observed in 28% of the fruit while combination browning made up 6% of the total browning. As in season one, only one fruit was observed with CO₂ browning.

Table 1 shows the influence of harvest maturity and evaluation time on browning incidence. Harvest maturity caused significant differences in total browning incidence and was influenced by evaluation time (p-value <0.0001). Diffuse and radial browning varied with different maturities at different evaluation times (p-value <0.0001 for both types of browning) (Table 1). CO₂ browning was not influenced by any of the factors in this season but combination browning statistically differed significantly over time (p=0.011) (Table 1). A higher browning incidence was observed in fruit harvested at post-optimum maturity over all the evaluation times compared to fruit harvested at optimum maturity, however, only significantly after the SL storage period. Generally, the highest incidence of browning was observed after 7m CA + 6w RA + 7d SL with maximum total browning of 32%, diffuse browning of 22% and combination browning of 10% (Fig. 2). Further statistical analysis was not conducted on combination browning due to low incidence (5 fruit).

All four types of internal flesh browning (diffuse, radial, CO₂ and combination) were observed in 2015 (Fig. 1) and diffuse browning was the dominant type of internal flesh browning. It has already been established that 'Cripps' Pink' is susceptible to these types of internal flesh browning (Castro et al., 2007; Crouch et al., 2015a,b; James and Jobling, 2008; James et al., 2007; Jobling, 2002; Majoni et al., 2013; Mitcham et al., 2004) as well as the bulge type browning (Bergman et al., 2012). The bulge type of internal flesh browning was never observed in this study which may possibly mean that 'Rosy Glow' is not prone to this type of browning or not in the Elgin production region. It is evident in this study that 'Rosy Glow', like 'Cripps' Pink', is susceptible to diffuse browning, radial browning, CO₂ browning as well as combination (diffuse + radial) browning as predicted by (Dall, 2007). This confirms that 'Rosy Glow' may not be any different from its parent cultivar, 'Cripps Pink', except for the colouring of the peel (Langford, personal communication). Nonetheless, it is worthy to note that of 2,240 fruit that were evaluated in the two seasons only 127 exhibited internal flesh browning.

Two harvest maturities were evaluated in order to determine optimal harvest maturity for long term CA storage for 'Rosy Glow' in this trial. Again, it has already been amply demonstrated in studies all over the world that harvest maturity at which 'Cripps Pink' apple fruit is harvested, plays a very crucial role in how well it stores (Castro et al., 2007; Crouch et al., 2015a,b; East et al., 2004; James and Jobling, 2008; Jobling, 2002; Jobling et al., 2005; Majoni et al., 2013; Mitcham et al., 2004; Moggia et al., 2015). Coupled with harvest maturity, storage time (also known as evaluation time) has been reported to exacerbate a fruit's susceptibility to the internal flesh browning disorder (Bergman et al., 2012; Butler, 2015; Crouch et al., 2014; Jobling, 2002; Majoni et al., 2013; Moggia et al., 2015). Crouch et al., (2014) as well as Moggia et al., (2015) indicated that diffuse browning in 'Cripps Pink', while worsening with post optimum harvest, was also progressive in shelf-life. Reports show that radial browning is also influenced by harvest maturity but did not seem to be progressive in shelf-life (Crouch et al., 2014) in these studies as the incidence may have been too low to conclusively establish this. Both reports were confirmed in this study, however, radial browning, just like diffuse browning, was progressive in shelf-life in the second season of this study (Fig. 2) when more fruit were used and were significantly higher in post optimally harvested fruit.

It is therefore concluded that, the 'Rosy Glow' apple cultivar may be susceptible to all four types of internal flesh browning. The incidence of internal browning in 'Rosy Glow' may be mitigated by harvesting fruit at an optimum harvest maturity (30% - 40% starch). The progression of radial browning with shelf-life in this season needs to be investigated further to determine whether or not, radial browning in 'Rosy Glow' may be progressive. The difference in browning percentages, from one season to the other are also acknowledged, for which it is recommended that, seasonal variations, orchard as well as preharvest influences of the internal browning disorder in 'Rosy Glow' be investigated and large number of fruit evaluated in order to ensure meaningful statistical differences.

Starch breakdown (%)

The starch breakdown for optimally harvested fruit differed significantly from fruit harvested post-optimally. The aim was to harvest optimum fruit between 30-40% starch breakdown and post-optimum fruit >50% starch breakdown. The optimum fruit had an actual average starch breakdown of 38%, whereas the post-optimum fruit had an average starch breakdown of 47%. The two harvest maturities differed significantly in internal browning, and differences in maturity and quality indices, are further discussed in the following sections.

Background colour (colour chart and hue angle)

The background colour of fruit harvested at both maturities remained within the internationally acceptable standards (cream pale-green but not yellow background) (Hurndall and Fourie, 2003) over all evaluation points except for post-optimally harvested fruit after the SL period (Table 2). Optimally harvested fruit retained a significantly greener background colour (measured by colour chart and hue angle) compared to post-optimally harvested fruit after all three storage and evaluation times.

Firmness (kg)

Firmness was highest (9.3 kg) in fruit harvested at optimum maturity and significantly different from fruit harvested at post-optimum harvest maturity (8.7 kg). Fruit harvested at optimum maturity maintained a similar firmness after the three storage times (CA, RA and SL) and they did not statistically differ from one another except, from the firmness at harvest, which was significantly higher by 1 kg. The firmness of post-optimally harvested fruit deteriorated between the 6 week RA and the shelf-life storage evaluation (0.5 kg) as was the case between harvest and after CA storage (1.0 kg). The firmness of post-optimally harvested fruit did not differ after the CA and the RA storage evaluations. (Table 2). The difference in firmness after the shelf-life period was 1.2 kg between optimally and post-optimally harvested fruit. However, both maturities remained within the acceptable international export standards for 'Pink Lady' (7.0 kg) (Hurndall and Fourie, 2003) even after the shelf-life period.

Total soluble solids, (% TSS)

Even though TSS of fruit harvested at both maturities did not differ significantly at harvest, there were significantly different between the two harvest maturities after storage and evaluation (Table 2). Fruit harvested at optimum maturity had a significantly but slightly higher TSS level at evaluation time 7m CA but was significantly lower at evaluation times 7m CA + 6w RA and 7m CA + 6w RA + 7d SL. Fruit harvested at both maturities maintained TSS above the recommended 13% level over all storage evaluation times except at 7m CA + 6w RA where optimum maturity fruit had a slightly lower TSS level (12.7%).

Titrateable acidity (TA %)

There was no specific pattern in the acidity of fruit from both harvest maturities. Table 2 shows that acidity of fruit harvested at post-optimum maturity recorded a significantly higher acidity at harvest, yet, optimum harvested fruit at evaluation time 7m CA and 7m CA + 6w RA recorded a significantly higher acidity than post-optimum fruit. Fruit harvested at both maturities had a similar titrateable acidity level at 7m CA + 6w RA + 7d SL.

Trial 2: Storage temperature, duration and 1-MCP (SmartFresh™) treatment**Internal browning**

A total of 84 (1.8%) out of 4800 fruit (Fig. 3A) were affected by the flesh browning disorder in this season which is lower than what was observed in the first season. The first incidence of internal flesh browning in this season was observed at evaluation time 5m CA + 6w RA (14 fruit). Table 3 shows the distribution of the incidence of the internal flesh browning after storage evaluation times as well as the dominant browning type at that time.

Three types of the internal browning disorders were observed namely diffuse browning, CO₂ browning and combination browning, the most common being diffuse browning (65 fruit = 77%) (Fig. 3B). CO₂ browning accounted for 16% (13 fruit) and combination browning affected 6 fruit (7%) (Fig. 3B). There was no radial browning in this season but, interestingly, fruit were found with a combination of radial and diffuse browning in them. A different kind of combination browning was also observed at evaluation time 7m CA + 6w RA + 7d SL, but only affected 1 fruit.

Table 4 shows how the different types of browning as well as the total overall browning statistically differed with the factors and or, their interaction. According to Table 4, total browning, was significantly influenced by 1-MCP treatments at different storage temperatures and storage / evaluation times. Figure 4 shows that total browning was highest at evaluation time 7m CA + 6w RA + 7d SL in untreated control fruit stored at temperature 2 °C, and was significantly higher than total browning at all other storage / evaluation times. Total browning was significantly higher in control fruit stored at 2 °C at the onset of browning (5m CA + 6w RA) but at evaluation time 7m CA + 6w RA control fruit stored -0.5 °C rather exhibited a significantly higher total browning. 1-MCP treated fruit, rather than control fruit, exhibited a significantly higher total browning at -0.5 °C during evaluation time 7m CA + 6w RA + 7d SL but, at the same evaluation time, total browning was much higher in the control fruit stored at 2 °C. In general, total browning occurrence was higher in control fruit and at 2 °C (Fig. 4).

Diffuse browning dominated the season reaching levels slightly over the allowable percentage (1%) that is stipulated by the International Pink Lady Association (IPLA) standard. Figure 3B shows that 65 fruits were affected by the diffuse browning type of the disorder and that is 1.4% of the total number of fruit evaluated for this trial in the 2015 season. According to Table 4, diffuse browning was significantly influenced by 1-MCP treatments at different storage temperatures over different storage / evaluation times. Diffuse browning was highest in control fruit stored at 2 °C during evaluation time 7m CA + 6w RA + 7d SL (18%) and it was significantly higher than diffuse browning at all other evaluation times (Fig. 4). Incidence of diffuse browning was observed at all the evaluation times

beginning from 5m CA + 6w RA to 7m CA + 6w RA + 7d SL, except at 5m CA + 6w RA + 7d SL. At 5m CA + 6w RA, no diffuse browning was observed in control and 1-MCP treated fruit stored at temperature -0.5 °C, but fruit stored at 2 °C exhibited diffuse browning with no significant difference between control and 1-MCP treated fruit. Only 1-MCP treated fruit exhibited the diffuse browning disorder at 7m CA in both temperatures. Nonetheless, they neither statistically differed significantly from the control fruit nor at the different temperatures. Control fruit stored at -0.5 °C developed a significantly higher diffuse browning during 7m CA + 6w RA. Diffuse browning was observed at 7m CA + 6w RA in both temperatures in control as well as in 1-MCP treated fruit did not differ significantly from one another. Both treatments of 1-MCP exhibited diffuse browning in both storage temperatures at 7m CA + 6w RA + 7d SL. However, while diffuse browning observed in control fruit stored at temperature -0.5 °C was zero, the highest incidence of diffuse browning was seen in control fruit at 2 °C at this same time and differed significantly from the 1-MCP treated fruit at 2 °C and -0.5 °C (Fig. 4).

Carbon dioxide browning was also identified as one of the browning types in the season. According to Fig. 3B, thirteen (13) fruit were affected by this type of browning incidence and Table 4 shows that 1-MCP application and storage / evaluation time significantly influenced CO₂ browning. Further statistical analysis was not conducted on the data, nevertheless, more (9 fruit) carbon dioxide browning was observed in control fruit than there was in 1-MCP treated fruit (4) throughout the season.

There were a few incidences of combination browning. It was observed at evaluation time 7m CA + 6w RA + 7d SL, in 1-MCP treated fruit stored at 2°C. Two kinds of this browning type were observed: radial browning + diffuse browning in one fruit and radial browning + carbon dioxide browning in one fruit. Radial browning + carbon dioxide browning in one fruit combination type is new and unreported, however, it was observed only in one (1) fruit. Further statistical analysis, was not conducted on combination browning due to the low fruit number.

Control fruit were affected with the incidence of browning at both temperatures more than it affected 1-MCP treated fruit in this season. No radial browning on its own in one fruit was observed in the second season.

Table 5 shows that factors evaluation time, temperature and 1-MCP treatment affected the quality and maturity of 'Rosy Glow'. However, starch breakdown, which was influenced only by the interaction of storage / evaluation time and 1-MCP, due to starch levels breaking down shortly after harvest. Results for further statistical analysis conducted on individual maturity and quality parameters are discussed below.

Background Colour (Colour chart)

Background colour was similar for fruit harvested for all treatments (Fig. 5) and remained similar for all treatments at 3m CA and after 3m CA + 6w RA. Control fruit yellowed significantly more than 1-MCP treated fruit at 2 °C when evaluated at 3m CA + 6w RA + 7d SL while at -0.5 °C, both 1-MCP treated and control fruit exhibited similar ripening rates according to background colour. Yellowing was not significantly different between the control and 1-MCP treated fruit at both temperatures at 5m CA. However, while control and 1-MCP treated fruit exhibited similar background colour -0.5 °C, control fruit ripened significantly lower at temperature 2 °C at 5m CA + 6w RA (Fig. 5). The background colour was the most yellow in the control fruit stored at -0.5 °C for 5m CA + 6w RA + SL and it significantly differed from the background colour at all evaluation times. The background colour change

from green to yellow was significantly higher in the control non-1-MCP treated fruit at both storage temperatures at 7m CA + 6w RA + SL. No significant difference was observed between the control and 1-MCP treated fruit at temperature -0.5 °C during the same evaluation time (Fig. 5) except at the SL periods, where 1-MCP treated fruit remained similar at both temperatures, while control fruit consistently maintained better at -0.5 °C comparative to control fruit stored at 2 °C. Overall ripening in this season, however, remained within acceptable limits as stipulated in the IPLA standards, with only the control untreated 1-MCP fruit stored at 2 °C after 7m CA + 6w RA + 7d SL reaching a yellow colour of 4.0 on the colour chart and generally would occur after fruit had been bought and possibly been consumed.

Firmness (kg)

Firmness was the highest at harvest and did not differ significantly between treatments at harvest (Fig. 6). At 3m CA, control (untreated 1-MCP) fruit stored at -0.5 °C recorded a significantly lower firmness when all other fruit maintained a similar firmness. 1-MCP treated fruit exhibited a significantly higher firmness at both storage temperatures at evaluation times 3m CA + 6w RA and 3m CA + 6w RA + 7d SL. Fruit evaluated in the seventh month of CA (7m CA) behaved strangely (Fig. 6). Control untreated fruit recorded a significantly lower firmness at -0.5 °C compared to 2 °C. The same was observed at 7m CA + 6w RA. 1-MCP treated fruit exhibited a significantly higher firmness compared to untreated fruit and were mostly similar at both temperatures and all other evaluation times (Fig. 6). However, firmness of all the fruit met the acceptable IPLA standards (7.0 kg) in this season as in the previous.

Total soluble solids (TSS %)

TSS at harvest was higher in the fruit used for the 1-MCP treatment for both temperatures. Both the control fruit used for storage at 2 °C and -0.5 °C were harvested below 13% with fruit that was stored at -0.5 °C having a lower TSS compared to fruit that was to be stored at 2 °C. The reason for these differences at harvest are unknown. However, during storage evaluations at 3m CA + 6w RA and up to 7m CA + 6w RA + 7d SL had TSS levels higher than 13%. TSS was slightly but significantly higher in 1-MCP treated fruit stored at -0.5 °C but, did not differ significantly at 2 °C after 3m CA (Fig.7). There was no significant difference between control and 1-MCP treated fruit stored at -0.5 °C but control fruit stored at 2 °C had a significantly higher TSS after 3m CA + 6w RA. Fig. 7 shows that after 3m CA + 6w RA + 7d SL no significant difference was observed between control and 1-MCP treated fruit at the two different temperatures, however, higher TSS levels were recorded for fruit stored at -0.5 °C. Both control and 1-MCP treated fruit exhibited a similar level of TSS for both storage temperatures at 5m CA and 5m CA + 6w RA. There was no significant difference between the two 1-MCP treatments at -0.5 °C, but 1-MCP treated fruit had significantly higher TSS at storage temperature 2°C. Also, control fruit showed a significantly lower TSS at 2°C when there was no significant difference between control and 1-MCP treated fruit at -0.5 °C and 1-MCP treated fruit at 2°C (Fig. 7). At 5m CA + 6w RA + 7d SL, 1-MCP treated but not control fruit, recorded a significantly higher TSS at both temperatures and there was no significant difference between TSS level observed in 1-MCP treated fruit at both temperatures. There was no significant difference at 7m CA between 1-MCP treated and control (untreated fruit) at both temperatures. 1-MCP treated fruit recorded a significantly higher TSS at -0.5 °C but no significant difference was observed at 2 °C after evaluation time 7m CA + 6w RA (Fig. 7). After 7m CA + 6w RA + 7d SL, 1-MCP treated fruit

had a significantly lower TSS at -0.5 °C but recorded the highest TSS for fruit stored at 2 °C which significantly differed from control fruit stored at the same temperature at the same time (Fig. 7). Generally, treatment fruit maintained TSS within the IPLA acceptable limit at both temperatures throughout this season.

Titrateable acidity (TA %)

TA was highest at harvest (0.64%) and lowest after 7m CA + 6w RA + 7d SL at 2 °C without 1-MCP treatment (0.32%) (Fig. 8). After 3m CA + 6w RA + 7 d SL 2 °C stored control fruit had the lowest TA compared to other treatments and just above the 0.40% cut-off lower limit. After 5m CA + 6w RA untreated -0.5 °C stored fruit as well as treated and untreated 2 °C stored fruit also reached TA levels close to the lower limit cut-off of 0.40%. After shelf-life of the 5m CA storage period both -0.5 °C and 2 °C untreated control fruit were below the 0.40 % lower limit. After 7m CA all treatment TA values were above the lower limit however, after the additional 6w RA period, both untreated, -0.5 °C and 2 °C stored fruit were below the recommended lower TA limit. After this shelf-life period all fruit were either on the lower limit at 0.4% TA or below (0.32 % for 2 °C untreated fruit).

Starch (%)

Starch breakdown at harvest in this season was high and approximately at the upper limit (40%) for fruit at harvest according to IPLA standards. According to table 5, starch breakdown of fruit in this season was significantly influenced by 1-MCP and storage / evaluation time. Fig. 9 shows that, starch breakdown was similar in fruit used for the control (untreated) and 1-MCP treated fruit at harvest but, at 3m CA, 1-MCP treated fruit exhibited a slightly but significantly higher starch breakdown comparative to control fruit. Starch breakdown was 100% after all evaluation periods from 3m CA + 6w RA for all treatments.

1-MCP has been established to help maintain apple fruit quality and inhibit or even prevent disorders associated with long term storage, especially in CA, where the level of oxygen is low and may be sensitive to CO₂ (Bergman et al., 2012; Majoni et al., 2012; Sisler and Blankenship, 1996; Sisler and Serek, 2003; Watkins, 2006). 1-MCP treatment in this study interacted with both temperature and evaluation time to maintain 'Rosy Glow' quality and it was observed that in general, 1-MCP treated fruit fared better in storage, mainly after 5m CA, compared to 1-MCP untreated fruit. However, uncharacteristic of 1-MCP treatments the differences were relatively small and variable in many cases for this trial except for internal browning where 1-MCP treated fruit did not have as higher level of internal browning.

The effect of temperature was not very clear due to the fact that the two storage temperatures used in the study gave contrasting results in different seasons. Reports from studies already conducted are not strict on which temperature must be used. Jobling, (2002) suggested that storing 'Cripps Pink' fruits at temperatures higher than -0.5°C (0°C to 3°C) reduced internal browning but (Hurndall and Fourie, 2003) also recommended -0.5°C for long period storage of 'Cripps Pink'. It seem therefore that storage temperature is influenced by many other factors, for example, composition of storage atmosphere (Jobling, 2002) and harvest maturity (Majoni et al. 2012).

Diffuse browning is undoubtedly the dominant type of browning observed, implying that, 'Rosy Glow' may be more susceptible to the diffuse type of browning. All the other types of browning observed (radial, CO₂ and combination) need to be investigated further to ascertain their risk of development. The principal factor reported to influence diffuse

browning development is chilling injury (Jobling, 2002; Bergman et al., 2012; Majoni et al., 2012; Butler, 2015), however, it is also reported that preharvest factors such as Growing Day Degrees above 10°C, soil, climate and maturity may take a toll on the susceptibility of 'Cripps Pink' apples to browning (Butler, 2015; Jobling, 2002). Harvesting fruit at optimum maturity is very likely to reduce the susceptibility of 'Rosy Glow' to diffuse browning. In this study interestingly 2 °C at the end of storage had higher levels of diffuse and than that of -0.5 °C stored fruit and 1-MCP treated fruit. This may indicate the interaction of senescence and chilling injury and may help explain differences in seasons depending on the oxidative state of the fruit.

The incidence of browning in both seasons was very low. Data from the previous season should be seen with caution as it seems that browning occurred during storage, levelled out but decreased again after a longer period of storage for diffuse browning which is known to be present also after shelf-life in 'Cripps' Pink'. This while in the second season, we see more diffuse browning with longer storage times and in the shelf life period. The explanation would be that the number of fruit used in 'Rosy Glow' trial were increased in the second season, and trials done in a location, which was more likely to develop a higher incidence of internal browning. For this reason, 2015 season data, is reported and used for making recommendations. However, results should be confirmed for a third season with higher numbers to confirm storage temperature results between 2 °C and -0.5 °C as they differed between the seasons and were different from 'Cripps' Pink' findings.

Trial 3: Tree age

Internal browning

Two hundred and forty-six fruit (9.6%) out of 2,560 fruit (Fig. 10A) were observed with four types of internal browning (diffuse, radial, CO₂ and combination) (Fig. 10B) throughout the trial in 2015. Sixteen fruit were affected after 7m CA, eighty-one fruit after 7m CA + 6w RA and one hundred and forty-nine (199) fruit after 7m CA + 6w RA + 7d SL. Diffuse browning affected 148 fruit (60.2% of the total browning) making it the dominant browning type. Radial browning affected 60 fruit (24.4%), CO₂ browning affected 9 fruit and combination browning affected 29 fruit, 3.7% and 11.8% of the total browning, respectively (Fig. 10B).

Eight orchards were used in this season for this trial and they were each susceptible to internal browning (Fig. 11). All orchards exhibited all the types of browning except CO₂ browning which was seen in all but in fruit from two orchards (Chiltern 23 and De Rust) (Table 6). Here again, it is worthy to note that two orchards, both in their 4th leaf in 2015, which lay side by side on the Chiltern farm, exhibited very different levels of incidence of internal flesh browning (Fig. 11). A new type of combination browning, made up of radial browning and CO₂ browning, was observed this season in two fruit from the Chiltern 24 orchard (Table 6).

Results showed that storage / evaluation time but not tree age (4th vs 7th leaf), influenced total internal browning (Pr>F: <0.0001), diffuse browning (Pr>F: <0.0001), radial browning (Pr>F: <0.0001) as well as combination browning (Pr>F: <0.0001) (Table 7). CO₂ browning was influenced by the interaction of storage / evaluation time and tree age (Pr>F = 0.033) (Table 7) but affected few (9) fruit. Figure 12 shows how total browning, diffuse browning, radial browning as well as combination browning incidence varied with evaluation time after 7 months of CA storage, RA and SL. All but combination browning increased with time reaching their highest level at after SL. Radial browning at 7m CA was also not significantly different from radial browning at 7m CA + 6w RA. No further statistics was performed on CO₂ browning due low prevalence level.

Table 8 shows the results of the effect of orchard or farm and storage or evaluation time on total browning and the different types of browning observed in the season. According to Table 8, total browning and all the browning types except CO₂ browning, were influenced by the interaction of orchard and evaluation time factors. According to Fig. 13, all orchards except Glen Brae showed varying browning percentages over storage / evaluation times. Total browning percentages increased generally with time in fruit from all the orchards even though some increments were not significant for some orchards. The highest incidence of browning was seen in fruit harvested from the Chiltern 24 orchard and it differed significantly from observation made in all other orchards at all storage times except from the Graymead orchard after the RA as well as the SL evaluations. Glen Brae fruit had a similar browning percentage at the different evaluation times but Chiltern 23, De Rust and Glen Fruin all exhibited similar browning percentages (4-6%) after 7m CA + 6w RA (and significantly higher percentages after SL (11-16%) (Fig. 13). Southfield fruit had no browning after 7m CA + 6w RA, but did not differ significantly from Chiltern 23, De Rust, Glen Fruin and Glen Brae but, total browning increased to similar levels of these farms after the shelf-life period. Chiltern 24 and Texel fruit total browning both increased significantly from the 7m CA to the RA and again after the SL period, while Graymead fruit increased in total browning between the 7m CA and RA period but did not increase more after the SL period.

Chiltern 24 exhibited significant increase in total browning percentage with time and was similar to Graymead after CA and after shelf life (SL) but significantly lower than Graymead after RA. Chiltern 24 had significantly higher than the remaining orchards at all storage times (Fig. 13). Graymead apart from being similar to Chiltern 24 after CA and shelf-life, exhibited browning percentages significantly higher than other orchards at all storage times. No browning incidence was recorded for the Texel orchard after CA, but Texel exhibited browning percentage similar to Chiltern 24 and Graymead after RA and SL, respectively (Fig. 13) and was significantly higher than Chiltern 23, De Rust, Glen Brae, Glen Fruin and Southfield after RA and SL. All other orchards recorded similar browning percentages and were significantly lower at all storage evaluation times (Fig. 13).

Table 8 shows that diffuse browning was influenced by the orchard and storage time interaction. The highest incidence of diffuse browning (33%) was recorded after shelf life in fruit from the Texel orchard and it differed significantly from all orchards at all times, even though the diffuse browning incidence was relatively low (6%) and statistically comparable to lowest browning incidence after the 7m CA + 6w RA evaluation (Fig. 14). Graymead and Chiltern 24 also had a higher incidence of diffuse browning compared to fruit from other orchards after the SL period (20-23%) but also after the 7m CA + 6w RA evaluation (15 and 25 %, respectively). Although diffuse browning (7-9%) was already recorded for Chiltern 24 and Graymead orchard's fruit after 7m CA this incidence was not significantly different to other farms with no browning at that time. Graymead fruit recorded a similar diffuse browning percentage after 7m CA + 6w RA and after SL although browning increased significantly from 7m CA evaluation. Whereas fruit from Chiltern 23, Chiltern 24, Texel and Glen Fruin recorded similar diffuse browning percentages after RA and CA storage but a significantly higher percentage after SL. Even though Texel exhibited the highest diffuse browning on one evaluation after SL, Chiltern 24 and Graymead recorded higher diffuse browned fruit throughout the storage evaluations (42).

Radial browning according to Table 8, differed with the interaction of orchard and storage or evaluation time. Radial browning was first observed after 7m CA + 6w RA in this season in fruit from three orchards (Chiltern 24, Graymead and Texel) but, after SL, it was

observed in all the orchards (Fig. 15). The highest incidence of radial browning was observed for Chiltern 24 after SL and then Graymead fruit which did not differ significantly from each other. There was a general increase in the radial browning incidence in all the orchards from after 7m CA +6w RA to after SL (Fig. 15). Figure 15 also shows that, radial browning incidence after RA was similar in all the orchards except in for Graymead, where radial browning incidence was significantly higher. At evaluation time 7m CA + 6w RA + 7d SL, radial browning was lowest in Texel and it was significantly different from radial browning incidence in Chiltern 24, Graymead and De Rust but not from the remaining orchards (Fig. 15). Radial browning incidence in De Rust fruit was however, similar to Chiltern 23, Glen Brae, Glen Fruin and Southfield, significantly lower than Chiltern 24 and Graymead but, significantly higher than Texel.

Texel exhibited the highest incidence of combination (Radial + Diffuse) browning after RA and it differed significantly from all orchards after RA and SL. Chiltern 23, Chiltern 24 and Graymead show a similar level of combination browning (Radial + Diffuse) incidence after RA and they were significantly higher than that of Glen Brae, Glen Fruin and Southfield at that same time (Fig. 16). Combination browning (Radial + Diffuse) incidence in De Rust after RA was similar to that of all other orchards except Texel and no combination browning (Radial + diffuse) was observed in Chiltern 23, Glen Brae, Graymead and Texel after shelf life. A similar level of combination browning was observed in the remaining four orchards (Fig. 16). CO₂ browning observed in this season was neither influenced by storage nor evaluation time nor orchard according to Table 8.

Ground colour differences and starch breakdown

Table 9 indicates the effect of the interaction between evaluation time and tree age on fruit background colour and starch breakdown. It shows that background colour was significantly higher in both 4th and 7th leaf fruit after SL as expected compared to after CA and RA evaluation times. Seventh leaf fruit exhibited a significantly higher / yellower background colour as compared to the 4th leaf fruit for this evaluation time (Table 9). Background colour was similar for fruit from both tree ages at harvest and after 7m CA + 6w RA but varied significantly after 7m CA and after 7m CA + 6w RA + 7d SL. Starch breakdown was complete (100%) for fruit from both tree ages after 7m CA and according to Table 9, 4th leaf fruit had a significantly higher level of starch breakdown at harvest compared to the 7th leaf fruit, although both were still within the IPLA described optimum level of 30-40%.

According to Table 10, background colour was influenced by the interaction of orchard and storage / evaluation time. Figure 17 shows that, all the orchards, except De Rust and Texel were slightly greener after CA storage, however it must be noted that this is highly unlikely and colour chart indices were very similar and LSD values extremely small leading to significant differences which the human eye may not have been able to interpret at such small intervals. Also the colour chart works in values of 0.5 and LSD was 0.1. After 7m CA fruit ground colour from all farms seemed very similar but fruit from Glen Fruin, Graymead and Texel were slightly but significantly yellower. After 7m CA + 6w RA all fruit were slightly but significantly yellower except for fruit from Chiltern 24 which retained the greener colour and Glen Fruin that retained it slighter yellower colour obtained after 7m CA. After the SL evaluation Graymead and Texel were the most yellow (4.5) although they were similar at 7m CA + RA to Glen Fruin and Glen Brae fruit. However, fruit from these farms seemed to slightly more yellow from harvest right through to SL evaluations. Chiltern 24 fruit although being slightly less yellow after 7m CA + RA compared to other fruit after the SL

evaluation was also more yellow and comparable to De Rust and Glen Fruin with Glen Fruin reaching the colour chart value of 4.0. Chiltern 23 did not yellow at the same rate compared to Chiltern 24 after SL, which was interesting as they were harvested at very similar background colours and Chiltern 24 seemed less yellow after the RA period. Glen Brae fruit had the least yellow background colour and was similar to that after harvest.

Table 10 indicates that, starch breakdown was influenced by orchard and storage time. Figure 18 shows that differences in starch breakdown levels existed at harvest for the various orchards and that after the 7m CA storage no starch was left. Chiltern 23 was harvested at the highest starch breakdown (53%) levels, which was in the post-optimum range. In that season starch breakdown did not occur fast and then all of a sudden changed from the Friday to the Monday when maturities in the orchards were measured. All other farm starch breakdown levels were harvested below 40% (within the optimum range) and significantly different to that harvested from Chiltern 23. Graymead, Southfield and Texel had a similar level of starch breakdown at harvest ($\pm 30\%$) and although De Rust fruit starch breakdown levels were significantly higher compared to Texel and Southfield fruit, they were not significantly different to fruit harvested at Graymead. Glen Fruin was slightly more advanced in starch breakdown (below 40%) compared to Graymead but, was not significantly different to fruit harvested at De Rust. Glen Brae had the lowest starch breakdown at harvest ($\pm 24\%$) which may explain the retention of background colour after shelf-life of fruit from this farm.

Firmness, TSS and TA

According to Table 11, firmness, TSS and TA significantly changed over evaluation time as could be expected in ripening fruit. Firmness and TA were highest at harvest but decreased significantly over time and were lowest at 7m CA + 6w RA + 7d SL. TSS increased from harvest at 13.0% to 14.7% after 7m CA and 7m CA + 6w RA and was the highest after SL, although it was not significantly different to TSS after CA storage. Firmness and TSS were also slightly but significantly affected by the main effect tree age (Table 11). Fruit from the 7th leaf trees had a significantly higher firmness but lower TSS level while 4th leaf trees' fruit recorded a slightly lower firmness and higher TSS level. Although these differences were significant differences were very small.

Fruit firmness over harvest, storage and evaluation times differed for various orchards (Table 12). At harvest Graymead was the firmest followed by fruit from Southfield and Glen Brae (Fig. 19). Fruit from Chiltern 23 and 24, Glen Fruin and Texel were similar in harvest firmness and did not differ significantly. Chiltern 24 was slightly firmer than Chiltern 23 fruit and not significantly different from Glen Brae fruit firmness at harvest. De Rust had the lowest firmness after harvest but did not differ significantly from Texel fruit. After 7M in CA strangely a firmer harvest orchard Chiltern 24 fell below the IPLA standard as was Texel fruit and continued to decrease after the respective 6w RA and 7 day SL storage periods. Chiltern 23, Graymead, Glen Brae and Southfield however, maintained high level of firmness above the IPLA standard of 7.0 kg throughout the storage periods and SL. After 7m CA + 6w RA De Rust and Glen Fruin also had a firmness on or just below the IPLA standard which was also maintained on or below the IPLA standard after SL. Graymead recorded a significantly higher level of firmness that differed from all the orchards at all the evaluation times except after RA, where it was similar to firmness measured for Glen Brae.

Orchards also showed different TSS levels over time (Table 12). At harvest almost all orchards' fruit fell on the IPLA standard of 13% TSS or just below (Graymead and

Southfield) (Fig. 20) except for Glen Brae and Texel fruit that were higher than 14% TSS. Glen Brae and Texel maintained a TSS level significantly higher than all other orchards and similar from after CA through to SL (Fig. 20). Glen Brae recorded the highest TSS levels at all evaluation times and differed significantly from Texel and the other orchards. After 7m CA onwards all orchards attained the TSS level of above 13%.

TA changed significantly over harvesting and storage / evaluation time for various orchards (Table 12). At harvest all orchard TA levels fell within IPLA standards of 0.8-0.4 %TA (Fig 21). Glen Brae and Texel had the highest TA (below 0.7%) after harvest and after 7m CA. Chiltern 23 and then Glen Fruin had the lowest TA values at harvest with 0.5 and 0.52, respectively. After 7m CA Glen Fruin fruit had the lowest TA of 0.4% and after the RA period fell below 0.4%. Chiltern 23 also had a low TA of just below 0.4% after the RA period. After the 7m CA +6w RA + SL evaluation only Glen Brae and Texel TA was above 0.4% (Fig. 21).

Principal component analysis – relation of maturity indices and orchard to browning incidence

Figure 22, describes 71.6% of the variation in orchards for total browning and the quality parameters measured at harvest (harvest maturity data). It can be seen that Graymead and Chiltern 24 associated positively with firmness, total browning and background colour as they were in the same quadrant. Texel and Glen Brae positively associated with TA and TSS but, negatively with starch breakdown while Chiltern 23 and Southfield rather negatively associated with TSS and TA but, positively with starch breakdown (Fig. 22). Glen Fruin and De Rust did not show any clear association with any of the quality parameters at harvest nonetheless, Glen Fruin can be said to have a slight association with starch breakdown. Glen Fruin and De Rust were both located in a quadrant that can be said to indicate negative correlation with firmness, total browning and background colour. Table 13 shows that starch breakdown and TA were negatively correlated (80%). Background colour was weakly correlated to total browning. In Fig. 17 after 7m CA +6w RA + 7d SL Graymead and Texel were the most yellow and had the high levels of diffuse browning related to maturity even though other maturity parameters at harvest did not give an indication of ripening rate at harvest. Chiltern 24 with similar browning incidence to Graymead did not have the same high levels of yellowing but was yellower compared to Chiltern 23 and Glen Brae but did not differ to De Rust which had a low level of diffuse browning compared to Chiltern 24, perhaps explaining the weaker correlation for browning and background colour.

Four browning types were observed (Diffuse, radial, CO₂ and combination browning) and dominant of them is diffuse browning. 'Rosy Glow', according to this study may be more susceptible to diffuse browning and it may be progressive in SL. Due to the inconsistency of the incidence of radial and CO₂ browning, further investigations are required to ascertain the susceptibility of 'Rosy Glow' to these types of internal flesh browning. 'Rosy Glow' is susceptible to the combination type of browning, as is 'Cripps Pink' according to Butler, (2015). Tree age did not play a role in the development of the internal browning incidence observed on 'Rosy Glow' in the two seasons and may therefore not play a role in 'Rosy Glow' susceptibility to browning, however, there is the need to conduct investigations using tree ages of wider difference to be able to confirm this. Orchard factors, especially maturity

of fruit, interacting with storage and evaluation time point was seen to have greatly influenced the development of the browning disorder in 'Rosy Glow' in this study. A weak correlation was found with orchards with browning and yellowing after storage and shelf-life. Ripening rate differences of which background colour is often a first indication should be investigated further in relation to browning in particular diffuse browning which is known to be influenced with maturity (both seasons). It is also recommended that further investigations be conducted into determining the specific orchard factors that influence the differences in internal browning development of 'Rosy Glow'.

d) CONCLUSIONS

1. Four internal browning types (Diffuse browning (DB) Radial browning (RB), CO₂ browning (CO₂B) and combination browning (CB)) were identified in all three trials, with DB being the most dominant.
2. There was enough evidence of harvest maturity effect on internal browning incidence as investigated in trial one ($p=0.020$). DB was influenced by the interaction of all three factors, 1-MCP, temperature and evaluation time, and so was combination browning. Carbon dioxide browning however, was influenced by evaluation time only.
3. Tree age did not significantly affect incidence of internal browning. Analysis showed that interaction between evaluation time and orchard differences rather had influence on all the browning types observed.

References:

- Bergman, H., Crouch, E.M., Crouch, I.J., Jooste, M.M., Majoni, T.J., 2012. Update on the possible causes and management strategies of flesh browning disorders in 'Cripps' Pink' apples.
- Butler, L., 2015. Internal flesh browning of 'Cripps' Pink' apple (*Malus domestica* Borkh.) as influenced by pre-harvest factors and the evaluation of near infrared reflectance spectroscopy as a non-destructive method for detecting browning. MSc(Agric) Thesis. Faculty of AgriSciences, Department of Horticultural Sciences, Stellenbosch University.
- Castro, E., De, Biasi, B., Mitcham, E., Tustin, S., Tanner, D., Jobling, J., 2007. Carbon Dioxide-induced Flesh Browning in Pink Lady Apples. *J. Amer. Soc. Hort. Sci.* 132, 713–719.
- Crouch, E.M., Jooste M., Majoni, T.J., Crouch, I.J. and Bergman, H., 2015a. Harvest Maturity and Storage Duration Influencing Flesh Browning in South African 'Cripps' Pink' Apples. *Acta Hortic.* 1079, 121-127.
- Crouch, E., Butler, L., Majoni, J., Theron, K., Jooste, M., Lötze, E., 2015b. Harvest maturity, soil type, tree age and fruit mineral composition in browning susceptibility of 'Cripps' Pink' apples. Annual IPLA Symposium, Monticello Casino, Chile (Accessed, Jan 2016).
- Dall, P., 2007. Pink Lady® 1, 1–7.
- East, A.R., Maguire, K.M., Jobling, J., Tanner, D.J., Mawson, A.J., 2004. The Effect of Harvest Date on Incidence of Pink Lady™ Apple Postharvest diseases and Disorders, in: International Conference Postharvest Unlimited Downunder 2004 687. pp. 347–348.
- Hunter, R.S., 1942. Photoelectric tristimulus colorimetry with three filters. *JOSA* 32, 509–538.
- Hurndall, R., Fourie, J., 2003. The South African Pink Lady™ handbook. South African Pink Lady Assoc.
- James, H., Brown, G., Mitcham, E., Tanner, D., Tustin, S., Wilkinson, I., Zanella, A., Jobling, J., 2007. 3. Browning in Pink Lady™ apples: Research results have helped to change market specifications for blush colour which is an added bonus for growers. AP04008 Contin. Flesh Browning Cripps Pink Proj. to Validate Recomm. 63.

- James, H., Jobling, J., 2008. The Flesh Browning Disorder of 'Pink Lady'TM Apples. New York fruit Q.
- Jobling, J., 2002. Understanding Flesh Browning in Pink Lady Apples 1–4.
- Jobling, J., Brown, G., Mitcham, E., Tanner, D., Tustin, S., Wilkinson, I. and Zanella, A., 2004. Flesh browning of Pink LadyTM apples: why do symptoms occur? Results from an international collaborative study. *Acta Hortic.* 682, 851–858.
- Majoni, T.J., Jooste, M., Crouch, E.M., 2013. The Effect of 1-MCP and Storage Duration on the Storage Potential and Flesh Browning Development on 'Cripps ' Pink ' Apples Stored under Controlled Atmosphere Conditions. *Acta Hortic.* 1007, 49–56.
- McGuire, R.G., 1992. Reporting of objective color measurements. *HortScience* 27, 1254–1255.
- Mitcham, E., Tanner, D., Tustin, S., Wilkinson, I., Zanella, A., Jobling, J., James, H., Brown, G., 2004. Flesh browning in Pink LadyTM apples: research results have helped to change market specifications for blush colour which is an added bonus for growers, in: International Conference Postharvest Unlimited Downunder 2004 *Acta Hortic.* 687, 175–180.
- Moggia, C., Pereira, M., Yuri, J., Torres, C., Hernandez, O., Icaza, M., Lobos, G., 2015. Preharvest factors that affect the development of internal browning in apples cv. Cripp's Pink: Six-years compiled data. *Postharvest Biol. Technol.* 101, 49–57.
- Sisler, E.C., Blankenship, S.M., 1996. Methods of counteracting an ethylene response in plants. US Pat. 5, 988.
- Sisler, E.C., Serek, M., 2003. Compounds interacting with the ethylene receptor in plants. *Plant Biol.* 5, 473–480.
- Thai, C.N., Shewfelt, R.L., 1990. Peach quality changes at different constant storage temperatures: empirical models. *Trans. ASAE* 33, 227–0233.
- Watkins, C.B., 2006. 1-Methylcyclopropene (1-MCP) based technologies for storage and shelf life extension. *Int. J. Postharvest Technol. Innov.* 1, 62–68.

Tables and Figures

Table 1. Univariate test p-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for Evaluation time (T), harvest maturity (H) and the interaction between harvest maturity and Evaluation time (TxH). Fruit were harvested on Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

Source	Total Browning	Diffuse Browning	Radial Browning	Carbon Dioxide	Combination
Evaluation Time (T)	< 0.0001	< 0.0001	< 0.0001	0.395	0.011
Harvest Maturity (H)	< 0.0001	< 0.0001	0.0000	0.319	0.240
Time*Maturity (TxH)	< 0.0001	< 0.0001	< 0.0001	0.395	0.248

Table 2. Maturity indices measured for two harvest maturities at evaluation times: after harvest, after 7 months of controlled atmosphere storage (CA) at -0.5 °C, and after an additional 6 weeks of regular atmosphere storage (RA) simulating the shipping and stock rolling period and 7 days of shelf-life storage (SL) at ambient temperature (≈ 20 °C). Fruit were harvested at Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

Evaluation time	Maturity	Starch Breakdown (%)	Background Colour (Chart index)	Background Hue Angle (°)	Firmness (kg)	TSS (%)	TA (%)
Harvest	Optimum	38c	3.2d	95.2 b	9.3a	13.3e	0.64b
	Post-Optimum	47b	3.2d	94.2 b	8.7b	13.5cde	0.66a
7 months CA	Optimum	100a	3.1de	102.5 a	8.4c	13.7bc	0.49c
	Post-Optimum	100a	3.4c	78.9 c	7.7d	13.4de	0.46d
7m CA + 6wRA	Optimum	100a	3.1e	102.8 a	8.3c	12.7f	0.47c
	Post-Optimum	100a	3.5b	76.9 c	7.6d	13.9ab	0.42d
7m CA + 6w RA + 7d SL	Optimum	100a	3.5b	96.0 b	8.3c	13.6cd	0.36e
	Post-Optimum	100a	4.0a	70.5 d	7.1e	14.1a	0.35e
Source		Pr>F					
Evaluation Time		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Maturity		0.027	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Evaluation Time*Maturity		0.003	< 0.0001	< 0.0001	0.000	< 0.0001	< 0.0001

Table 3. Incidence of internal browning with occurring storage and evaluation time and dominant type of browning per the specific evaluation time of 'Rosy Glow' apples after 5 and 7 months of controlled atmosphere storage (1.5 % O₂, 1.0 % CO₂) and 6 weeks under regular atmosphere 5 and 7 months + 6 weeks RA) and 7 days at ambient (5 and 7 months + 6 weeks RA + 7 days SL). Data represents the mean of fruit stored at -0.5 °C and 2 °C. Fruit were harvested on Damar farm in the Vyeboom area of the Western Cape in the 2015 season.

Evaluation Time	Number of Brown Fruit	Dominant Browning Type
5 months CA	0	-
5 months CA + 6 Weeks RA	14	Diffuse
5 months CA + 6 Weeks RA + 7 days SL	0	-
7 months CA	8	Diffuse
7 months CA + 6 Weeks RA	19	Diffuse
7 months CA + 6 Weeks RA + 7 days SL	43	Diffuse

Table 4. Univariate test p-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for Temperature (Temp), Evaluation time (Time), 1-MCP treatment factors and their interactions. Fruit were harvested on Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

Source	Total Browning	Diffuse Browning	Carbon Dioxide	Combination
TEMP	0.003	0.022	0.142	0.011
1-MCP	0.023	0.119	0.142	0.011
Time *TEMP	< 0.0001	< 0.0001	0.116	< 0.0001
Time *1-MCP	0.005	0.099	0.013	< 0.0001
TEMP*1-MCP	0.023	0.054	0.769	0.011
Time *TEMP*1-MCP	< 0.0001	< 0.0001	0.063	< 0.0001

Table 5. Significance of maturity indices measured for 'Rosy Glow' apples by main and interactive effect of treatments (Temperature (Temp: -0.5 °C and 2 °C) and 1-MCP (SmartFresh™) (1-MCP: treated (and untreated) over specified storage / evaluation time (Time) (3, 5, 7m of Controlled Atmosphere (CA) + 6w regular atmosphere (RA) and 7 days at ambient). Fruit were harvested from Damar Farms in the Vyeboom area of the Western Cape in the 2015 season.

Source	Background Colour	Starch	Firmness	TSS	TA
Time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TEMP	0.588	0.399	0.023	0.001	< 0.0001
1-MCP	< 0.0001	0.089	< 0.0001	0.002	< 0.0001
Time*TEMP	0.009	0.749	< 0.0001	< 0.0001	< 0.0001
Time *1-MCP	< 0.0001	0.037	< 0.0001	< 0.0001	< 0.0001
TEMP*1-MCP	0.030	0.308	0.409	0.028	< 0.0001
Time *TEMP*1-MCP	< 0.0001	0.143	< 0.0001	< 0.0001	< 0.0001

Table 6. Number of brown fruit and browning type of 'Rosy Glow' apples that were harvested in 2015 at optimum maturity (30-40% starch breakdown) from two tree age groups (4th and 7th leaf) from eight orchards / farms (4 for each age group) and stored at -0.5 °C. 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 the subsequent 7 day shelf-life period at ambient temperature.

Age (Leaf/Years)	Farm/ Orchard	Browning Type					SUM		
		Diffuse browning (D)	Combination browning (R+C)	Combination browning (D+R)	Radial browning (R)	CO ₂ damage (C)	Brown fruit (#)	Non-brown fruit (#)	Total fruit (#)
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	305	315
7	Graymead	43	0	3	22	4	72	248	320
7	Glen Fruin	8	0	2	4	1	15	310	325
7	Texel	32	0	7	3	1	43	277	320
Total		87	0	14	32	7	140	1140	1280

Table 7. Univariate test p-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for Evaluation time (Time) and Tree Age (4th and 7th leaf) as well as their interaction. Fruit were harvested from eight different orchards in the Grabouw/ Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature.

Source	Total Browning	Diffuse Browning	Radial Browning	Carbon Dioxide	Combination
Time	< 0.0001	< 0.0001	< 0.0001	0.114	< 0.0001
Tree Age	0.076	0.081	0.643	0.087	0.854
Time *Tree Age	0.244	0.119	0.254	0.033	0.885

Table 8. Univariate test p-values indicating significance at the 0.05% level on the type of browning percentage of 'Rosy Glow' apples for evaluation time (time) and orchard/farm as well as their interaction. Measurements were made on fruit of two different tree ages (4th and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period (SL) at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

Source	Total Browning	Diffuse Browning	Radial Browning	Carbon Dioxide	Combination
Orchard	< 0.0001	< 0.0001	< 0.0001	0.170	0.227
Time	< 0.0001	< 0.0001	< 0.0001	0.108	< 0.0001
Orchard*Time	< 0.0001	< 0.0001	< 0.0001	0.079	0.006

Table 9. Significance of maturity and quality indices measured for 'Rosy Glow' apples by main and interactive effect of treatment (Tree Age and Evaluation time). Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period (SL) at ambient temperature after which they were evaluated.

Evaluation time	Tree Age	Background Colour	Starch
Harvest	4 th leaf	3.3 cd	38 b
Harvest	7 th leaf	3.4 c	33 c
7months CA	4 th leaf	3.0 e	100 a
7months CA	7 th leaf	3.3 d	100 a
7months CA + 6 weeks RA	4 th leaf	3.3 cd	100 a
7months CA + 6 weeks RA	7 th leaf	3.4 c	100 a
7months CA + 6 weeks RA + 7 days SL	4 th leaf	3.7 b	100 a
7months CA + 6 weeks RA + 7 days SL	7 th leaf	4.2 a	100 a
Source	Pr>F		
Time	< 0.0001		
Tree Age	< 0.0001		
Time*Tree Age	< 0.0001		

Table 10. Significance of orchard and evaluation time as well as their interaction in background colour, starch breakdown (%) firmness (kg), TSS (%) and TA (%) measured for 'Rosy Glow' apples. Fruit were harvested from eight different orchards in the Grabouw / Elgin and Vyeboom as well as Villiersdorp areas of the Western Cape in the 2015 season.

Source	Background Colour	Starch	Firmness	TSS	TA
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 11. Firmness (kg), total soluble solids (TSS %) and titratable acidity (TA %) measured for 'Rosy Glow' apples by main effect of Evaluation Time and Tree age and their interaction. Fruit were harvested from eight different orchards in the Grabouw/ Elgin, Vyeboom and Villiersdorp area of the Western Cape in the 2015 season. Fruit were stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life (SL) period at ambient temperature.

Factor	Firmness (kg)	TSS (%)	TA (%)
<u>Evaluation time:</u>			
Harvest	8.8 a	13.3 c	0.61 a
7 months CA	7.7 b	14.7 ab	0.46 b
7 months CA + 6 weeks RA	7.4 c	14.6 b	0.44 c
7 months CA + 6 weeks RA + 7 days SL	7.2 d	14.9 a	0.37 d
<u>Tree Age:</u>			
7 th leaf	7.8 a	14.2 b	-
4 th leaf	7.7 b	14.6 a	-
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 12. Significance of maturity and quality indices measured for 'Rosy Glow' apples by Orchard and Evaluation time interaction. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp of the Western Cape in the 2015 season.

Source	Background Colour	Starch	Firmness	TSS	TA
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 13. Correlation matrix of the correlation between total browning percentage and quality parameters at the 0.05% significance level of 'Rosy Glow' apples. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

Variables	Background Colour	Starch	Firmness	TSS	TA	Total Browning
Background Colour	-	-0.177	0.452	0.333	0.354	0.559
Starch	-	-	-0.332	0.303	-0.801	-0.069
Firmness	-	-	-	0.348	-0.010	0.394
TSS	-	-	-	-	0.621	-0.179
TA	-	-	-	-	-	0.203
Total Browning	-	-	-	-	-	-

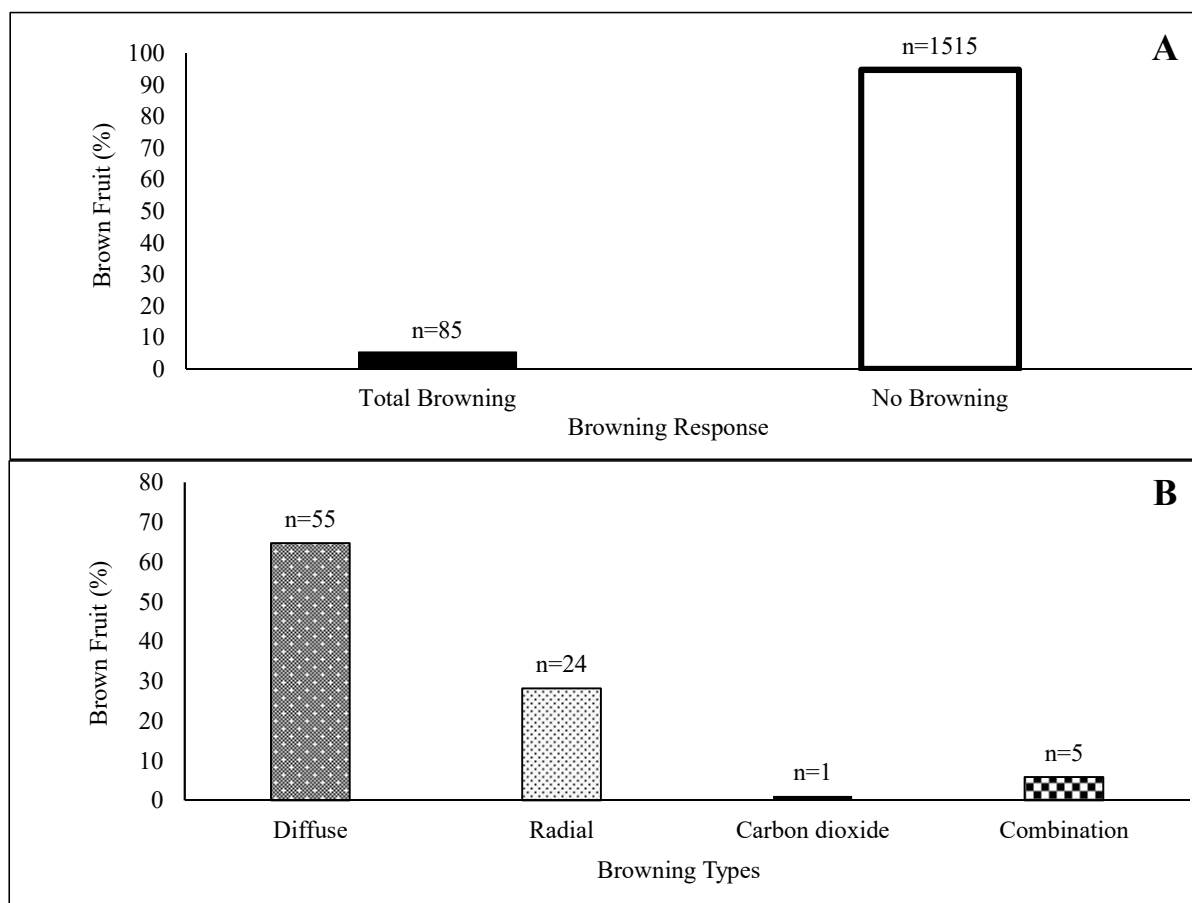


Figure 1. Browning incidence (%) in number of fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples that were harvested in 2015 at two harvest maturities. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (1.5 % O₂; 1 % CO₂), followed by 6 weeks in regular atmosphere at -0.5 °C and a 7 day shelf-life period at ambient temperature. Fruit were harvested on Damar farm in the Vyeboom area of the Western Cape in the 2015.

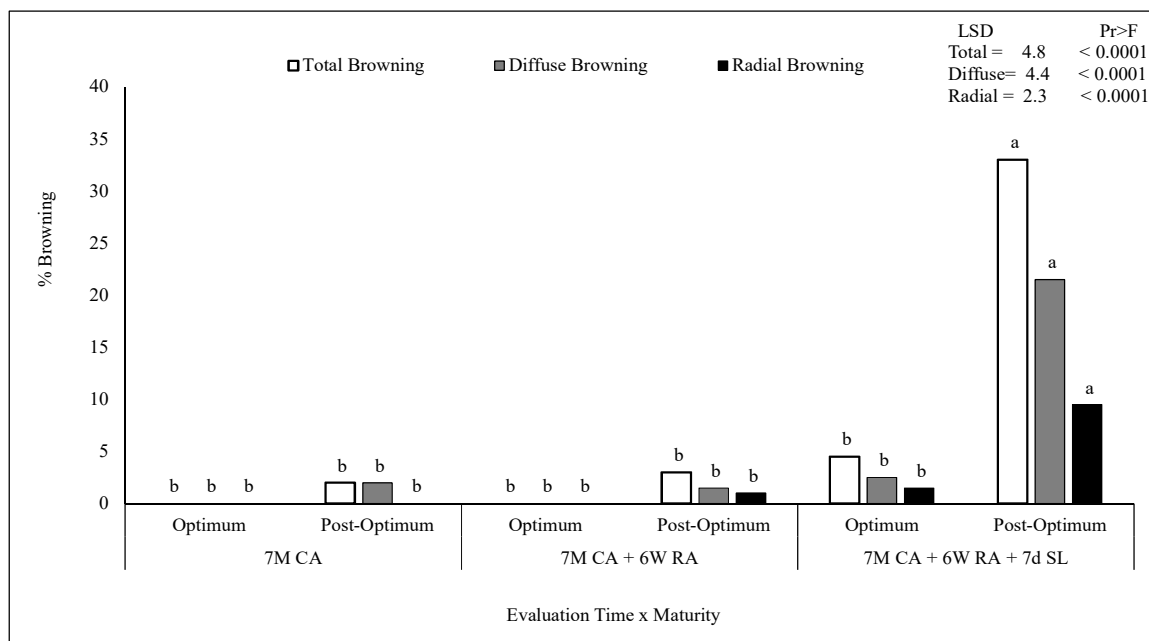


Figure 2. Percentage total, diffuse and radial browning by interaction between evaluation time and maturity comparison of 'Rosy Glow' apples. Fruit were harvested on Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

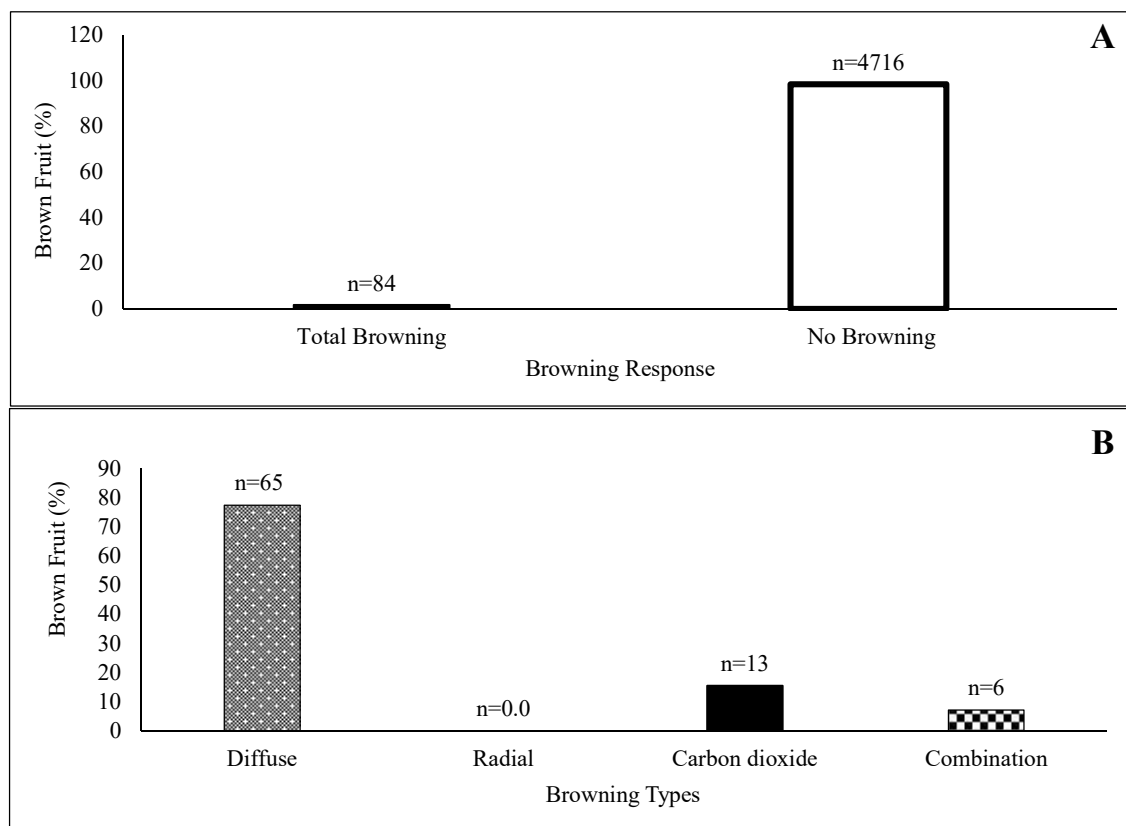


Figure 3. Browning incidence (%) in number of fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples that were harvested in 2015 at optimum harvest maturity. Measurements were made on fruit that were stored for 3 months, 5 months and 7 months in controlled atmosphere (1.5 % O₂; 1 % CO₂) at -0.5 °C, each followed by 6 weeks in regular atmosphere at -0.5 °C and a 7 day shelf-life period at ambient temperature. Fruit were harvested on Damar farm in the Vyeboom region of the Western Cape in the 2015.

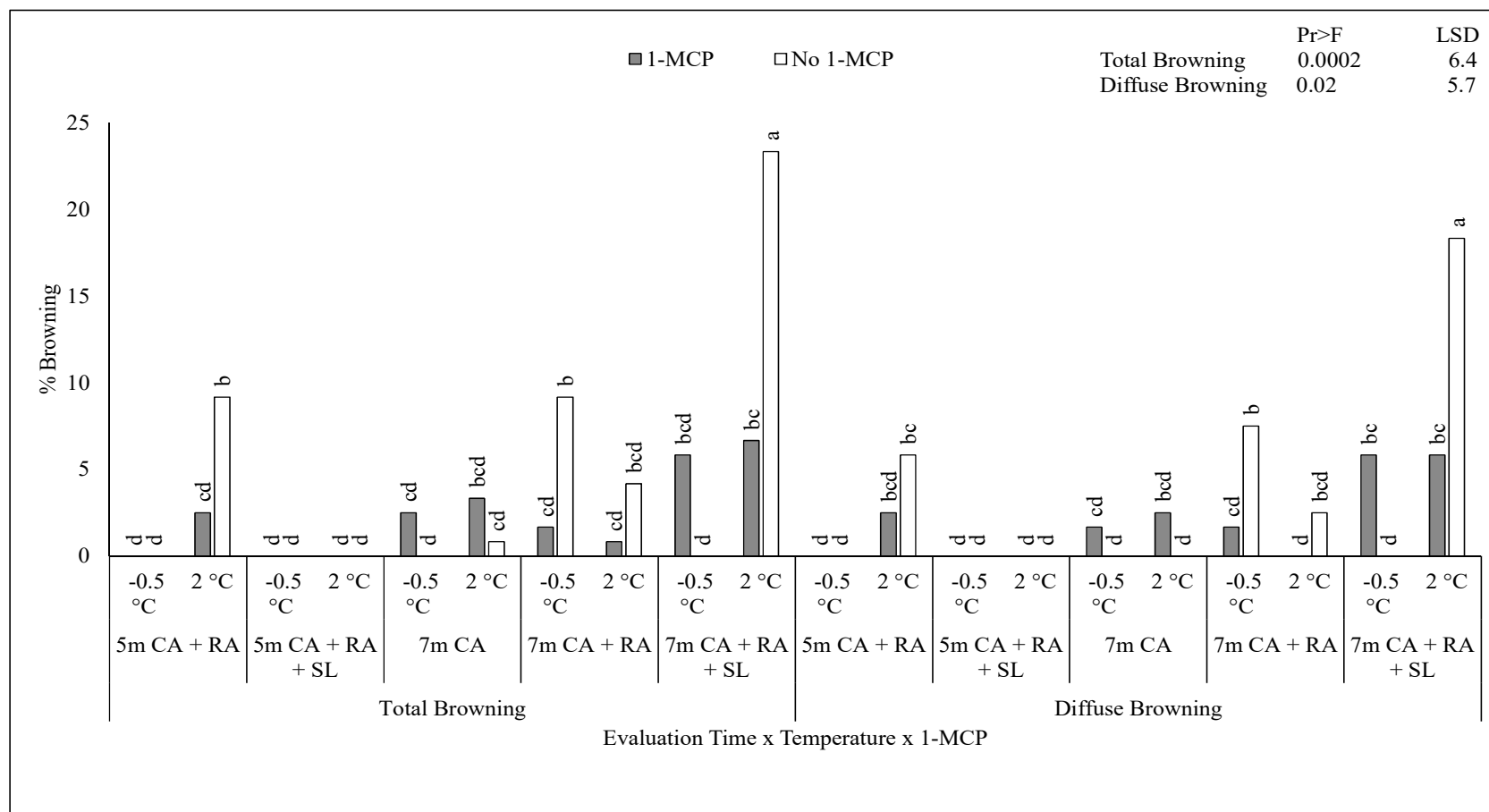


Figure 4. Percentage Total and Diffuse browning by interaction between Evaluation Time, Temperature, and 1-MCP multiple comparison of 'Rosy Glow' apples. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C) over specified evaluation time (3, 5, 7m of Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 week regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature). Fruit were harvested on Damar farm in the Vyeboom region of the Western Cape in the 2015 season.

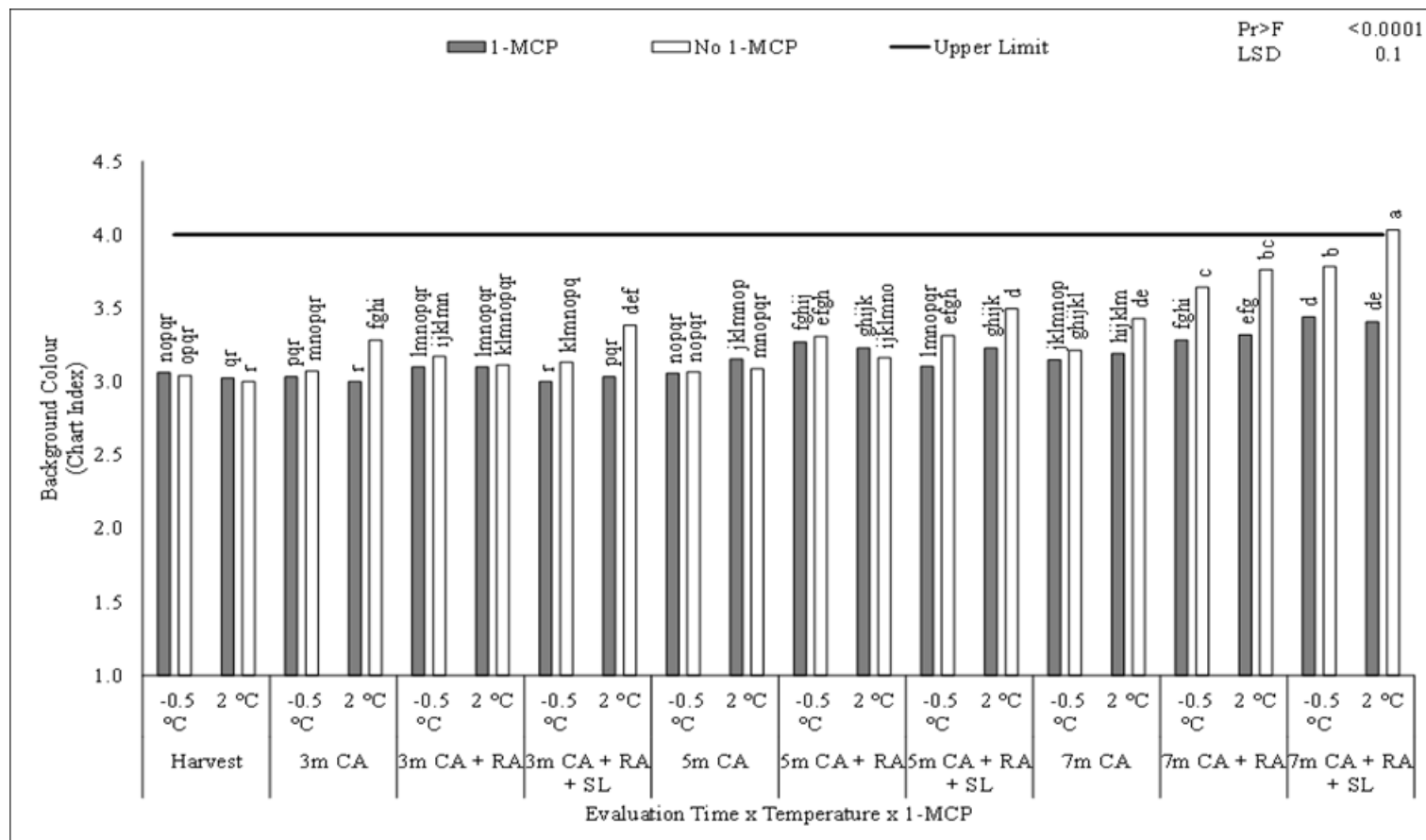


Figure 5. Multiple comparison test of interaction between Evaluation Time, Temperature and 1-MCP on background colour (chart) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C.) over specified evaluation time (3, 5, 7m of Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 week regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature). Fruit were harvested From Damar Farms in the Vyeboom region of the Western Cape in the 2015 season.

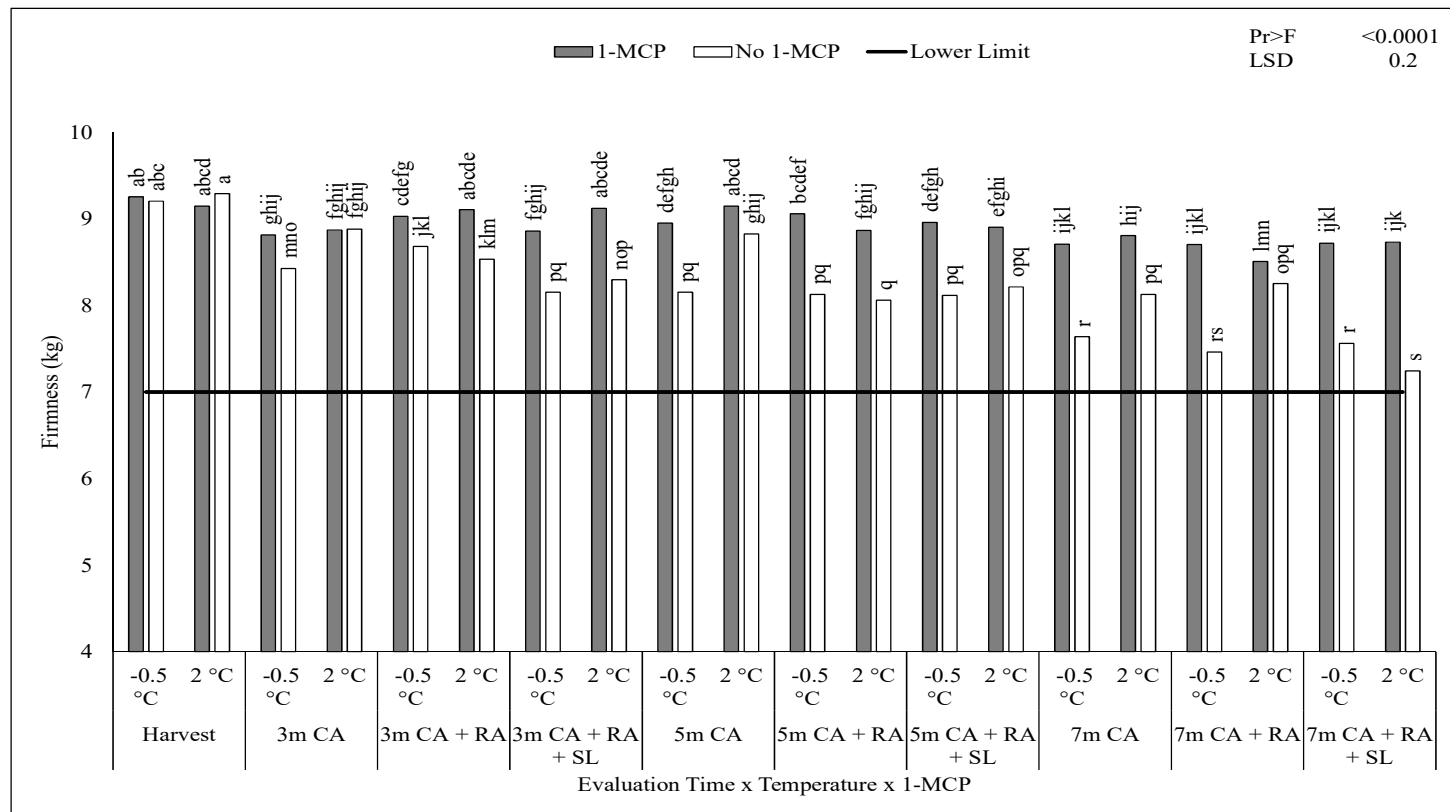


Figure 6. Multiple comparison test of interaction between evaluation time, temperature and 1-MCP on firmness (kg) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C.) over specified evaluation time (3, 5, 7m of Controlled Atmosphere (CA (1.5% O₂ and 1% CO₂)) + 6w regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature). Fruit were harvested From Damar Farms in the Vyeboom region of the Western Cape in the 2015 season.

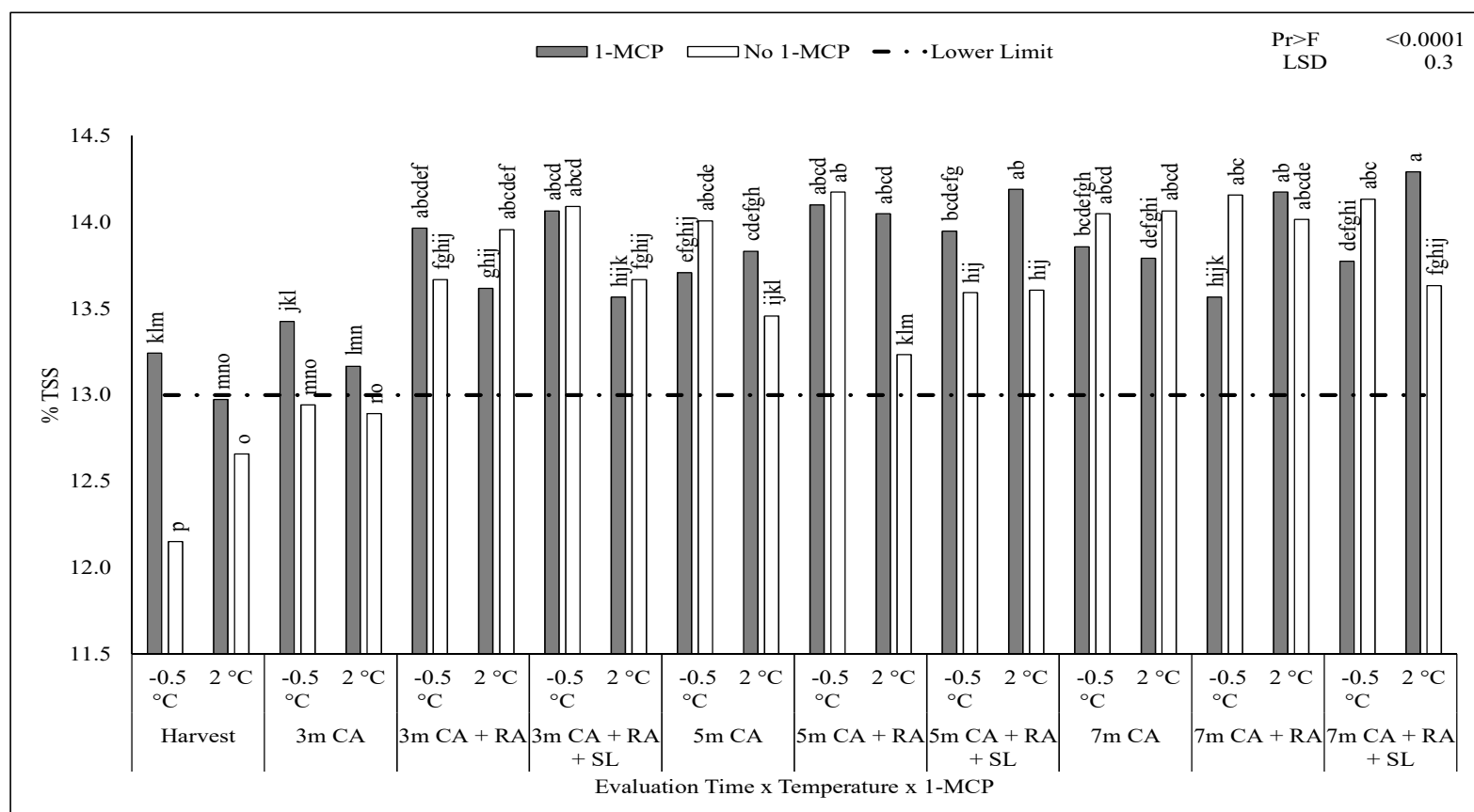


Figure 7. Multiple comparison test of interaction between evaluation time, temperature and 1-MCP on TSS (%) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C.) over specified evaluation time (3, 5, 7m of Controlled Atmosphere (CA (1.5% O₂ and 1% CO₂)) + 6w regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature). Fruit were harvested From Damar Farms in the Vyeboom region of the Western Cape in the 2015 season.

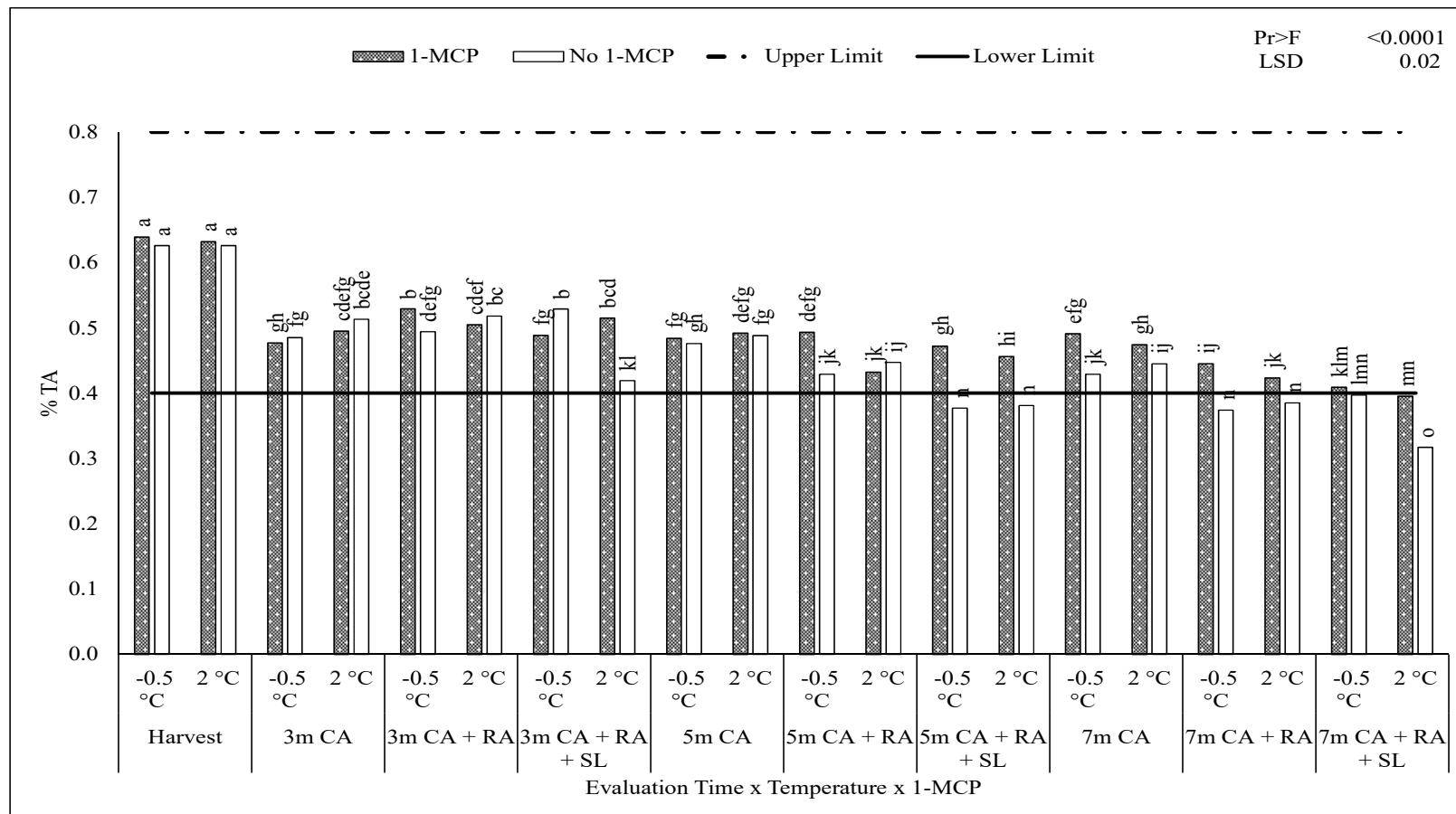


Figure 8. Multiple comparison test of interaction between evaluation time, temperature and 1-MCP on TA (%) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C.) over specified evaluation time (3, 5, 7m of Controlled Atmosphere (CA (1.5% O₂ and 1% CO₂)) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature). Fruit were harvested From Damar Farms in the Vyeboom region of the Western Cape in the 2015 season.

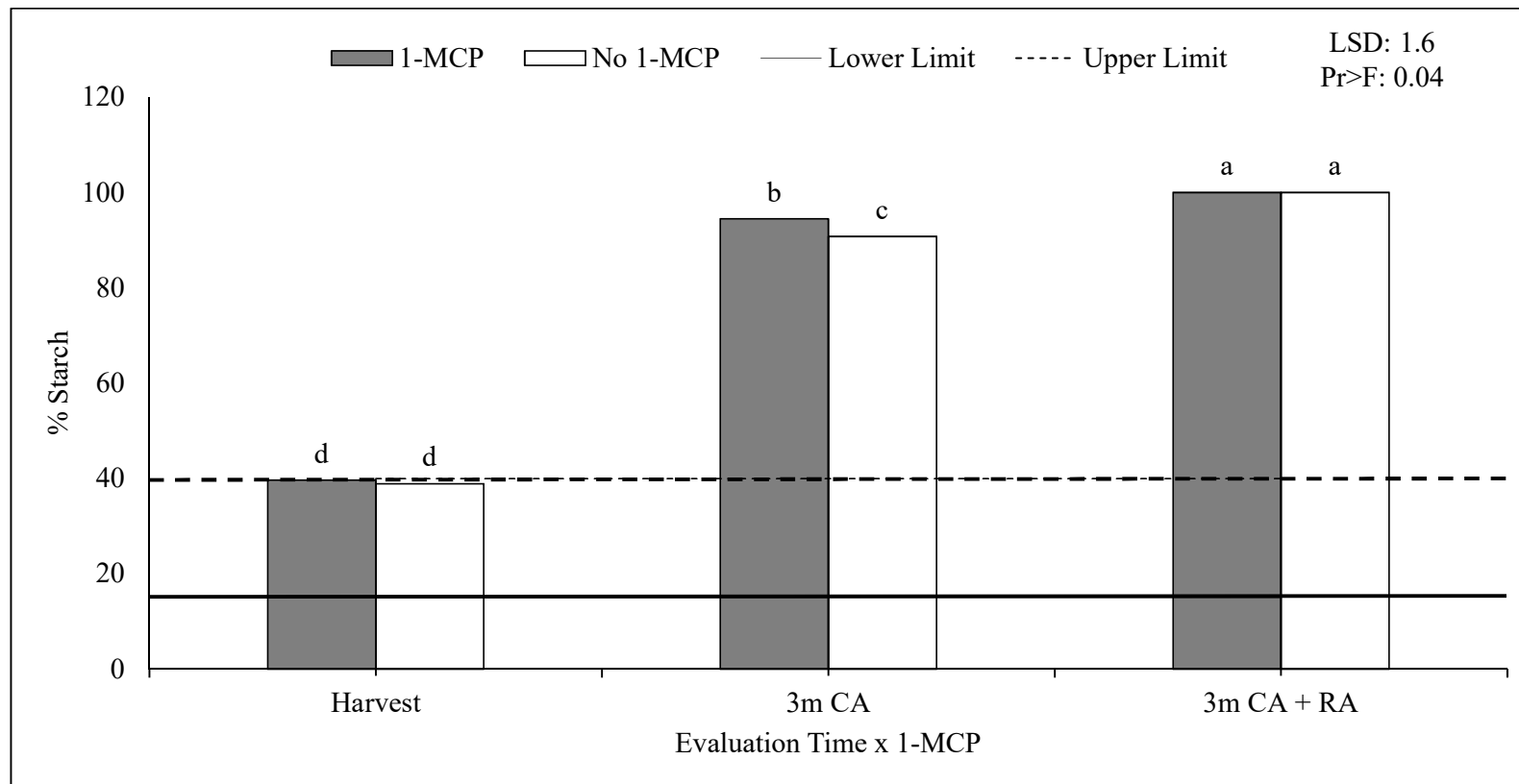


Figure 9. Multiple comparison test of interaction between evaluation time and 1-MCP on starch (%) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit that were treated with or without 1-MCP and stored at two different temperatures (-0.5 °C and 2 °C.) over specified evaluation time (3, 5, 7m of Controlled Atmosphere (CA (1.5% O₂ and 1% CO₂)) + 6w regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature). Fruit were harvested From Damar Farms in the Vyeboom region of the Western Cape in the 2015 season.

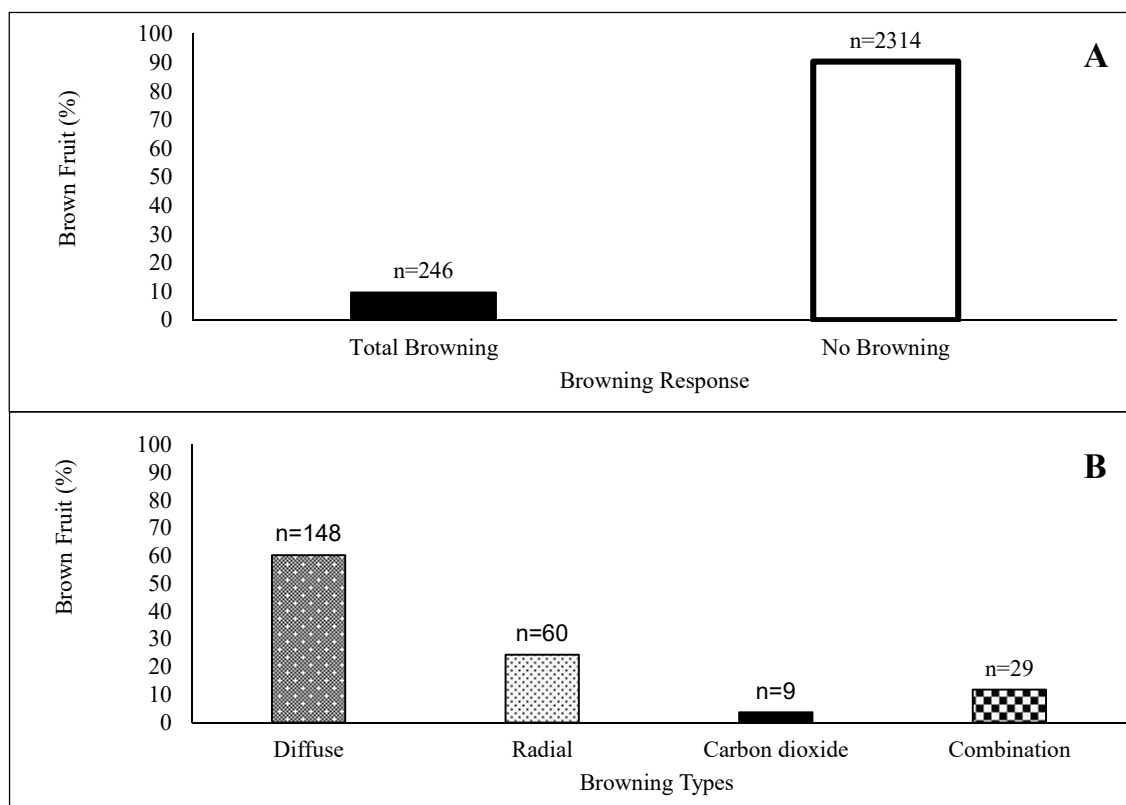


Figure 10. Browning incidence (%) in number of fruit (A) and browning incidence (%) per browning type (B) in 'Rosy Glow' apples that were harvested in 2015 from two tree ages. Measurements were made on fruit that were stored for 7 months in controlled atmosphere (1.5 % O₂; 1 % CO₂), followed by 6 weeks in regular atmosphere at -0.5 °C and a 7 day shelf-life period at ambient temperature. Fruit were harvested from eight orchards in the Elgin region of the Western Cape in the 2015.

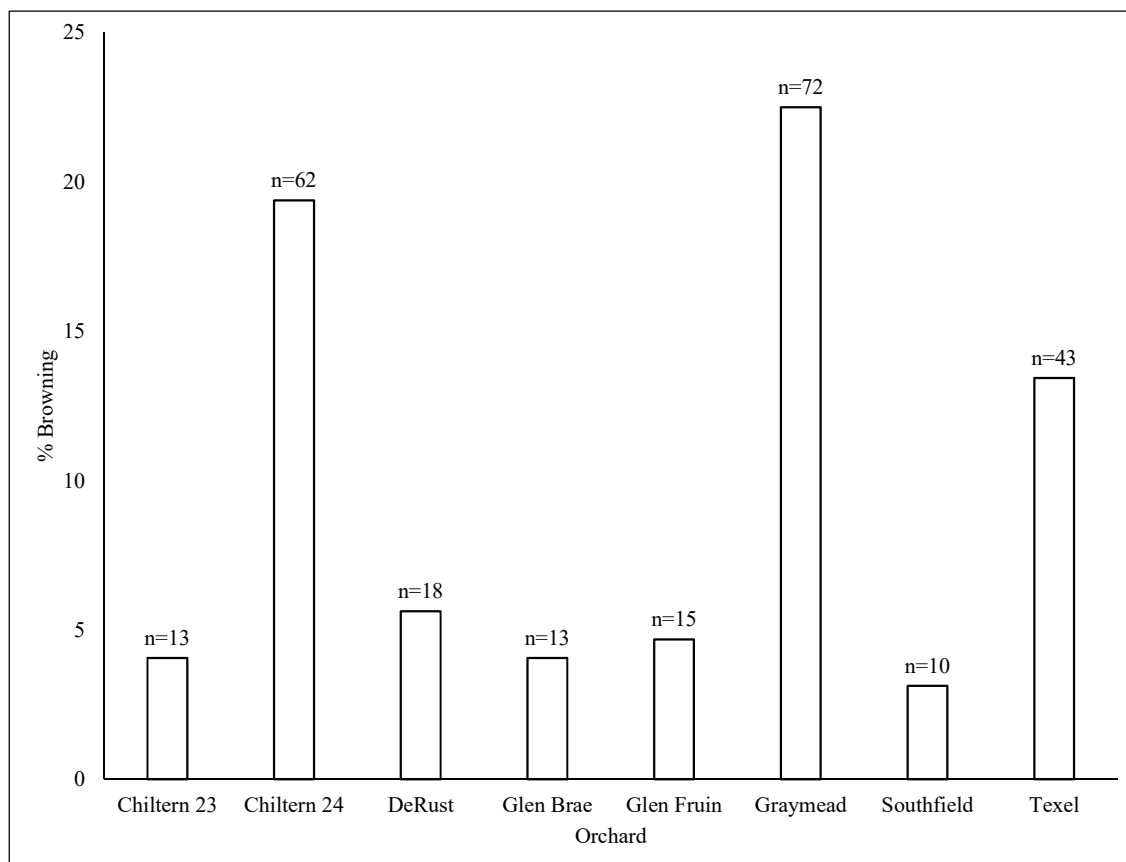


Figure 11. Browning incidence (%) per orchard / farm for 'Rosy Glow' apples harvested at optimum maturity (30%-40% starch breakdown). Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

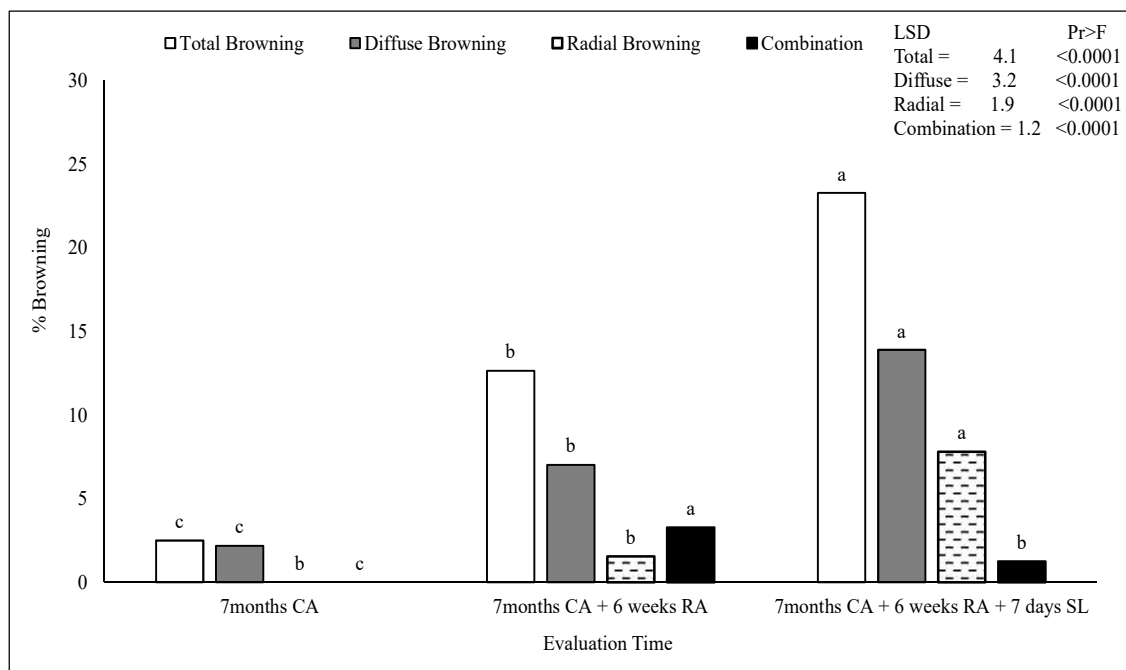


Figure 12. Percentage Total, Diffuse, Radial and Combination browning as influenced by time of evaluation by multiple comparison of 'Rosy Glow' apples. Fruit were harvested from eight different orchards (Fig. 11) in the Elgin/Grabouw, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

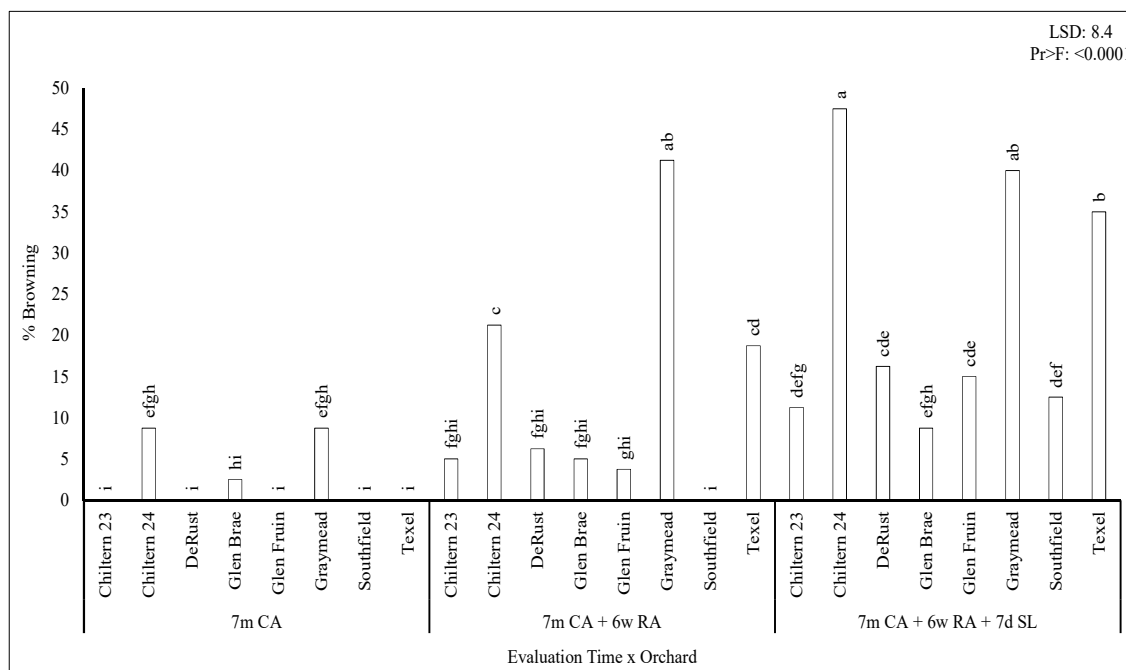


Figure 13. Percentage Total browning by evaluation time and orchard interaction multiple comparison of 'Rosy Glow' apples. Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA (1.5% O₂ and 1% CO₂)) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/ Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

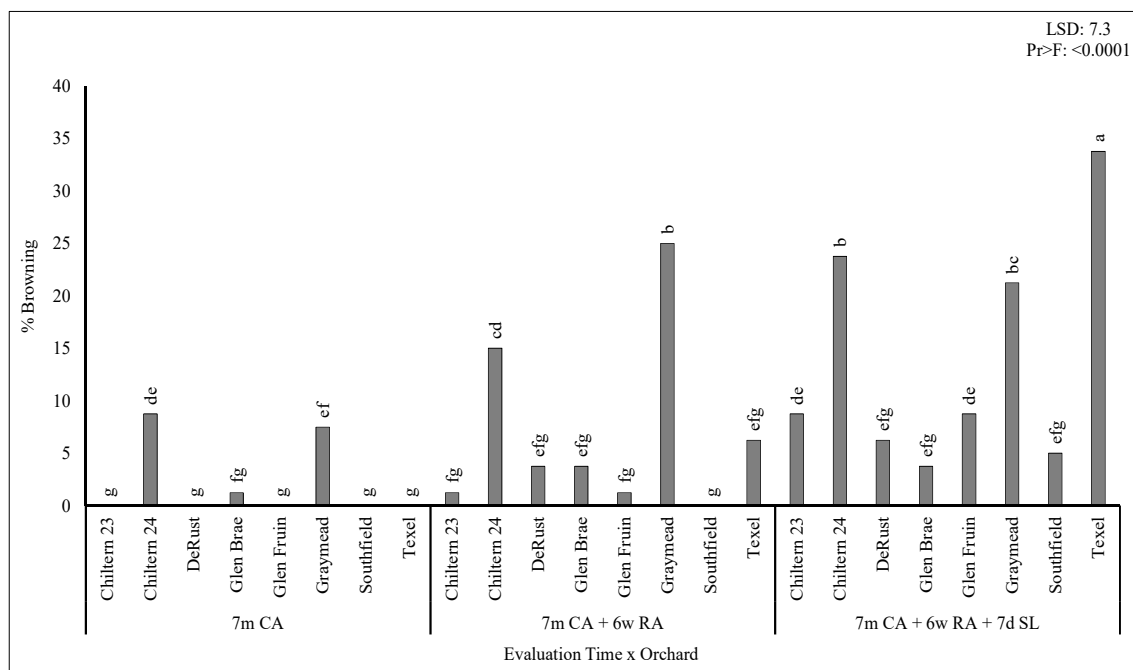


Figure 14. Percentage Diffuse browning by evaluation time and orchard interaction multiple comparison of 'Rosy Glow' apples. Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6w regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

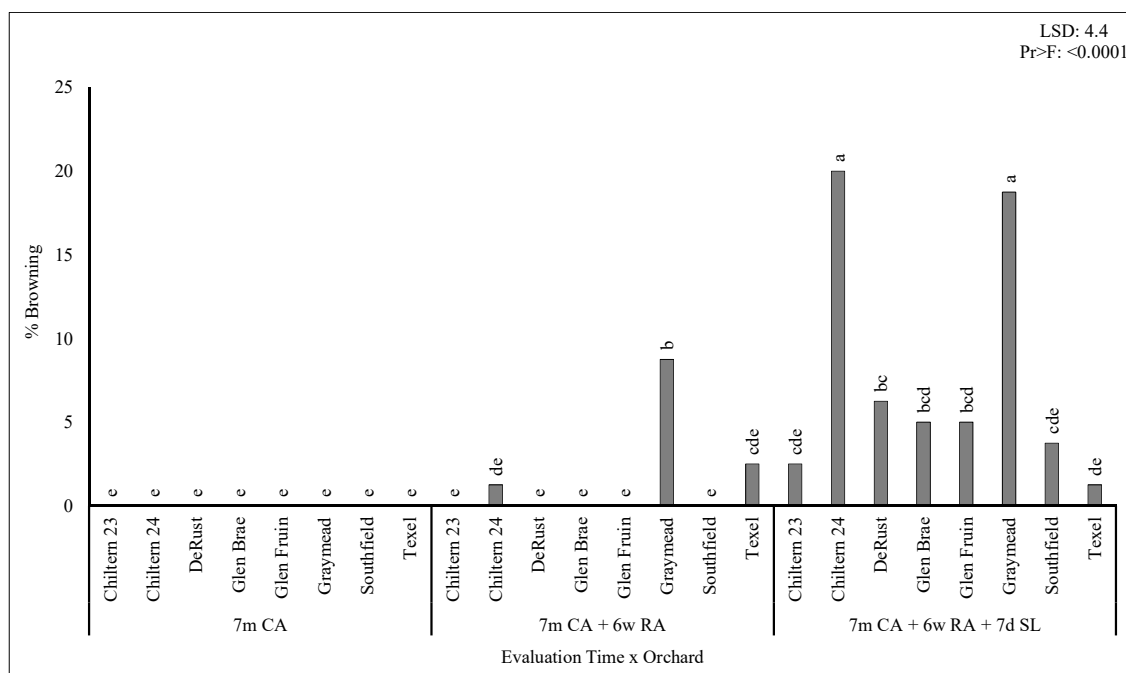


Figure 15. Percentage Radial browning by evaluation time and orchard interaction multiple comparison of 'Rosy Glow' apples. Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6w regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

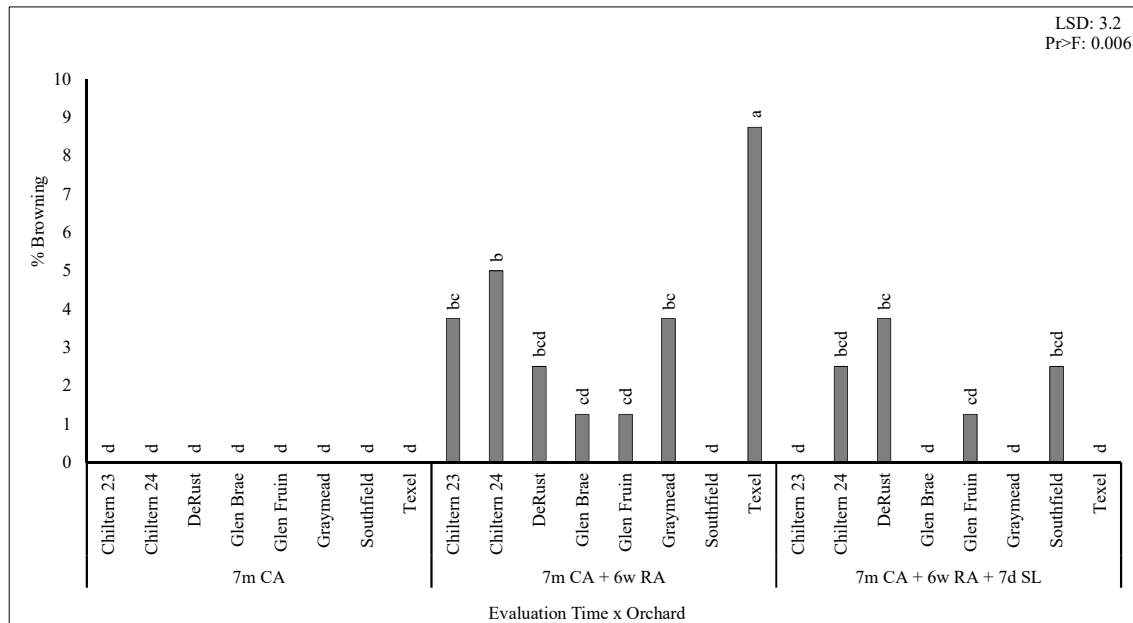


Figure 16. Percentage Combination browning by evaluation time and orchard interaction multiple comparison of 'Rosy Glow' apples. Measurements were made on fruit of two different tree ages (4years and 7years) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6w regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/ Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

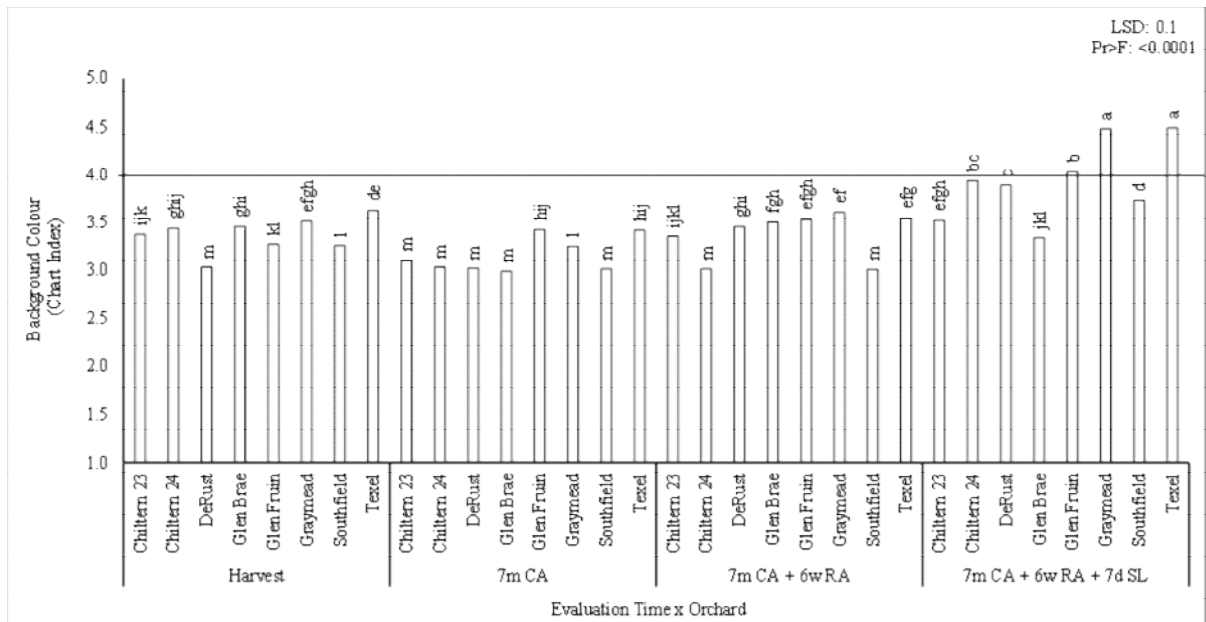


Figure 17. Multiple comparison test of interaction between evaluation time and orchards on background colour (chart) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit of two different tree ages (4years and 7years) and stored at 0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

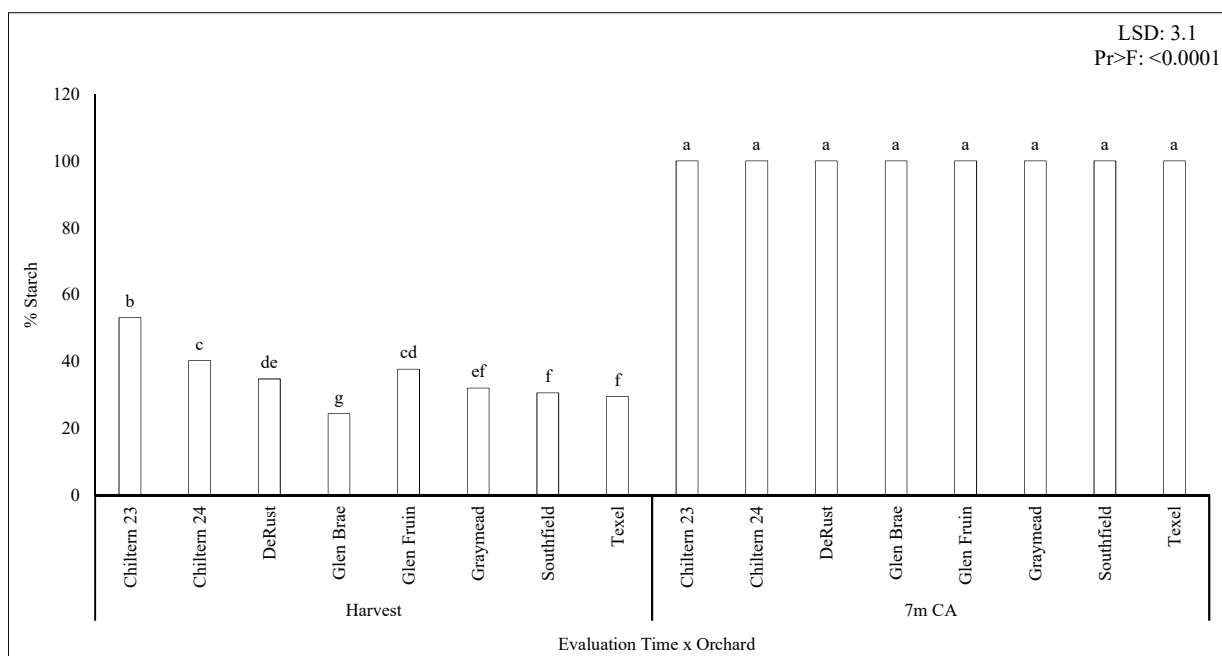


Figure 18. Multiple comparison test of interaction between evaluation time and orchards on starch (%) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 week regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

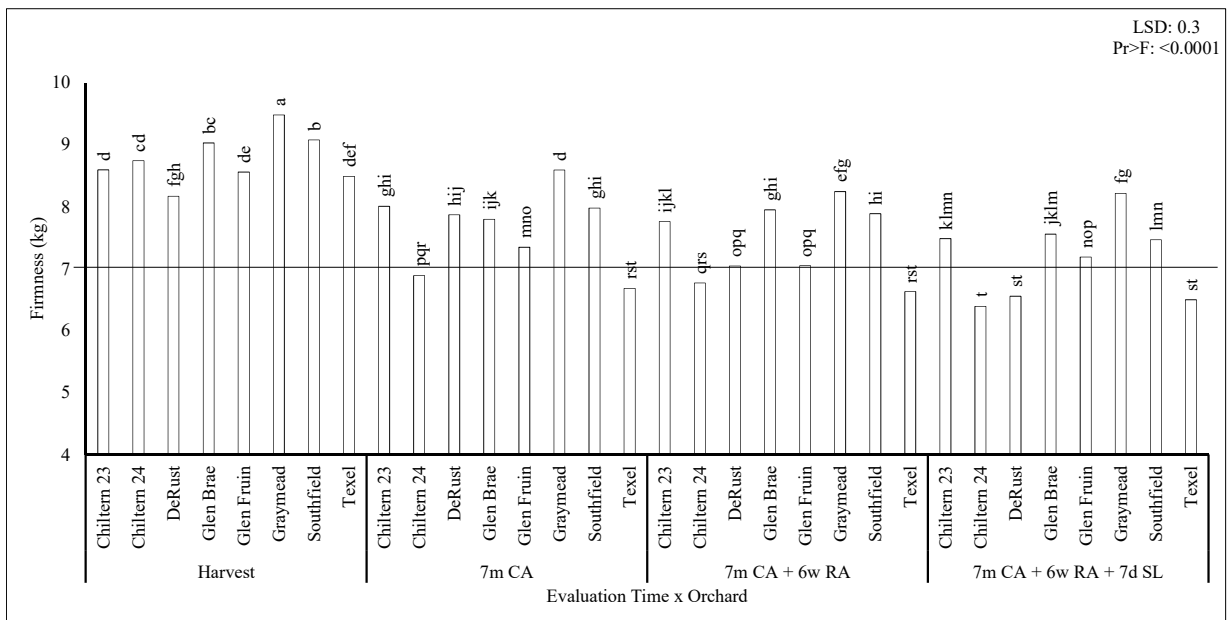


Figure 19. Multiple comparison test of interaction between evaluation time and orchards on Firmness (kg) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit of two different tree ages (4years and 7years) and stored at -0.5 °C for 7months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 week regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom and Villiersdorp areas of the Western Cape in the 2015 season.

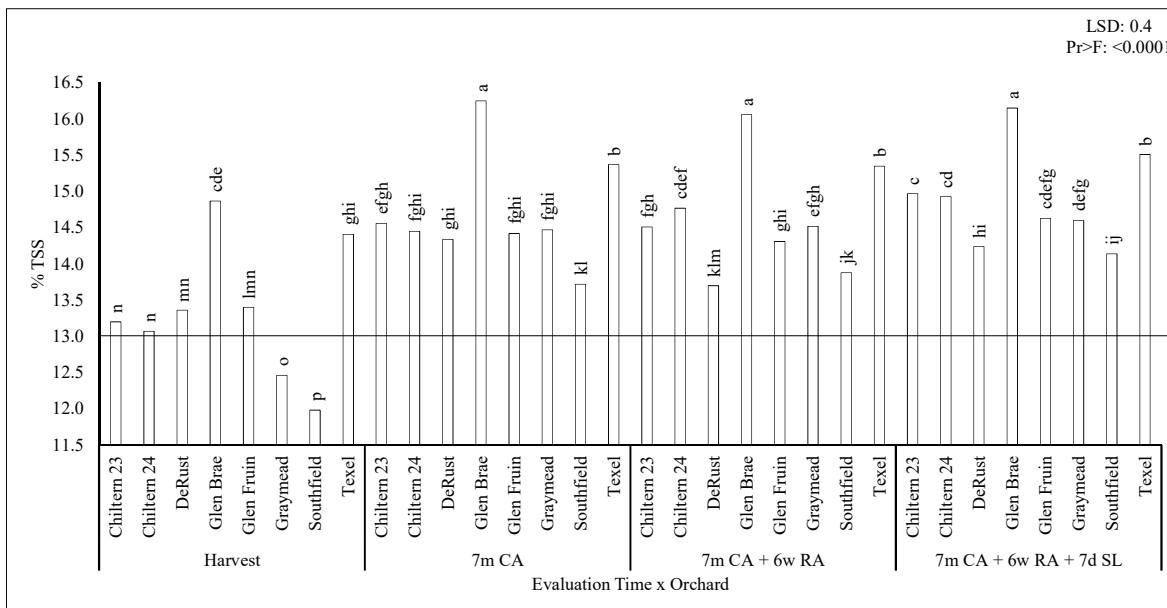


Figure 20. Multiple comparison test of interaction between evaluation time and orchards on TSS (%) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 week regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom, Villiersdorp areas of the Western Cape in the 2015 season.

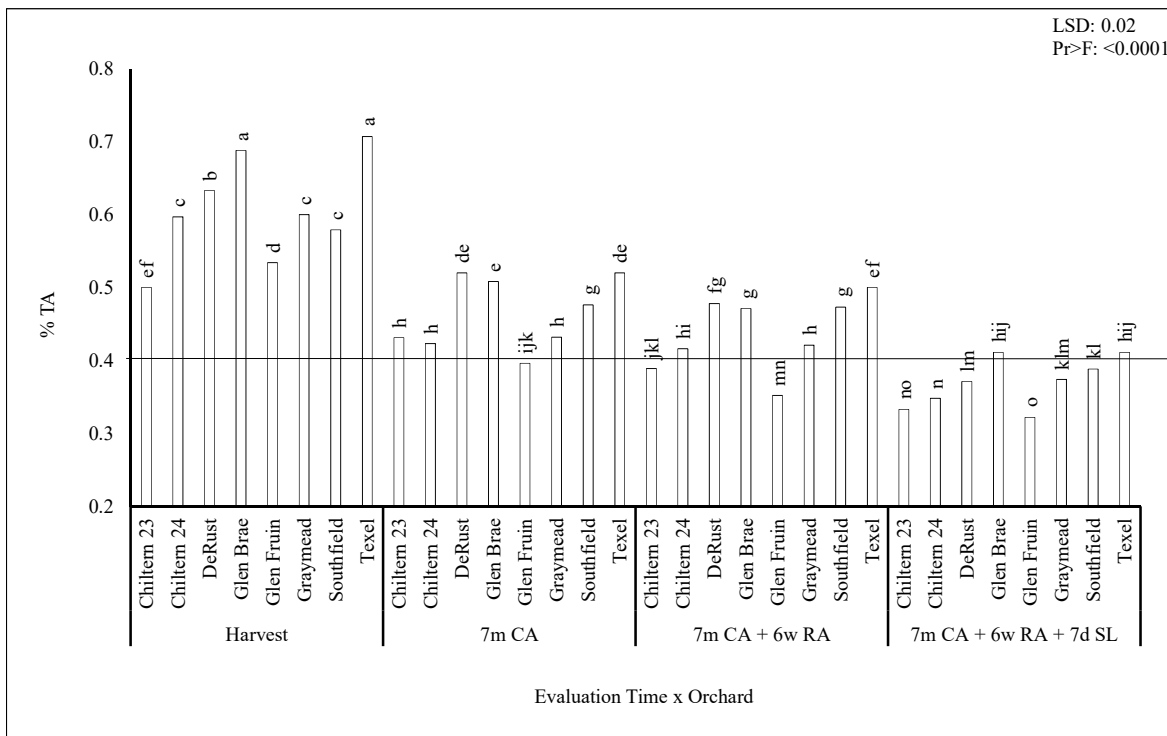


Figure 21. Multiple comparison test of interaction between evaluation time and orchards on Titratable acids (%) in 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA) (1.5% O₂ and 1% CO₂) + 6 weeks regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom, Villiersdorp areas of the Western Cape in the 2015 season.

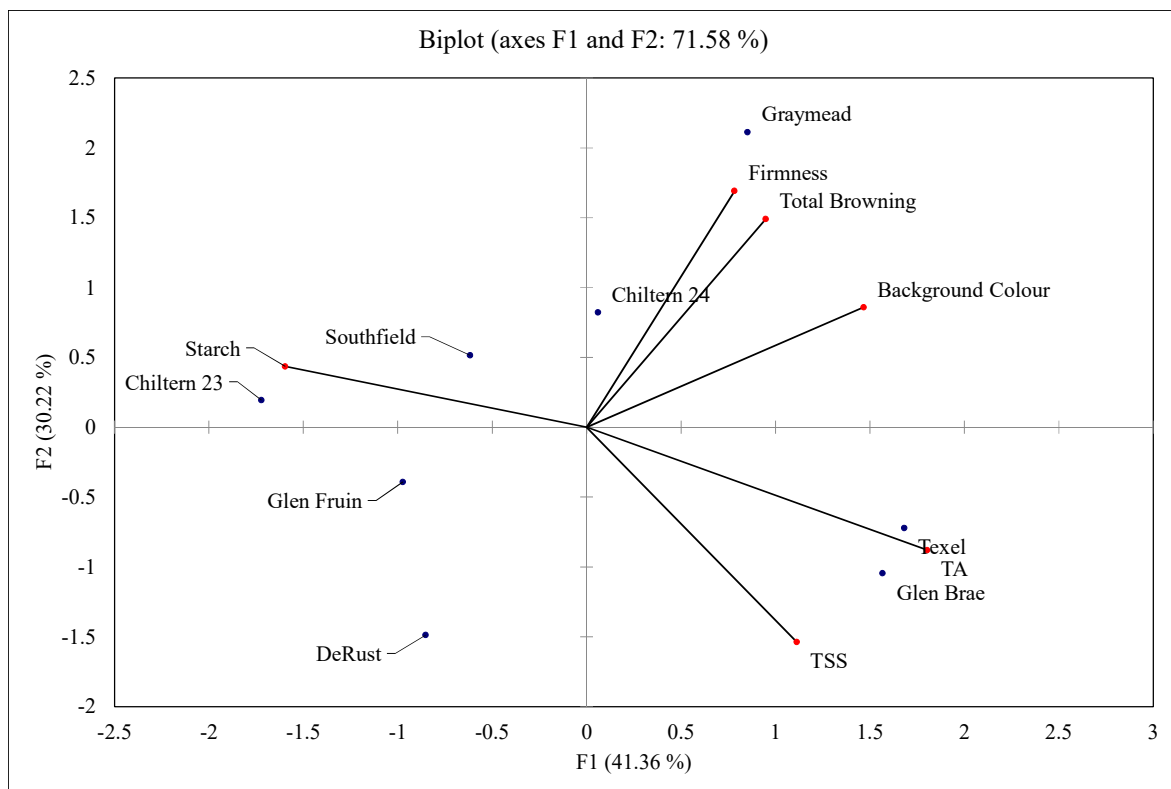


Figure 22. Association between total browning percentage, quality parameters as well as orchards of 'Rosy Glow' apples that were harvested in 2015 at optimum maturity. Measurements were made on fruit of two different tree ages (4th leaf and 7th leaf) and stored at -0.5 °C for 7 months under Controlled Atmosphere (CA (1.5% O₂ and 1% CO₂)) + 6w regular atmosphere (RA) and a subsequent 7 day shelf-life period at ambient temperature. Fruit were harvested from eight different orchards in the Grabouw/Elgin, Vyeboom, Villiersdorp areas of the Western Cape in the 2015 season.

6. ACCUMULATED OUTPUTS

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

Storage protocol for 'Rosy Glow' / recommendations.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Presentations, recommend / discuss with packhouses when contacting researcher. Refer to ACTA HORT paper / and the final report or MSc Thesis.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Total cost to industry throughout the project
Masters Students				
James Wonder Doe	Ghanaian	MSc(Agric)	Final MSc year	R 235 587

This document is confidential and any unauthorised disclosure is prohibited

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

Doe, JM, Schoeman, L, Crouch, EM (2018). Effect of harvest maturity, storage condition and duration, as well as tree age, on internal flesh browning and quality of 'Rosy Glow' apples grown in South Africa. ACTA HORT accepted – expected publication 2018.

e) PRESENTATIONS/PAPERS DELIVERED

Doe, JW, Crouch EM. (2016). Harvest and postharvest factors influencing internal browning and fruit quality of 'Rosy Glow' apples. Presentation at the SASHS Combined Congress in Bloemfontein. Jan.

(Award: James Wonder Doe was awarded the Best MSc Presentation Prize as well as the Best Overall Presentation at this conference and has received a R10 000 contribution towards presenting at an international conference.)

Crouch, EM, Butler, L, Majoni, T, Jooste, M, Lötze, E Theron, Doe, JW Crouch, I, Bergman, H, Viljoen, D. (2016). Internal browning of 'Cripps' Pink' and 'Rosy Glow' apples. South African Pink Lady Association AGM Technical Presentations. Top Fruit, Simondium. 9 Nov.

Doe, J.M., Schoeman, L., Crouch, E.M. (2017). Effect of harvest maturity, storage condition and duration, as well as tree age, on internal flesh browning and quality of 'Rosy Glow' apples grown in South Africa. VII International Conference on Managing Quality in Chains (MQUIC), Stellenbosch, South Africa. 4-7 Sept.

Crouch, EM. (2017). Pre- and Post-Harvest factors influencing internal browning of 'Cripps' Pink' apples in South Africa. Australia Pear Limited, Postharvest Seminar, Adelaide, 17 Jan.

7. PERSONS PARTICIPATING IN THE PROJECT

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	Cost to Project R
E.M. Crouch	PhD (Agric)	-	W	F	Stellenbosch University	PL	
L. Schoeman	PhD (Agric)		W	F	Stellenbosch University	Co-supervisor	
S. Arendse	Matric	-	B	F	Stellenbosch University	TA	15928
E. Hendrickse	St 8	-	B	F	Stellenbosch University	TA	15928
R. Poole	Matric	-	B	F	Stellenbosch University	TA	15928
A. Swarts	Matric	-	B	M	Stellenbosch University	TA	15928
K. vd Merwe	BTech	-	W	M	Retired ARC PH manager	Coll	-

⁽¹⁾Race
 B = African, Coloured or Indian
 W = White

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⁽²⁾Gender F = Female
 M = Male

⁽³⁾Position Co = Co-worker (other researcher at your institution)
 Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
 PF = Post-doctoral fellow
 PL = Project leader
 RA = Research assistant
 TA = Technical assistant/ technician

8. BUDGET

a) BUDGET FOR THE COMING YEAR

N.A.

b) ANNUAL BUDGET FOR PROJECT RECEIVED FROM INDUSTRY TO DATE (OR THE PREVIOUS 5 YEARS)

YEAR		SAAPPA	TOTAL
2014	Running+bursary	293633	293 633
2015	Running+bursary	448736	448 736
2016	Bursary	90167	90 167

Industry allocated project
number

PHI allocated project
number

EVALUATION BY INDUSTRY

This section is for office use only

Project number

Project name

Name of Sub-
Committee*

Comments on project

Committee's recommendation (Review panel in the case of PHI)

- Accepted.

☐

- Accepted provisionally if the sub-committee's comments are also addressed

☐

Resubmit this final report by _____

- Unacceptable. Must resubmit final report.

☐

Chairperson _____

Date _____

*SUB-COMMITTEES

Winetech

Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;

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Version 2015

Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit

Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer

Peer Work Groups: Post-Harvest; Horticulture; Soil Science; Breeding and Evaluation; Pathology; Entomology