Feasibility of closed ventilation and automatic ventilation for sea freight of *Proteaceae* cut flower stems

By

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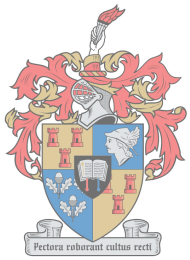

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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DEDICATION

My heroine and mother, Lois T. Njerere Matsikidze. My living proof that God can send a woman to do a man's job.

My late hero and father, Edwin P. Matsikidze. A man who mastered the art of turning nightmares into sweet dreams.

My blood and brother, Kudzai G. Matsikidze. Never worry about the enemies that come from behind you, I have your back.

SUMMARY

Global trends aimed at advancing sea freight technology and reducing carbon emissions have led to the invention of automated reefer technologies. This development brings the challenge of having to determine product physiological limits that are vital for the implementation of automatic ventilation technology on South African produced *Proteaceae* cut flower stems.

A study was conducted to determine the respiration rates, lower O_2 limits and CO_2 toxicity tolerance limits of *Proteaceae* cut stems in order to assess the feasibility of using automatic ventilation (AV+) vs. conventional fixed open-air exchange (AirEx) ventilation shipping of *Proteaceae* cut stems. In a closed ventilation system (representing unvented conditions as is possible with AV+ technology) which contained a mixed load of *Proteaceae* products in 45-55 % free air, it was observed that the O_2 level fell to approximately 8.5 % whilst the CO_2 level rose to about 10 %, when a HarvestWatch™ dynamic controlled atmosphere (DCA) system was used for gas sampling. Using a handheld gas analyser as an alternative sampling method revealed that under these conditions O_2 levels fell below 2 %, and CO_2 levels rose to above 17 %. Although the O_2 dropped considerably, it was still above the Lower Oxygen Limit (LOL), which ranged from 0.09-0.33 % O_2, 0.08-0.41 % O_2 and 0.08-0.48 % O_2 for *Leucadendron*, *Leucospermum* and *Protea* products respectively.

At 5 °C, the respiration rates were between 15.11-48.07 mL $CO_2.kg^{-1}.h^{-1}$ for *Leucadendron*, 19.06-45.44 mL $CO_2.kg^{-1}.h^{-1}$ for *Leucospermum* and 10.76-27.24 mL $CO_2.kg^{-1}.h^{-1}$ for *Protea*. Closed ventilation, low O_2 and high CO_2 atmospheres generally resulted in mass loss that was lower than or comparable to AirEx. The inflorescence and leaf colour changes in *Proteaceae* products stored in closed ventilation, low O_2 and high CO_2 treatments were commercially not significant. There were no signs of low O_2 and/or high CO_2 damage on the stems stored under closed ventilation. The inflorescence and leaf visual quality of *Proteaceae* products stored in closed ventilation was generally better than that of stems stored in AirEx. *Leucadendron, Leucospermum* and *Protea* stems stored in high CO_2 treatments had comparable or better quality than stems stored in AirEx. However, exposure to 15 % CO_2 for 21 d reduced longevity of some products. In *Leucadendron*, the response to AirEx, DCA and 2 % O_2 on flower head visual quality was variable and the treatments were equally effective in maintaining leaf visual quality. Flower and leaf visual quality and longevity was comparable between the AirEx and low O_2 treatments in *Leucospermum* and *Protea*.

Monosaccharides plus oligosaccharides were the most abundant sugars, followed by polysaccharides, and starch was the least abundant in *Leucadendron*, *Leucospermum* and *Protea* stems. Differences in polysaccharide and starch content were minor, between AirEx and low O_2 treatments, also between AirEx and high CO_2 treatments. Lipid peroxidation was comparable between AirEx and high CO_2 atmospheres. The AirEx, DCA and 2 % O_2 treatments had an insignificant effect on total phenolic content of products.

Further research is recommended under commercial conditions in AV+ type reefers, where automatic ventilation should be set to maintain a minimum of 2 % O_2 and maximum of 15 % CO_2 concentration during long-term sea freight shipping of *Proteaceae* cut flowers to ensure optimum product quality throughout the cold chain for an extended vase life.

OPSOMMING

Internasionale tendense gemik op die bevordering van seevragbehoueringstegnologie asook om koolstofvrystelling te beperk het gelei tot die vooruitgang van automatiese reefer tegnologie. Hierdie ontwikkeling bied die uitdaging om die fisiologiese beperkings van snyblomprodukte vas te stel wat krities is vir die implementering van automatiese ventilasie (AV+) tegnologie op Suid-Afrikaans geproduseerde *Proteaceae* snyblom stele.

'n Studie is uitgevoer om die respirasietempos, laer O_2 limiete (LOL) asook CO_2 toksiteitstoleransie limiete van *Proteaceae* snyblomstele te bepaal om sodoende die toepaslikheid van die gebruik van automatiese ventilation (AV+) teenoor die konvensionele vaste oop-lug stelsel (AirEx) ventilasie te evalueer. In 'n geslote sisteem (wat nie-geventileerde toestande soos moontlik met AV+ tegnologie voorstel) met 'n gemengde vrag van *Proteaceae* produkte in 45-55 % vry lug, is waargeneem dat die O_2 vlakke tot ongeveer 8.5 % gedaal het, terwyl die CO_2 vlakke tot net bokant 10 % gestyg het, wanneer 'n HarvestWatch™ dynamiesbeheerde atmosfeerstelsel (DCA) gebruik was om gasse te monitor. Wanneer 'n draagbare gas analiseerder as 'n alternatiewe moniteringsmetode gebruik is, is waargneem dat met hierdie metode O_2 vlakke tot onder 2 % geval het, terwyl die CO_2 vlakke tot bokant 17 % gestyg het. Alhoewel die O_2 aansienklik geval het, was dit steeds bokant die LOL wat gewissel het vanaf 0.09-0.33 % O_2, 0.08-0.41 % O_2 en 0.08-0.48 % O_2 vir *Leucadendron*, *Leucospermum* en *Protea* produkte onderskeidelik.

By 5 °C is respirasie waardes van tussen 15.11-48.07 mL $CO_2.kg^{-1}.h^{-1}$ vir *Leucadendron*, 19.06-45.44 mL $CO_2.kg^{-1}.h^{-1}$ vir *Leucospermum* and 10.76 27.24 mL $CO_2.kg\text{-}^{1}.h^{-1}$ vir *Protea* aangeteken. Geslote ventilasie, lae O_2 en hoë CO_2 atmosfeer het oor die algemeen laer of soortgelyke massa verlies getoon as waargeneem met AirEx. Die bloeiwyse en loof kleurveranderings van *Proteaceae* produkte wat in geslote ventilasie, lae O_2 en hoë CO_2 behandelings gestoor is, was nie kommersieël betekenisvol verskillend van mekaar nie. Daar was geen teken van lae O_2 en/of hoë CO_2 skade aan stele gestoor in geslote ventilasie nie. Die bloeiwyse en loof visuele kwaliteit van *Proteaceae* produkte gestoor in geslote ventilasie was oor die algemeen beter as dié van stele wat in AirEx gestoor is.

Leucadendron, *Leucospermum* en *Protea* stele onder hoë CO_2 behandeling het vergelykbare of beter kwaliteit getoon as stele gestoor AirEx. Blootstelling aan 15 % CO_2 vir 21 d het egter die langslewendheid van sommige produkte negatief beïnvloed. In *Leucadendron*, was die impak van AirEx, DCA en 2 % O_2 op die bloeiwyse se visuele kwaliteit

variërend, maar meestal was behandelings ewe effektief om blaarkwaliteit te handhaaf. Blom- en blaar visuele kwaliteit en langslewendheid was vergelykbaar tussen die AirEx en lae O_2 behandelings in *Leucospermum* en *Protea*.

Monosakkariede asook oligosakkariede was die mees verteenwoordigende suikers, gevolg deur polisakkariede, met stysel in die laagste hoeveelheid waargeneem in *Leucadendron*, *Leucospermum* en *Protea* stele. Verskille in polisakkariede en stysel inhoud was weglaatbaar tussen AirEx en lae O_2 behandelings, asook tussen AirEx en hoë CO_2 behandelings. Lipied peroksidase was vergelykbaar tussen produkte blootgestel aan AirEx en hoë CO_2 atmosfeer. Die AirEx, DCA en 2 % O_2 behandelings het ‘n nie-betekenisvolle effek op die totale fenoliese inhoud van die produkte gehad.

Verdere studies word aanbeveel onder kommersiële toestande in AV+ tipe reefers waar automatiese ventilasie gestel word om ‘n minimum van 2 % O_2 en ‘n maksimum van 15 % CO_2 konsentrasie te handhaaf binne vragbehouering gedurende lang-termyn seevrag verskeping van *Proteaceae* snyblom stele om optimale produk kwaliteit te verseker regdeur die koue ketting vir met ’n verlengde vaaslewe.

PREFACE

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. Each paper was prepared as a scientific paper for submission to *Postharvest Biology and Technology*. Repetition or duplication between papers might therefore be necessary.

LIST OF PUBLICATIONS AND CONFERENCE PRESENTATIONS

Matsikidze, S. N. Huysamer, M. Botes, A. and Hoffman, L. 2017. Helping Cape Flora find its sea legs. *PHI Innovate*. Page 71-75. Available from: http://www.capeflorasa.co.za/wp-content/uploads/2017/04/PHI-Innovate-2017-WEB-p72-75.pdf

Matsikidze, S. N. Huysamer, M. Botes, A. and Hoffman, E.W. Feasibility of closed ventilation and automatic ventilation for sea freight of *Protea* cut flowers. *Acta Horticulturae*. [In Press] Presented at the 2nd International Ornamental Symposium (ISHS). Available from: http://www.ishs2017stellenbosch.co.za/

Matsikidze, S. N. Huysamer, M. Botes, A. and Hoffman, E.W. Dynamics of long-term cold storage conditions for fynbos (*Leucadendron*) cut flowers as relevant for sea freighting. Oral presentation at the *Combined Congress* (SA Society for Horticulture Sciences), Klein Kariba, Bela, 23-27 January, 2017.

Matsikidze, S. N. Huysamer, M. Botes, A. and Hoffman, L. Dynamics of long-term cold storage conditions for fynbos (*Leucadendron*) cut flowers as relevant for sea freighting. Oral presentations at the 2nd South African *Post-harvest Innovation Symposium*, Spier Conference Centre, 21-22 November 2016.

Matsikidze, S. N. Huysamer, M. Botes, A. van der Merwe, K. and Hoffman, L. Dynamics of long-term cold storage conditions for fynbos (*Leucospermum*) cut flowers as relevant for sea freighting. Poster presentation at the 2nd South African *Post-harvest Innovation Symposium*, Spier Conference Centre, 21-22 November 2016. Award: 3rd prize for the poster category.

Matsikidze, S. N. Huysamer, M. Botes, A. and Hoffman, L. Dynamics of long-term cold storage conditions for fynbos cut flowers as relevant for sea freighting. Oral presentation at the 2017 *Cape Flora SA Technical Field day*, Klein Joostenberg, Stellenbosch, 7 March 2017.

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GENERAL INTRODUCTION

1. A switch from air- to sea freight as the new preferred option for flower exports

Seasonality in the production and utilisation of perishable products, coupled with natural climatic variation, are the backbone of all horticultural trade (Cook, 2002). Agricultural products are often transported over long distances before they reach the final consumer, as is the case with ornamentals from Southern Africa. The term ornamentals usually refers to plants produced for their flowers and/or foliage, also to include potted flowering and foliage plants (Reid, 2009). Up until recently, cut flowers were almost exclusively transported by air, due to the high perishability of the product and the existence of an effective global flower production and distribution system. A fast transport system is required to reach distant markets, since the production regions are mostly situated in developing countries which have cheaper labour and land (Reid, 2009).

Immediately prior to the economic recession of 2008, the New Agriculturist (2007) estimated the total annual increase in global cut flower trade to be at 20 %, but Rabobank (2015) more recently reported that the cut flower exports from low-cost cut flower exporting countries became volatile from 2009 onwards. Rabobank (2015) speculated that growth in the floriculture trade is unlikely to increase in the traditional major markets (i.e. Europe, U.S.A, Japan) in the next decade, although there is potential for increased trade in countries with strong economic growth. Furthermore, export data indicate that there has been a downward trend in total flora exports from South Africa (SA) since the 2002/2003 season (PPECB 2008, 2010 as cited by Reinten et al., 2011).

The decrease in exports from SA has mostly been blamed on increased air transport costs and awareness of the high carbon footprint of air freighted goods (Crous et al., 2013; Reinten et al., 2011). Worse still, the control of atmospheric conditions during air freight is poor as multiple cold chain breaks often occur (Zeltzer et al., 2001). In addition, Maphaha (2014) and Westra (2009) identified limiting air freight volume allocations as one of the main restrictions in expanding cut flower and bud exports from SA**.** Up to 90 % of global intercontinental flower exports are transported by air (Lutes, 2014), but high jet-fuel costs and advances in cooling technology are stimulating a shift to sea freight, especially for imports into Europe. As a result, there has been an increase in the volume of flowers that are transported by sea from South

America and Africa (Reid and Jiang, 2012; Sechler, 2013) and approximately 15 % of flower exports from Colombia are now via sea (Rabobank, 2015).

Flower volumes transported by sea are likely to increase dramatically as more research is being directed towards optimization of sea freight conditions for intercontinental trade. Sea freight allows transportation of larger volumes of flowers and, better storage environment manipulation (Stephens et al., 2003), and has already proved to be partially successful (Westra, 2009). Furthermore, sea freight costs are nearly half those of air freight, which is an important consideration for price-conscious traders (Sechler, 2013). However, since some flower products are still regarded as unsuitable for sea freight, air freight is unlikely to be phased out soon. After taking into account all the benefits of sea freight, it may seem inevitable for Cape Flora (also referred to as Cape Fynbos) flower exporters to favour full adoption of sea freight. However, unlike potted plants, some Cape Flora cut flower products have a much reduced storage and post-storage vase life. This means that these high-value products have to reach the consumer in the shortest possible time to ensure that high quality is maintained.

Moreover, flowers by design are comprised of different tissue types which makes their post-harvest life more complicated (Halevy and Mayak, 1981; Reid, 2009). Thus, both cut flowers and intact ornamentals are highly complex plant organs and loss of quality of stems, leaves or flower parts may easily result in rejection at the market (Reid, 2009). Reid and Jiang (2012) conferred that the most essential components of quality are freshness and vase life, with both aspects being highly dependent on optimum postharvest handling.

2. Sea freight options available to Cape Flora within the South African export context

Indigenous floral products from SA and Australia should meet the market's expectations in order for them to maintain and improve their industry's reputation and market share (Gollnow et al., 2014), and this requires innovative freight and storage technologies. Reid and Jiang (2012) noticed a general reduction in vase life of cut flowers and foliage, especially in North America, and a lower per capita consumption of cut flowers in the USA, as compared to other developed countries. The authors attributed this to changes in markets and transportation, which have not taken advantage of advances in postharvest technologies to counteract vase life challenges posed by storage time and/or temperature effects.

Cape Flora cut flowers are transported by means of the conventional fixed open-air exchange vents (AirEx) system during sea freight. The reefer containers are typically ventilated

at a fixed rate of 15 000 L fresh air per hour to avoid anaerobic conditions and ethylene build up (M. Huysamer 2015, pers. comm. 30 March). Cooling of high volumes of warm, moist air to shipping temperatures between 1°C and 4°C causes condensation and ice formation on the cooling coils, which requires occasional defrosting cycles. During defrosting cycles, temperature control is absent and this may in addition, promote desiccation of flowers over long storage periods. AgriHort Communications (2014) highlighted the risk of over-ventilation that arises from using an AirEx system. Therefore, the use of AirEx on flowers over long periods of cold storage can pose the potential for a postharvest disaster, with regard to leaf blackening and chilling injury, which are both conditions observed in Cape Flora that are considered to be promoted by moisture loss (Ferreira, 1983; Paull and Dai, 1990).

While a ventilation rate of 15 000 L h^{-1} is effective for fruit storage, chances are high it is not ideal for Cape Flora products. This may be due to the differences in the physiology and ethylene sensitivity of the products (L. Hoffman 2015, pers. comm. 30 March) and lack of information on lower O_2 limits (LOL) and CO_2 toxicity tolerance limits of Cape Flora products. Choice and implementation of atmosphere control technology is dependent on commodity characteristics (MOL, 2015). Thus, the rate of fresh air change should be directed by the minimum O_2 and maximum CO_2 tolerance limits of the product in question. Unfortunately, the air exchange dynamics of Cape Flora cut flowers, a relatively new floral crop under long-term cold storage shipment, are largely unknown to the exporters and industry. The Cape Flora industry is, therefore, unable to utilise automatic ventilation technology, due to the absence of information on LOL and CO_2 toxicity tolerance limits during long-term cold storage.

3. Advances made in sea freight with automatic ventilation technology

The AV+ technology was developed by Maersk Container Industry (MCI), a major sea freight services provider (Moller, 2014). The technology monitors CO_2 and O_2 levels during shipment and automatically adjusts the amount of fresh air injected into the reefer container, depending on the set lower O_2 limit and maximum acceptable CO_2 for the commodity (Moller, 2014; Port Technology International, 2014). Controlling the ventilation ensures that the right amount of air required is injected into the reefer container, thereby reducing chances of unnecessary cooling, with lowered energy inputs and associated costs. Furthermore, strict control of the warm, moist air injected causes reduced dehydration of the produce in transit (Moller, 2014). According to Moller (2014), automatic ventilation technology is the next standard technology for all reefer containers, therefore, Cape Flora exporters will inevitably

also rely on and benefit from it in the nearby future. This imminent change in sea freight technology requires several urgent investigations into aspects of Cape Flora flower gas exchange dynamics under long-term cold storage, which currently limits the utilisation of the technology.

Firstly, however, the feasibility of closed ventilation during long-term (21 days) sea freight needs to be ascertained. Secondly, knowledge of the LOL and CO_2 toxicity tolerance limits for Cape Flora cut products is essential prior to implementing automatic ventilation technology. This will give insight on the lowest level to which O_2 can be allowed to drop before anaerobic respiration occurs (LOL) and the maximum acceptable CO_2 amount (toxicity level) before tissue damage occurs.

4. Prospects for Cape Flora cut flower exporters

Consumer demands and trade expectations are becoming increasingly sophisticated and variable. As a result, the requirement for sustainable production and distribution is also on the increase (Rikken, 2011). Cut flower industries internationally have to be technologically dynamic and innovative in their production, handling and marketing approach in order to keep up with market expectations. The indigenous South African Cape Flora cut flowers, though still considered a new floricultural crop, are very much part from this global marketing trend. Although postharvest respiration control in flowers is currently mainly achieved through temperature manipulation, more attention should be devoted towards other methods like dynamic controlled atmosphere (DCA) storage, or modified atmosphere packaging (MAP).

5. Conclusion

This study aims to provide a greater insight to the questions brought forward by the Cape Flora cut flower industry, which to date have prevented the adoption of automatic ventilation technology and the more frequent use of sea freight as an alternative to air freight.

The objectives of this study are:

- To measure respiration rate at 5 and 15 °C and determine the levels to which O_2 depletes and CO_2 accumulates in a closed ventilation system and the viability of long-term closed ventilation storage of *Proteaceae* cut flower products.
- To determine the lower O_2 limits for a range of *Proteaceae* cut flower products using DCA technology and long-term cold storage in low O_2 atmosphere.

- To determine the CO_2 toxicity tolerance limit for a range of *Proteaceae* cut flower products through long-term cold storage in high CO_2 atmosphere.

It is beyond doubt that the optimisation of postharvest quality of cut flowers relies on effective integration of several preservation technologies. However, as stated by Macnish et al. (2009), the commercial interest in controlled atmosphere (CA) and MA techniques is likely to remain low in the ornamental industry, until their effectiveness is proven across a wide range of high value products. This study intends to provide suitable evidence to recommend or dismiss the feasibility of commercial implementation of closed ventilation - a form of passive modified atmosphere - and automatic ventilation technologies. In addition, it may serve as baseline information for the development of a comprehensive protocol and handling manual advising South African Cape Flora exporters on the use of sea freight for their range of products.

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PAPER 1: SEA FREIGHT AS A SUSTAINABLE OPTION WITHIN THE EXPORT VALUE CHAIN OF CAPE FLORA CUT FLOWERS

Abstract

The global trade in indigenous South African cut flowers continues to grow despite it being regarded as a relatively new industry. Increased interest in the *Proteaceae* cut flower products from the Cape Flora Kingdom has seen a marked increase in export volumes of the products due to their striking appearance, genetic diversity and perceived long vase life. The Cape Flora cut flower industry is export oriented, with Europe being the largest market. Increase in airfreight costs and efforts to reduce the carbon footprint have seen the gradual development of interest in sea freight. The use of sea freight for cut flowers exported from South Africa to overseas markets requires use of lower temperatures to reduce respiration rate and a longer transport duration, which both increase the risk of quality loss during transit. Thus, postharvest treatments and sound storage techniques aimed at maintaining the harvesting quality become a necessity when using sea freight. The development of automated reefer ventilation techniques has also exposed the lack of vital information on the gas tolerance limits of the products. There is need to assess the current handling and storage practices, and identify the future prospects, in order to allow adoption of new reefer technologies and improvement of the current storage techniques. Unfortunately, little literature is available on the gas tolerance limits for Cape Flora cut flowers and there are no sea freight protocols currently that are specifically designed for these products. As a result, there are huge variations in handling and storage practices among exporters. This review intends to highlight the nature and market for the Cape Flora cut flowers, identify the challenges encountered during long-term cold storage and the techniques used to delay quality loss and the importance of storage atmosphere as a remedy quality loss due to long-term cold storage.

Key words: Cape Flora, CO_2 toxicity, dynamic controlled atmosphere, long-term cold storage, lower O_2 limit, *Proteaceae*

1. Introduction

Cut flower production is predominantly restricted to regions with sufficient arable land having the required resources for agriculture, where labour costs is low and with suitable climates (Zeltzer et al., 2001). As a result, flowers are produced mainly in Central America,

Africa, Israel, and the Far East. However, currently the major flower markets include Europe, North America and Japan, where the standard of living, and consequently the flower consumption per capita, is relatively high (Worldatlas, 2017; Zeltzer et al., 2001). Thus, when areas of production are not close to the final markets, effective levels of global marketing are instrumental.

Several native South African floral species, many which are endemic to the Cape Floral Kingdom, have long fascinated both local and international horticulturists, leading to their exploitation for commercial purposes. A continued keen interest in these products has earned some of them, such as *Gladioli* and *Freesia*, positions amongst the top 10 products found on the European and other international floricultural markets (Bester et al., 2009). Similarly, many other South African produced Cape Flora products over the last three decades have become increasingly more important to the international floricultural industry. This is mainly due to their large striking inflorescences and/or brightly coloured foliage, along with a perceived long vase life (Coetzee and Littlejohn, 2001). However, the required intercontinental transfer of these highly perishable products from South Africa to its various regional, but also distant overseas markets, demands prolonged cold storage, which are likely to put significant pressure on the ability to maintain a long vase life (Ekman et al., 2008).

The appearance, quality and longevity of cut flowers are dependent on a range of factors of which the most important would be: cultivar characteristics, cultivation conditions, optimum harvesting time, suitable transportation conditions and appropriate handling practices (Teixeira da Silva, 2003). Upon arrival at the market, postharvest quality of cut flowers is a continuum from production to the consumer, within a value chain where proper cold chain management is critical. Poor temperature control and high freight costs which are regularly associated with air transport are among the challenges that have prompted the switch to sea freight (Reid and Jiang, 2012).

While effective cold storage may be an easier goal to achieve over short distances, extended periods of cold storage are likely to promote quality loss and reduce vase life due to the extended time between harvest and sale (Reid and Jiang, 2012). Worse still, some flower importers, as part of a marketing strategy, choose to stock up weeks in advance of special flower events and holidays (Reid and Jiang, 2012), which further extends the cold storage period requirement. As a way of solving these challenges, the cut flower industry is required to develop innovative and cost effective methods within storage technology, but also with respect to vase life improvement. The *Protea* industry in SA is undergoing rapid changes with regard to improved transport methods and innovative export technologies (Kras, 2010).

The interests and demands of producers and consumers in new products are dynamic. This requires phytosanitary regulations, evaluation parameters and storage conditions to evolve accordingly with product development, in order to meet the quality and longevity requirements of the ever-changing flower industry (Teixeira da Silva, 2003). The value of flowers to an exporter lies in the possibility of a successful sale, while the buyer realizes flower value in acceptable longevity and high quality (Teixeira da Silva, 2003). Macnish et al. (2009) proposed controlled atmosphere (CA) as a method of improving the longevity of cut flowers due to its effectiveness in lowering respiration, pathogen activity and senescence. The exploration of new varieties of flowers with improved postharvest life may assist in fulfilling the consumers' demand for high quality (Teixeira da Silva, 2003). Controlled atmosphere technology has been highly successful in maintaining fruit quality during storage (Hoehn et al., 2012) and is usually associated with the low temperature storage, and other post-harvest treatments. Better yet, if the minimum O_2 and maximum CO_2 stress limits are known, dynamic controlled atmosphere (DCA) technology could be used in monitoring the storage atmosphere composition during sea freight.

The following review aims to provide better insights into the applicability and feasibility of various storage and transport options of Cape Flora products through evaluating the possible use of CA and other related technologies, identifying the current commercial practices and challenges, and identifying future prospects for long-term cold storage of Cape Flora products.

2. Uniqueness of Cape Flora cut flowers

Numerous South African plants are globally renowned as sources of genetic material for cut flowers and as a result, several of them have been hybridized, and distributed globally. The uniqueness of plants from the Cape Floral Kingdom, which is the smallest and most diverse plant kingdom, has drawn the attention of the international market (Reinten et al., 2011). Among these plants are flowers from the Fynbos biome, which contains about 9000 species, of which approximately 69 % are considered endemic (Goldblatt and Manning, 2000). The *Proteaceae* family, which are indigenous to this biome, contains more than 60 genera and nearly 1400 species. Australia is home to over 800 species, while Africa claims about 330 species, most that are native to the Western Cape of South Africa. The structure of these plants may be highly variable to range from ground covering types with creeping stems, to those with

underground stems, while some exhibit vertical stems typical of shrubs and trees (Leonhardt and Criley, 1999).

Proteaceae plants generally have lignified, hard and leathery leaves typical of Mediterranean vegetation. This family is further characterised by the presence of proteoid roots, which are an interesting and unique adaptation to the soil and environmental conditions since their distribution is concentrated around areas with soils that are usually highly deficient in plant nutrients (Brits, 1984; Lamont, 1982; Shane et al., 2008). However, it is the striking coloured and interestingly textured inflorescences associated with the *Proteaceae* family that have captured the imagination of the international floricultural industry and resulted in increasing inter-continental trade.

3. South Africa Cape Flora cut flower trade

Reid (2009) noted that the high export value of cut flowers has the potential to fuel exponential rises in cut flower production in many countries. Although there is prospects for increased trade in countries with strong economic growth (Rabobank, 2015), Mamias (2015) highlighted that the growth in the floriculture trade which was observed in the 1990-2000s has levelled off. During all this economic turbulence, South Africa's primary export market, the Netherlands, continues to dominate the global flower trade industry (Maphaha, 2014), serving as a hub for flower exports from developing countries. It has a major advantage with respect to the distribution of flowers due to its proximity to several major importers (Sepúlveda, 2009).

The South African Cape Flora industry consists of both veld-harvested product as well as cultivated production. Veld-harvested flowers have traditionally been used for dried floral products as they were generally regarded to be low value products of inferior quality (Gerber and Hoffman, 2014). However, over the last five to eight years the global cut flower trade of wild-harvested Cape Flora products has been growing rapidly as the bulk of these products are incorporated as foliage fillers in bouquets, aimed in particular at the United Kingdom retail market. Cape Flora SA (2017a) reported the number of bouquets exported for the 2016/2017 season as follows: 377 528 bouquets with *Protea* as focal flower, 101 999 using *Leucospermum*, with 352 561 and 186 279 bouquets classified as either mixed greens or just mixed bouquets.

The leading commercial cultivated products are *Protea* (59 %), *Leucadendron* (17 %), *Leucospermum* (14 %), *Berzelia* (4 %), *Brunia* (3 %), with *Serruria* and *Erica*, among others, making up the remaining 3% (Cape Flora SA, 2017b; Conradie and Knoesen, 2010; Gerber

and Hoffman, 2014). The trade of Cape Flora has always been export-oriented with the local market up to 2010 being almost non-existent (Gerber and Hoffman, 2014). However, in recent years the interest in Cape Flora products within South Africa appears to be growing rapidly, both in the informal and retail market, though this trend has not formally been quantified (EW Hoffman, personal communication). For exports (Figs. 1, 2, 3), the European Union (EU) remains the leading market for Cape Flora and claims nearly 80% of the market share (Gollnow and Gerber, 2015; PPECB, 2013; Reinten et al., 2011). Malan (2015) warned that South African *Protea* flower sales as recorded by the PPECB are unable to provide reliable market information due to a lack of detailed local and foreign tree census and trading data. However, Gollnow and Gerber (2015) reported that exports of *Protea*, *Leucadendron*, *Leucospermum* and Greens in the 2014/2015 season from South Africa were based on cultivation from approximately 601, 206, 143 and 59 ha, respectively.

Despite the increase in trade of indigenous South African flowers to the Netherlands in the recent past, the Netherlands market is still dominated by commodity flowers from Kenya (26.7 %) and Ecuador (21.3 %) (Maphaha, 2014). Direct sales from SA to the United Kingdom (UK) have increased significantly to consume 33 % of the total *Protea* and Cape Fynbos products that are exported from SA, mainly due to an increased demand for bouquets (Reinten et al., 2011; Gollnow and Gerber, 2015). The rest of the South African floral export products are distributed via the Middle East or to Mediterranean countries (11.8 %), the Far East and Asia (6.2 %), to some African countries (4.9 %), with only small volumes being destined for the Americas (1 %). In 2002, Coetzee et al., reported that 70 % of flower exports from South Africa were Cape Fynbos products, however by 2010, PPECB export data (as cited by Reinten et al., 2011) showed that this figure had risen to 84 %.

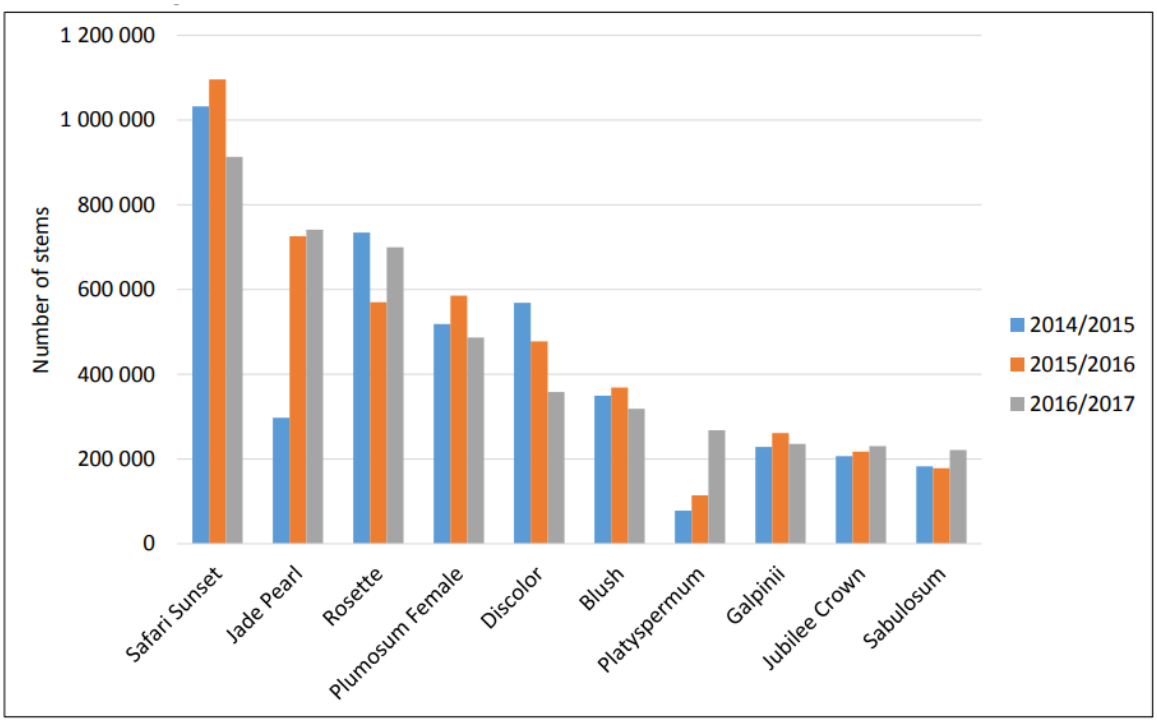

Fig. 1. *Leucadendron* stem exports from South Africa from 2014-2017 (CapeFlora SA, 2017a).

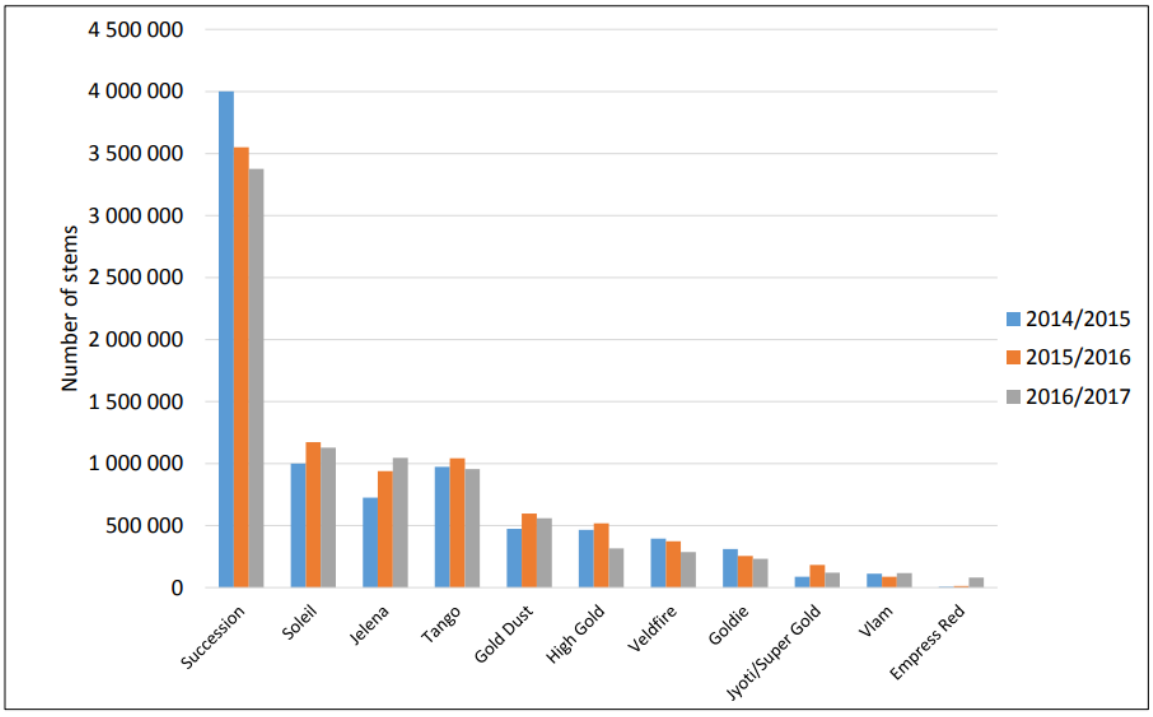

Fig. 2. *Leucospermum* stem exports from South Africa from 2014-2017 (CapeFlora SA, 2017a).

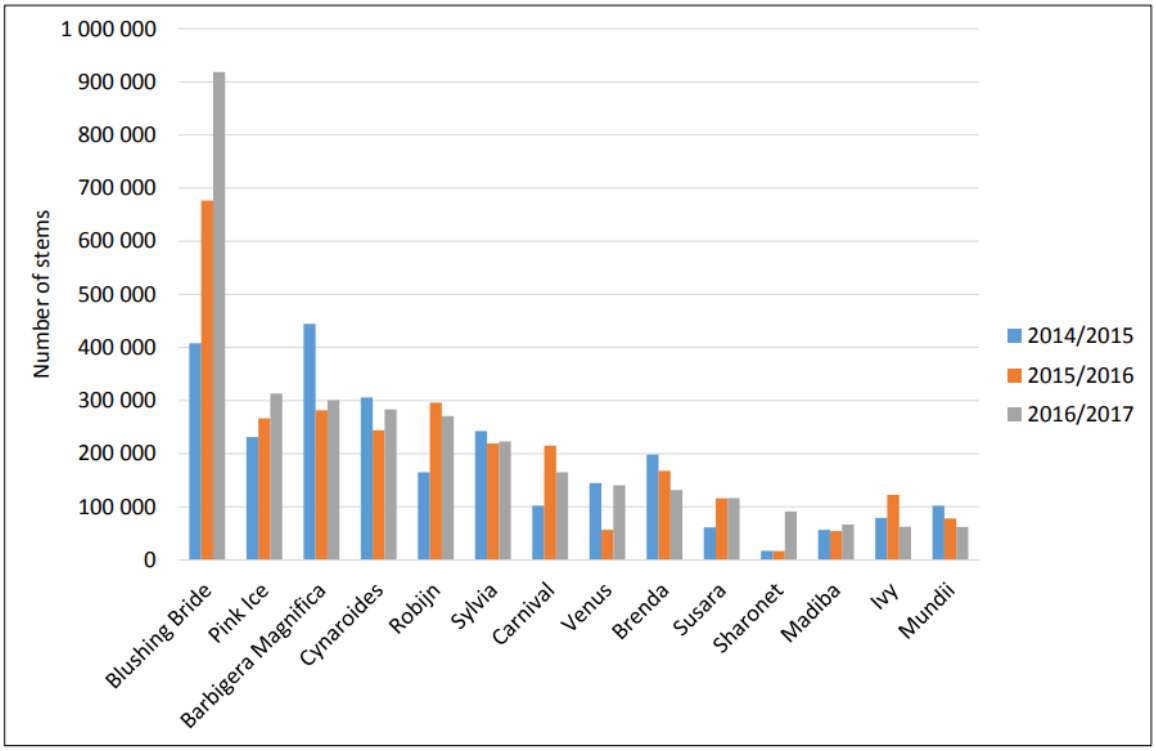

Fig. 3. *Protea* stem exports from South Africa from 2014-2017 (CapeFlora SA, 2017a).

4. Challenges associated with long-term storage of Cape Flora floral products

4.1 Harvesting maturity

Most *Protea* cultivars and species will complete their development and achieve the maximum vase life when harvested at the soft tip stage, just immediate prior to when the inflorescence bracts open to reveal the individual florets. In addition, *Protea* flowers are also harvested at the soft tip stage to avoid insect- and bird damage normally associated with flower opening. *Leucospermum* flowers are normally harvested based on the number of reflexed styles, with 30 to 50 % reflexed styles being the industry norm (Crous et al., 2013; Malan, 2012). Premature harvesting of *Leucospermum* inflorescences will most likely cause inability of styles to reflex (Eigenhuis, 1999; Faragher et al., 2010), while late harvesting will inevitably reduce the storage- and vase life (Malan, 2012). *Leucadendron* products tend to have a more flexible harvesting window that which are closely linked to when the desired colour development is achieved and may commence from the point of adequate leaf and shoot maturity onwards. Despite these broad guidelines, the ideal harvest maturity remains dependent on the consumer's preferences and the exports' expectations, which may in turn vary significantly with the amount of product available at the time within the market chain. The

ideal harvesting stage may differ between species and cultivars, however, the optimum harvesting maturity should always ensure that the harvested buds have the ability to fully develop after harvest, have maximum storability and with stems having the prospects of achieving a reasonable vase life.

Most commodity flowers such as roses, carnations and *Chrysanthemum* are mainly harvested in the bud stage, which allows bud opening to occur after storage or distribution (Reid, 2008). However, Halevy and Mayak (1981) cautioned that buds of some flower cultivars may not open properly if harvested too early. Cut flowers that are destined for local markets are commonly harvested when the bud is more open as compared to those intended for storage or long-distance shipment (Reid, 2008). Paull and Dai (1990) reported that stems of *Protea* that are harvested when the inflorescences are still immature are considered more susceptible to leaf blackening than those harvested at optimum inflorescence maturity. Ferreira (1986) and Joyce et al. (1995) seemed to be in agreement that high susceptibility to leaf blackening observed on early harvested stems is due the high respiration rates of developing inflorescences.

Early maturity harvesting would be advisable to counteract the effects of a long storage duration and to extend the vase life, as is the case for bud-harvested snapdragons (Reid, 2016). However, it may increase the risk of carbohydrate depletion due to higher respiration rates associated with a more immature harvesting stage. In addition to low temperature storage, other measures to reduce respiration, such as high CO_2 and low O_2 atmospheres, may be beneficial during long-term cold storage.

4.2 Harvesting conditions

Faragher et al. (2010) advised against harvesting of most cut flowers at the end of a hot day or during the hottest part of the day, for example, when temperature exceed that of 30 °C. The most ideal temperature condition for Cape Flora flower harvesting is when field temperatures are below 25 °C, with the softer, more fragile flowers such as *Serruria* to be harvested at even lower temperatures (Malan, 2012). For most Cape Flora products, despite the risk of harvesting wet stems when exposed to dew, flowers are normally harvested in the morning, possibly to avoid high day temperatures that promote water loss and to ensure sufficient time for grading and processing.

However, Faragher et al. (2010) and Malan (2012) agreed that the postharvest quality of *Protea* flowers is better maintained when they harvested later in the day as it reduces incidence of leaf blackening, which is accelerated by the presence of moisture on the leaves.

For *Protea* in particular, Malan (2012) encouraged harvesting in the afternoon since the leaves below the inflorescence are likely have higher carbohydrate reserves as compared to early in the morning. Hoffman et al. (2014) noted that harvesting in the afternoon reduced leaf blackening, by 40-60 % after 10 days of vase-life, regardless of stage in season. In addition, afternoon-harvested stems showed better uptake of pulsing solutions, which may be explained by either increased transpiration or osmotic potential of stems later during the day.

Both the weather preceding the time of harvest and the time of year at harvest have a direct impact on Cape Flora quality, specifically as pertaining to leaf blackening incidence (Malan, 2012). In Israeli-produced *Leucadendron*, chilling injury and leaf blackening that develops during sea freight is particularly prevalent in autumn-harvested stems (Philosoph-Hadas et al., 2010). South African produced 'Sylvia' that is harvested during spring shows a higher susceptibility to leaf blackening (Malan, 2012), possibly due to low carbohydrate reserves following winter (Hettasch et al., 2001). Sufficient sunlight exposure immediately prior to harvest may favour better stem quality, since stems harvested following three to four days of cloudy conditions are known from experience to be more vulnerable to leaf blackening.

In addition, leaf blackening of *Protea* stems is more prevalent under conditions where stems experience field temperatures greater than 30 °C, for at least two days, just before harvesting (Malan, 2012). Generally, high humidity and high temperature conditions prior to harvesting promote leaf blackening. To conclude: huge variations in the incidence of leaf blackening may result from differences in cultivars, growing- and harvesting conditions among producers, but are also linked to the time within the season and even to climatic conditions immediately prior to or at harvest.

4.3 Cultivar

Fragmented and/or lack of information that focuses on the direct effect of cultivar on postharvest life of different Cape Flora cut flowers greatly hinders the development of suitable product specific protocols for optimum postharvest management of these high-value ornamentals. Despite this, comparisons between products are made based on differences in responses to pathogens, physiological disorders, handling practices, vase life and environmental conditions, as is observed and recorded between both species and genera of Cape Flora products.

A study by Macnish et al. (2010) on roses provided firm evidence for the role of genetic variation when the average vase life of modern rose cultivars was recorded to vary from five to 19 days. The authors similarly noted that sensitivity to ethylene also varied between

cultivars, as five of the 38 cultivars tested were reported to be insensitive to ethylene. Macnish et al. (2010) further noted that in *Alstroemeria*, duration before petal drop and leaf yellowing differed greatly between lines produced by the same breeder.

In *Proteaceae* McConchie and Lang (1993b) reported that, similar to other cut flowers, the degree and rate of leaf blackening differed considerably between species, and even between clones within the same species (Paull and Dai, 1990). In the latter study, the authors observed that leaf blackening varied considerably within the four *Protea neriifolia* selections that were evaluated. Vulnerability to leaf blackening in *Protea* flowers varies between species, and according to Van Doorn (2001) appears to be most severe in *P. neriifolia*, *P. compacta*, *P. coronatea* and *P. eximia*. McConchie and Lang (1993b) also noted that hybrid cultivars, for example 'Pink Ice' (*P. compacta* x *P. susannae*) and 'Sylvia' (*P. eximia* x *P. susannae*), had inherited the susceptibility to leaf blackening from one of their parents.

Interestingly, unlike *Protea* and *Leucadendron*, *Leucospermum* products are not vulnerable to leaf blackening (Dai and Paull, 1997). This shows the importance of genetic variation with regard to postharvest disorders in determining and selecting suitable Cape Flora products for their ability to tolerate long-term cold storage and display an extended vase life. In accordance, Blomerus et al. (2010) proposed that breeders should concentrate on selecting for seedlings that are less prone to inheritable conditions, like susceptibility to leaf blackening, as a way of improving postharvest quality and vase life.

4.4 Leaf blackening

In 1983, Ferreira reported that some *Protea* species are affected by a rapid blackening of the foliage (leaf blackening) which occurs within a few days of harvest. Leaf blackening has been identified by the presence of limp and leathery leaves, which develop black spots or areas that normally results in the leaves turning black. Some researchers (Paull et al., 1980; Whitehead and de Swardt, 1982) have directly linked polymerization and oxidation of hydroxyl-phenols and tannins that follows loss of cell compartmentalisation with leaf blackening. Van Doorn (2001) proceeded to identify peroxidase and polyphenol oxidase (PPO) as the key enzymes behind blackening in plants.

Initial research (Paull et al., 1980; Whitehead and de Swardt, 1982) suggested that leaf blackening of *Protea* stems was caused by water stress. Contrary to this, Reid et al. (1989) demonstrated that leaf blackening was unaffected by reduced transpiration or increased water stress as covering the inflorescence with a plastic bag, which decreases water stress through reduced transpiration, did not reduce leaf blackening compared to stems held without bags. A

different mechanism was also proposed that explained that leaf blackening resulted from exhaustion of leaf carbohydrates as girdling below the inflorescence significantly reduced leaf blackening (Dai and Paull, 1995; Malan, 2012; Reid et al., 1989). Studies by Paull et al. (1980) showed that the removal of inflorescences lessened leaf blackening through reduced carbohydrate demand. Within a day of harvesting, up to a 70 % depletion in mobile leaf carbohydrates may occur in *Protea neriifolia*, due to the high respiratory demand of the inflorescence (Jones et al., 1995).

Leaf blackening increases under high temperature (Stephens, 2003; Stephens et al., 2001) and low light storage conditions, possibly due to increased use of carbohydrates during respiration (Ferreira, 1986; Stephens et al., 2005). Carbohydrate reduction in *Protea* leaves is linked to the strong sink created by the inflorescence due to the high amount of nectar produced (Ferreira, 1986; Paull and Dai, 1990). The nectar produced by *Protea* flowers mainly contains glucose, fructose, sucrose and xylose (Cowling and Mitchell, 1981; Van Wyk and Nicholson, 1995). Unfortunately, even the removal of the inflorescence or girdling below the inflorescence appears to be ineffective in reducing leaf blackening of some cultivars, as observed when *Protea eximia* flowers were stored under dark conditions (Bieleski et al., 1992). Currently the serious and persistent postharvest disorder, leaf blackening, offers major challenges to long-term cold storage of *Protea* products as would be required during sea freight as leaf blackening is distinctly aggravated under storage conditions.

4.5 Phytosanitation

A successful export consignment greatly depends on the exporter being able to meet the phytosanitary requirements of the importer to avoid produce rejection. A report by PPCEB (1998-1999) as cited by Crous et al. (2013) revealed that up to 1 % of total *Proteaceae* shipments have been rejected at the ports before leaving South Africa. Worse still, Crous et al. (2013) stated that even more stems may be rejected on arrival at the markets on grounds of phytosanitation. The high susceptibility of flowers to pathogens and diseases is elevated by their fragile tissues and nectar production which acts a source of nutrients for the pathogens (Reid, 2008). On top of that, storage temperature fluctuations, often experienced in air freight, promote condensation of water on the leaves which accelerates leaf blackening, and favours the development of postharvest pathogens (Reid, 2008).

One such prevalent and common pathogen in the cut flower industry that reduces both quality and vase life of flowers is *Botrytis cinerea* (Tshwenyane et al., 2012). This pathogen readily germinates in the presence of moisture, even at very low temperatures (Reid, 2008;

Agrios, 2005) and is difficult to control since it is able to survive even at near zero temperatures and the symptoms of infection are mostly not visible at harvest. Unfortunately, the use of high CO_2 for phyto-sanitation within floral products is a challenge since the fungistatic CO_2 levels that are required for effective control of the pathogen (approximately 15 %) sometimes are also damaging to flower petals, stems and leaves (Kader, 2004).

Due to the strict quarantine inspection on all stems that are imported, the occurrence of pathogens and diseases is undesirable since it may lead to product rejection and even result in the ban of imports from a particular supplier or country (Reid, 2008). Crous et al. (2013) noted that countries that have indigenous *Proteaceae* flora are apparently more exposed to harmful pests compared to those where *Proteaceae* are produced as exotic flowers. Fortunately, most pathogens can be controlled through improved sanitation protocols, temperature management, together with proper chemical and biological control. In *Proteaceae*, as in other cut flowers, genetic variation between cultivars also steers selection for disease resistant cultivars, along with that of appearance and consumer preference as major breeding criteria for the flowers industry (Reid, 2008).

4.6 Light

Cut flowers are normally transported under dark conditions, with exposure to light during storage or transport only a consideration for ornamental products that are highly prone to leaf yellowing due to lack of light. Reid (2008) reported that exposure to light may be necessary when transporting *Chrysanthemum*, *Alstroemeria* and *Lilium* that develop leaf yellowing when stored in the dark, especially at high temperatures. Several studies provided evidence that exposing *Protea* stems to light during storage or transport, may lessen the effect of some physiological disorders, including that of leaf blackening. Jacobs and Minnaar (1977) noted that *Protea neriifolia* exposed to a minimum light intensity of 25 $\mu mol.m^{-2}.s^{-1}$ or higher developed less leaf blackening than those kept in total darkness. Similarly, vase life evaluations by Newman et al. (1990) also led to the conclusion that *Protea* stems stored under light conditions developed leaf blackening less rapidly than stems kept in the dark. More recently, Hoffman and Du Plessis (2013) reported that long-term cold storage of potted *Leucospermum* under low light conditions at 6 °C, for three weeks, resulted in better stomatal control and vase life quality compared to plants stored in darkness.

McConchie et al. (1991) and Jones and Clayton-Greene (1992) also shared a similar idea since they claimed that storage in photosynthetically active radiation (PAR) light significantly reduced leaf blackening due to the ability to photosynthesise. A possible

explanation by Jones and Clayton-Greene (1992) suggested that presence of light permit photosynthesis to continue, thus keeping the carbohydrate levels high in the leaves and lowering the possibility of leaf blackening. Personal communications with farmers as reported by Van Doorn (2001) revealed that there is reduced leaf blackening in stems kept under incandescent light bulb in pack- and store rooms, possibly due to the red light emitted by these bulbs.

Red light saturates phytochrome pigments and prevents light-related physiological changes, like leaf yellowing, which are believed to be a result of the conversion of phytochrome from the P*r* to the P*fr* form in darkness (Van Doorn and Van Lieburg, 1993). In a more recent study Liu et al. (2015) observed that detached kale, cabbage, lettuce and spinach leaves that are continuously subjected to diurnal light/dark cycles during storage had greater tissue longevity than those stored under continuous light or continuous darkness. This was attributed to circadian clock functioning and rhythmic changes that occur when storage cycles of equal light and darkness exposure are used. No studies, similar to that conducted by Liu et al. (2015), has been reported on Cape Flora products to date.

5. Maintaining quality of Cape Flora cut flowers during long-term cold storage and transport

Two distinct stages can be identified in harvested cut flowers, namely flower bud growth, which is then followed by flower development to full opening (Halevy and Mayak, 1981). Handling practices for promoting cut flower longevity are therefore intended to serve two seemingly contrasting purposes; initially, growth promotion and eventually, retardation of metabolic processes that result in senescence. Reid (2008) identified ‘freshness’ and a long vase life as the chief aspects of cut flower quality, factors which are both dependent on optimum postharvest management. To achieve reduction of postharvest losses requires producers and handlers throughout the value chain to understand both the biological and environmental factors linked to deterioration, as well as the application of available postharvest technologies for delaying of senescence (Kader, 2004). Although the aim is not to totally arrest postharvest senescence of fresh flowers, their value depends on our ability to control it within certain limits to meet the expectation of the customer.

5.1 Respiration rate

A proper understanding of the harvested plant part's physiological needs enables producers and exporters to take the appropriate steps in ensuring quality maintenance of different products (Teixeira da Silva, 2003). In ornamentals, respiration occurs either through the conversion of glucose to pyruvate with being finally released CO_2 or through the oxidative pentose phosphate pathway (OPPP) (Teixeira da Silva, 2003). However, glycolytic conversion of pyruvate to acetaldehyde and ethanol may occur when the O_2 amount falls below the level that allows aerobic respiration.

The respiration rate of flowers is generally considered to be high when compared to that of other plant products (Crous et al., 2013), which is believed to be the main cause for the accelerated senescence that occurs at elevated temperatures (Çelikel and Reid, 2002). In addition to the proposed high respiration rate, the different parts of the *Protea* inflorescence is also considered to vary significantly according to the different positions of the florets, or with different stages of development of the florets, where respiration rate is considered highest in the developing florets (Dai and Paull, 1995; Ferreira, 1986). Stephens et al. (2003b) demonstrated that postharvest processing also affects the respiration rate of *Protea* cut flowers (Fig. 4).

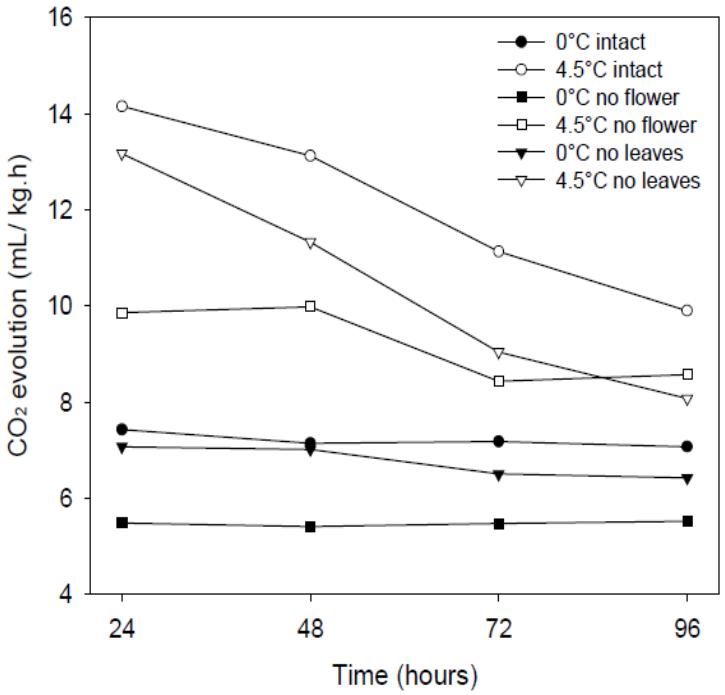

Fig. 4. Respiration rates of intact, decapitated and leaf stripped *Protea* cv. 'Sylvia' (*P. eximia* x *P. susannae*) stems after 12, 24, 48 and 72 h of storage at 0 and 4.5 °C (Stephens et al., 2003b).

The respiration rate of many cut flowers also changes during different stages of post-harvest life. The respiration rate rises to a maximum as flower head opening commences and gradually drops as flowers mature, where after it may sharply rises again over a brief period before finally decreasing (Coorts, 1973). The gradual decline in the respiration rate of cut flowers which is observed throughout the post-harvest life is hypothesised to be due to either a decline in respiratory substrates (Halevy and Mayak, 1981) or decrease in ATP (Beevers, 1976) due to oxidative phosphorylation and progressive uncoupling of electron transport.

Reid and Cevallos (2009) and Mattos et al. (2012) explained that floral crops show a general inverse relationship between respiration rates, the Q_{10} values, and the vase life. Cut flowers have extremely high rates of respiration and Q_{10} of most flowers usually ranges from 1.5 up to 7, between temperatures ranging from 0-10 °C and 10-20 °C (Reid, 2016). Saltveit (2016) classified respiration rates of horticultural products at 5 °C as follows (expressed as carbon dioxide production rates): very low at <5 $mg.kg^{-1}.h^{-1}$, low at 5-10 $mg.kg^{-1}.h^{-1}$, moderate at 10-20 $mg.kg^{-1}.h^{-1}$, high at 20-40 $mg.kg^{-1}.h^{-1}$, very high at 40-60 $mg.kg^{-1}.h^{-1}$ and extremely high respiration with value of >60$mg.kg^{-1}.h^{-1}$.

5.2 Storage temperature

Temperature control through rapid cooling and correct refrigeration is undoubtedly the most effective way of retarding postharvest senescence of cut flowers and is considered essential in quality preservation and vase life extension of cut flowers and foliage (Teixeira da Silva, 2003; Reid, 2016). Kader (2013) noted that at temperatures above optimum levels, product deterioration increases by two to three fold for every 10 °C rise in temperature. Due to the generally short vase life of flowers, low temperatures are essential to reduce respiration, without compromising on quality. Low temperature storage not only retards respiration and ethylene production, but also reduces the degradation of enzymes, inhibits pathogen activity and slows various processes related to growth and senescence (ASHRAE, 1994; Hardenburg et al., 1986; Marcos et al., 2014; Vieira et al., 2013). Bad temperature control and fluctuations in temperature however will accelerate quality loss (Halevy and Mayak, 1981; Tshwenyane and Bishop, 2011), possibly due to the development of condensation on leaves which promotes disorders such as leaf blackening and stimulates pathogen activity (Reid, 2008). Furthermore, temperature control is mostly absent during the short periods of the evaporator coil defrosting cycles in reefers used for sea freight (Vigneault et al., 2009).

Not all floricultural products are suitable for extended cold storage and transport due to their chilling sensitive nature, for example, anthuriums and gingers (Reid, 2014). If not

properly monitored, low-temperature storage can even be more detrimental than beneficial (Hardenburg et al., 1986; Sevillano et al., 2009). Still, low temperatures are currently the only and most effective option since high temperature storage promote wastage due to increased respiration rate (Eksteen et al., 1992; Stephens et al., 2001). Low temperatures may result in CI, which in *Leucospermum* and *Leucadendron* manifest as a darkened area on the leaves and petals, or as water soaked regions on the petals, and in severe cases, the collapse and desiccation of cells of the leaves and petals (Graham, 2005). Chilling damage occurs at temperatures above 0 °C, which can be as high as 8 °C and 12 °C for subtropical plants and tropical products respectively (Lyons, 1973). The severity of CI is influenced by a combination of storage temperature, storage duration and flower maturity (Goszczyńska and Rudnicki, 1988).

Cevallos and Reid (2000) showed that transportation temperatures of 5-10 °C for four to six days reduced vase life by up to 50 %. These findings agree with those of Reid (2001), who reported that the vase life of narcissus flowers stored at 10 °C for four days decreased by 30 % as compared to those stored at near 0 °C. *Chrysanthemum* stems that are stored at 5 °C have been reported to develop yellowing compared to those stored at 1 °C (Reid, 2016). Van Rooyen (2005) warned that the potential of CI development in fresh produce, particularly avocados, increases with an increase in extend of the cold storage period. This view was supported by Reid (2016) who reported the incidence of CI in *Chamaedorea* foliage to increase with storage duration at low temperature. Thus, cold storage time should preferably be short to maintain the best possible quality.

Sea freight to Europe takes a minimum of 21 days (including product accumulation prior to shipping) and this lengthy transportation period requires use of low temperature systems for quality preservation. Commercially recommended storage temperatures are normally between 0-1 °C for most flower cultivars (Çelikel and Reid, 2002; Macnish et al., 2009; Reid, 2001). According to Stephens et al. (2001) temperatures close to 0 °C may assist in the reduction of the incidence of leaf blackening, possibly due to the lowered respiration and consequently higher carbohydrate reserve status.

An addition benefit of the use of temperatures close to 0 °C can be found in the decrease of the occurrence of geotropic bending, such as in *Gerbera* flowers (Reid, 2001). Despite this, temperatures between 4-7 °C may be used for short-term storage and airfreight of Cape Flora products to avoid chilling damage (PPECB, 2013). *Proteaceae* products exported from Israel by sea are stored at 2 °C (Philosoph-Hadas et al., 2010), while those from SA are commonly shipped at temperatures between 1 and 4 °C (L. Hoffman 2015, pers.comm., 30 March; Ekman et al., 2008).

5.3 Air composition during long-term storage

As storage progresses, respiration contributes to CO_2 level rising and O_2 levels decreasing in a closed system. The level at which O_2 concentration decreases overall respiration rate, while still allowing aerobic respiration, greatly differs among horticultural commodities. Increasing the CO_2 level around some commodities reduces respiration, delays senescence, and retards fungal growth (Hardenburg et al., 1986). However, in low O_2 environments, increased CO_2 levels can promote fermentative metabolism (Saltveit, 2016). To avoid any toxicity to products, gas concentration control during storage is applied to maintain the O_2 concentration above the lower O_2 limit (LOL) to avoid hypoxia and keep the CO_2 concentration below the maximum limit for toxicity (Hodges et al., 2004).

According to Reid (2016) there have been relatively few reported benefits of CA storage of cut flowers, which may be a reason for the lack of extensive studies involving CA use on cut flowers. However, O_2 and CO_2 tolerance limits for various fruits and vegetables (Beaudry and Gran, 1993; Kader, 2014; Mattos et al., 2012) and for a limited number cut flowers (Macnish et al., 2009; Reid, 1997) have been documented. The symptoms of CO_2 phytotoxic damage (Fig. 5) and anaerobic respiration in Cape Flora products, are to a greater extent unknown since the toxicity tolerance limits for these products have not been explored. To compensate for this lack of knowledge, the conventional fixed-open-air-exchange ventilation (AirEx) system currently in use is set to ensure that the air composition is kept similar to that of atmospheric levels through a strong ventilation regime (AgriHort Communications, 2014), removing any possibility of O_2 or CO_2 related damage.

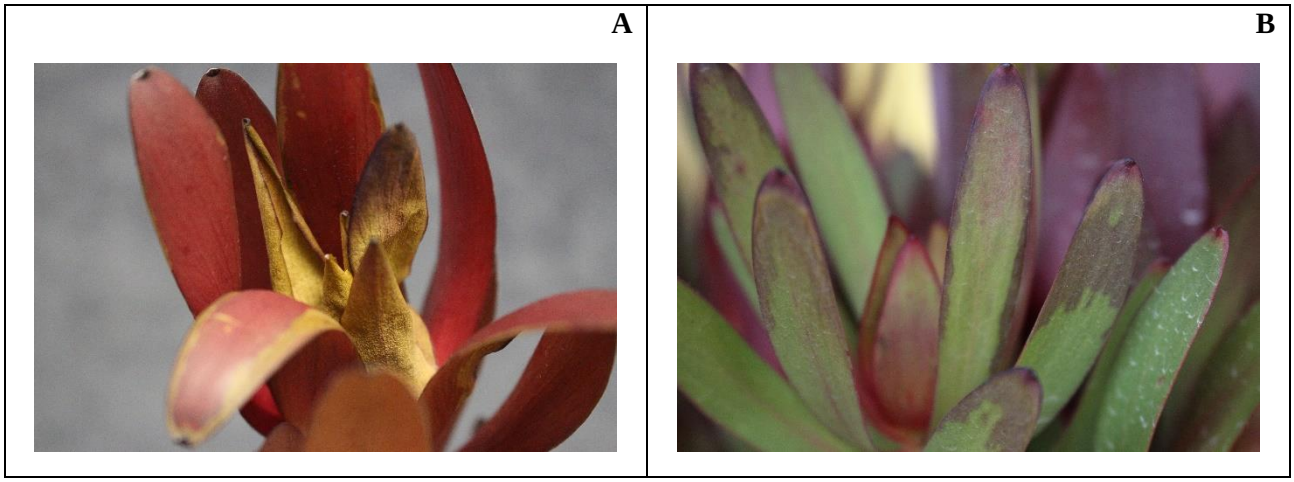

Fig. 5. High CO_2 damage on the involucral (5A) and mature foliage (5B) leaves of *Leucadendron* cv. 'Safari Sunset' after 21 d of storage at 1 °C in 20 % CO_2 (Matsikidze et al., unpublished data).

Proteaceae products are generally believed to be insensitive to ethylene gas (Reid, 2016), yet with little data to show or dispute this statement. However, Hoffman (2015, unpublished data) challenged this claim and proposed that some cultivars, such as *Protea* 'Venus' may actually show sensitivity to ethylene at 2 $mg.L^{-1}$ through discoloration of the bracts and by acceleration of the opening of the flower head. Several cut flowers produce ethylene when reaching maturity and with the onset of senescence. Some cut flowers, for example carnations, gypsophila and certain rose cultivars, are particularly sensitive and deteriorate rapidly when exposed to low concentrations of ethylene, where levels of 0.1 $mg.L^{-1}$ and above can cause damage in the vicinity of sensitive cut flowers (Reid, 2008).

Some of the most commonly used treatments used to effectively protect cut flowers from ethylene exposure include: ethylene blockers such as silver thiosulphate (STS) and 1-methylcyclopropene (1-MCP) or ethylene scrubbers like potassium permanganate ($KMnO_4$) or a new generation product such as palladium (Reid, 2008; Seglie et al., 2012; Smith et al., 2009; Terry et al., 2007). These treatments minimise the effects of either exogenous and/or endogenous ethylene. Storage and handling conditions of ethylene sensitive products should minimize the probability of ethylene exposure and provide adequate ventilation to remove any ethylene that may be present. Such ideal ventilation rates should prevent any build-up of unwanted gases without promoting water loss through overventilation.

5.4 Relative humidity (RH) and air circulation

Cut flower drooping and premature wilting of leaves and stems is directly associated with continuous water loss through transpiration and a decrease in water conductance in the stem (Teixeira da Silva, 2003). Water loss reduces vase life, therefore the use of technologies to limit water loss through storage under high RH atmospheres, as can be achieved through a combination of packaging and low temperature storage, have been explored to counteract the challenge of desiccation (Wills et al., 2007). Vapour pressure deficit (VPD), which is the difference in water content between the product and the surrounding air is often used as a parameter to predict expected moisture loss during long-term cold storage (Woolf et al., 2002). A storage atmosphere with a low relative humidity has a high VPD, and promotes moisture exchange from the produce into the surrounding air. Thus, VPD is inversely related to the RH of the air surrounding the produce (Kader, 2013). Mitchell (1992) reported that cold air holds significantly less water than warm air, which means that cold air has a low vapour pressure, explaining the efficacy in using low temperature to minimize water loss.

Apart from the diffusional resistance of the transpiration pathway, moisture loss from the produce is also affected by the difference in water vapour concentration between the leaf intercellular air spaces and the external bulk air (Wills et al., 2007; Taiz et al., 2015). Most fresh horticultural products require storage environments with a RH of 90-95 % for maximum shelf life (Reid, 2016; Vigneault et al., 2009). Incidence of wilting or shrivelling increases when produce experience low RH conditions. Vigneault et al. (2009) also highlighted that water-stressed products are more susceptible to pathogen infection, and are at higher risk of increased ethylene production, as the latter process is promoted by fungal growth (Williamson, 1950).

Reduction of air movement promotes a thicker boundary layer around the leaves and retards water loss from the cut stems. Recent reefer air circulation technology allows variable settings on the fan speed to reduce heat generation from the fans and lower energy consumption (MCI, 2016). Less air movement around the products promotes lower moisture loss and more uniformity in RH and temperature (Kader, 2013). The fans in the existing AirEx reefers currently used for transportation of Cape Flora cut flowers, however, operate at a constant high speed and the ventilation rate is fixed at 15 000 $L.h^{-1}$, which potentially increases the risk of excessive moisture loss.

5.5 Chemical treatments

Numerous chemical approaches are available to maintain the postharvest quality of cut flowers, but only few of them have been commercially implemented in the Cape Flora industry (Malan, 2012). Chemical treatments primarily have been developed to prevent disorders, reduce pathogen activity and alter plant physiology. One of the most common practices which is extensively used for ornamental quality maintenance is that of pulsing (Halevy and Mayak, 1981; Meir et al., 2007; Philosoph-Hadas et al., 2007; Stephens et al., 2005). According to Reid (2008) pulsing is the insertion (for a limited time period) of fresh cut flowers in a solution designed to prolong storage and vase life. Carbohydrate pulsing is a common practice that is usually used in combination with cold chain management to decrease carbohydrate exhaustion and extend vase life during storage and transport (Hardenburg et al., 1986).

Pulsing solutions serve to supply supplementary sugars, extend the vase life of ethylene-sensitive flowers, and discourage leaf yellowing. However, it is not a standard commercial practice to pulse all Cape Flora products, although sugar pulsing is used by some growers selectively during the season for some products to combat physiological disorders linked to carbohydrate levels (Malan, 2012). Postharvest carbohydrate exhaustion in leaves,

which is associated with assimilate requirements of the developing inflorescence, is believed to trigger leaf blackening in *Proteaceae* (Philosoph-Hadas et al., 2010).

Earlier studies (Dai and Paull, 1995) led to the conclusion that *Protea* species that are prone to leaf blackening may be pulsed overnight at 20-25 °C with 5 % sucrose or glucose to reduce severity of the condition. More recently, Stephens et al. (2003a) observed that glucose pulsing significantly reduced leaf blackening in 'Sylvia' proteas stored for 21 days at 1°C. The vase (1 and 2%) and pulsing (2%) glucose solutions also extended vase life of 'Cordi', 'Gold Dust', 'High Gold' and 'Succession' pincushions. Pulsing with lactulose, fructose and mannose has also been reported to result in improved vase life compared to unpulsed stems (Graham, 2005).

Philosoph-Hadas et al. (2010) reported that pre-storage pulsing of *Leucadendron* cv. 'Safari Sunset' stems with either 5 % sucrose or glucose, followed by addition of 2 % sucrose or glucose in the vase solution, reduced incidence of leaf blackening and desiccation after a prolonged period of sea transportation. Sucrose pulsing of 'Safari Sunset' stems preceding sea freight, raised carbohydrate levels in stem tissues, and thereby alleviated carbohydrate stress that resulted from the lengthy sea freight duration. However, several researchers (Dai and Paull, 1995; McConchie and Lang, 1993a; Stephens, 2003; Stephens et al., 2005) noted that use of exogenous sugar solutions has only been partially successful in reducing leaf blackening in some *Proteaceae* species, thus offering a possible explanation for its lack of adoption as a standard practice.

On the contrary, high concentrations of pulsing sugars may promote leaf blackening (Dai and Paull, 1995), collapse and damage especially under stress or light conditions (Malan, 2012; Shimizu-Yumoto and Ichimura, 2007). Shimizu-Yumoto and Ichimura (2007) applied sucrose pulsing on *Eustoma grandiflorum*, where after it was concluded that light exposure and low RH promoted higher sugar uptake during pulsing, due to the higher transpiration rates, and this excess uptake at an accelerated speed may lead to sugar toxicity.

6. Storage atmosphere modification

6.1 Potential use of CA technology in the cut flower industry

Kader (2004) described CA as a storage system that involves adjusting and maintaining an atmospheric composition that is unlike that of normal air (approximately 78 % N_2, 21 % O_2, and 0.03 % CO_2). It is considered that subjecting plant products to low O_2 (<8 %) and/or high CO_2 (>1 %) environments will lower respiration rate and decrease production of and sensitivity

to ethylene. Kader (2004) also explained that CA retards chlorophyll loss, and slows down the biosynthesis of anthocyanins and carotenoids, and oxidation of various phenolic compounds. However, Kader (2004) warned that beyond the maximum CO_2 concentrations and lower O_2 limit, respiration rate and ethylene production may be accelerated due to stress.

Thornton's (1930) studies on roses, using 5-30 % CO_2 storage atmospheres at different temperatures and different storage durations, were some of the first to demonstrate that CA may have the ability to prolong vase life of flowers. Later on, several researchers (Halevy and Mayak, 1981; Meir et al., 1995; Philosoph-Hadas et al., 2007) also claimed that CA storage inhibits decay development, leaf senescence, blackening, wilting, abscission and discolouration of cut flowers. *Leucadendron* cv. 'Safari Sunset' flowers that were kept at 15 % O_2 and 5 % CO_2 for 21 days at 2 °C were observed to have better quality and prolonged vase life than flowers kept under ambient air as the control (Philosoph-Hadas et al., 2010). It was reported that CA appeared to be more effective when used in combination with either 5 % sucrose or 5 % glucose pulsing since the pulsed stems had 0 % leaf blackening after four days of vase life, while non-pulsed stems had 50 % leaf blackening.

A disadvantage of CA is that products exposed to very low O_2 concentrations may undergo anaerobic respiration, while elevated CO_2 concentrations may also result in fermentative metabolism (Kader, 2004). In addition, changes in petal colour may occur under elevated CO_2 conditions, as caused by anthocyanin colour changes that occur in response to cellular pH changes (Staby, 1977). Stressful CA conditions are generally associated with a reduction of cytoplasmic pH and ATP levels, decreased pyruvate dehydrogenase activity and activation of pyruvate decarboxylase, alcohol dehydrogenase, and lactate dehydrogenase (Kader, 2004). These changes promote the excess accumulation of acetaldehyde, ethanol, ethyl acetate, and/or lactate, which are all detrimental to the quality of the produce subjected to CA levels that range outside the tolerance limit.

Romo-Parada et al. (1989) observed that elevated CO_2 levels might cause tissue damage related to increased amino acid leakage from the tissue, which signals membrane integrity loss. Similarly, in 1991, Romo-Parada et al. observed that high CO_2 atmospheres enhanced phospholipid and polyunsaturated fatty acid loss from the mitochondrial membranes of cauliflower florets. However, studies on roses, *Protea* cv. 'Sylvia' and other cut flowers stored in low O_2 and high CO_2 revealed similarities in quality to those stored in normal air (Macnish et al., 2009; Joyce and Reid, 1985; Stephens et al., 2003a). This discrepancy in results may be due to the variability in CA conditions and between cultivars and species (Thornton, 1930). Lack of incorporation of CA technologies in the cut flower industry has been attributed to both

the variability in product responses to CA and the high cost (Nowak and Rudnicki, 1990; Reid and Jiang, 2012). Lack of clear-cut, repeatable benefits will inhibit use of CA due to a negative cost-benefit analysis. Therefore, more research is needed before CA and MA techniques can be implemented for long-term cold storage of Cape Flora cut flowers.

6.2 Use of DCA as an advanced CA technique

Gasser et al. (2010) described the DCA storage technique as the lowering of the O_2 concentration of the storage atmosphere to levels near to, but above, the LOL. In DCA storage, the O_2 level is adjusted based on product response instead of using fixed gas concentrations as done under conventional CA storage (Veltman et al., 2003). In 2012, Wright et al. described the lower O_2 limit (LOL) as the environmental O_2 concentration at which cell metabolism changes from being primarily aerobic to anaerobic. According to Wright et al. (2010), the effectiveness of CA storage is potentially optimised through reduction of the O_2 concentration to levels close to the LOL. Since O_2 concentrations that are below the critical level may result in anaerobic respiration and ethanol production (Gasser et al., 2003), horticultural products should be kept at O_2 levels above the LOL to avoid quality loss.

Wright et al. (2012) explained that the LOL can be identified using the anaerobic compensation point (ACP), which is the O_2 level at which CO_2 production is lowest (Boersig et al., 1988). Another method involves using the respiratory quotient breakpoint (RQB), which is the point where there is a sudden rise in the ratio of CO_2 produced to the O_2 used (Gasser et al., 2010). Recently, other researchers (Burdon et al., 2008; Prange et al., 2007, 2005, 2003) followed a different approach and used DCA and flourometry to detect the LOL. Prange et al. (2003) noted that below the LOL, minimum fluorescence (*Fo*) increases and *Fv/Fm* (photochemical efficiency) decreases. Dynamic controlled atmosphere technology was initially developed for fruit and with time it has been extended to other commodities, as fruit quality loss under DCA storage is lower than under ultra-low O_2 storage (Gasser et al., 2010).

6.3 Modified atmosphere packaging (MAP) and the cut flower industry

Packaging decisions involve consideration of both the packaging container and the arrangement of the product in the container (Ekman et al., 2008; Reid, 2008). The design of the packaging container is important in restricting the depth of the flowers in the box, which are likely to reduce physical damage to the flowers. Moreover, flower heads can be arranged at both ends of the container for optimum use of packing space (Reid, 2008). Layers of paper are sometimes used to protect the flowers from injury, although the use of small pieces of paper

to protect only the flower heads appears to be a more viable option, due to the higher cooling efficiency of these flowers after packing (Reid, 2008). Some flower species require storage in an upright position to avoid quality loss from geotropic curvature (Reid, 2016), however Cape Flora cut flowers are usually dry packed horizontally in fibre-board boxes.

Mir and Beaudry (2004) described MAP as the practice of enclosing actively respiring commodities in polymeric film packaging material to alter the O_2 and CO_2 concentrations inside the packaging. A storage atmosphere that is low in O_2 and/or high in CO_2 alters the metabolism of both the product and the micro-organisms responsible for decay, and this may increase storage and shelf life. MAP systems create an optimal O_2/CO_2 atmosphere that is required to discourage processes like tissue browning and softening, chlorophyll loss and senescence (De Pascale et al., 2005). The resultant composition of the storage atmosphere within the package is influenced by the packaging material's permeability, product's respiratory characteristics, and the storage atmosphere conditions (Fonseca et al., 2002; Mir and Beaudry, 2016).

Although MAP has not been extended to Cape Flora cut flowers as a commercial practice, promising results have been obtained in studies done on other cut flowers. In 2005, De Pascale et al. used MAP boxes comprising of three layers, to evaluate the effect of MAP on *Gerbera*, *Lilium* and *Rosa* cut flowers after 10 days at 2 ^{o}C. It was reported that MAP reduced water- and quality loss. The findings by De Pascale et al. (2005) agreed with those by Patel and Singh (2009), who also observed that MAP provided better flower quality, with higher turgidity than the control flowers under normal packaging. De Pascale et al. (2005) concluded that MAP boxes reduced water loss, whilst the high CO_2 and low O_2 levels lowered metabolism, which was confirmed by the higher amounts soluble sugar in the petals of MAP-treated stems.

Grover et al. (2006) noted that use of polypropylene films during storage of Gladiolus cv. White Prosperity resulted in the least floret opening during the first 15 days of storage, lowered starch degradation during cold storage and promoted floral opening during vase life while maintaining high storage and transport quality of these cut flowers. Zeltzer et al. (2001) also reported that storage of roses for eight days at 2 °C, in a MAP box, lined with a differentially permeable polymer and tissue paper lamination, reduced moisture loss by 10 % and resulted in higher soluble carbohydrate content compared to the conventional shipping boxes.

Day (2001) advised successful passive MAP of fresh flowers relies on permeability of packaging material to gases and water vapour, which allows establishment of the ideal equilibrium modified atmosphere (EMA). A major problem in MAP use is the occurrence of

condensation on the inner surface of some packaging materials. At 10 °C and 99 % RH (inside package) condensation can easily occur when the packaging material's temperature drop by only 0.2 °C. Temperature fluctuations will therefore easily promote condensation when using MAP. However, the use of film surface treatments can cause droplet dispersion and the condensing water to form a thin, uniform film that is nearly invisible. Shirazi and Cameron (1992) also proposed the use of salts enclosed in permeable sachets as a way of reducing excess humidity inside MAP.

The O_2 and CO_2 threshold levels are dependent on cultivar, temperature and storage duration (Beaudry and Gran, 1993; Beaudry et al., 1992; Thornton, 1930), and it is vital that they are determined for every product when using MA techniques. It is important to select the ideal packaging material that allows optimal gas levels inside the MAP, since excessive CO_2, low O_2, and ethylene accumulation may cause flower damage (Halevy and Mayak, 1981). Kader (2014) warns that passive modified atmosphere is slow and most of the benefits of MA may be lost as a result of this. However, the benefits of closed- and reduced reefer ventilation, leading to higher CO_2 and lower O_2 atmospheres are worth investigating as an alternative MA technology.

Reid (2008) warned that using transportation boxes that have pre-formed cooling vents instead of sealed vents promotes fast warming when the box is kept at high temperatures, during cold chain breaks. It is therefore advised to rather use boxes with a simple sealable flap system, where the flaps are sealed following pre-cooling and the flowers are thus less exposed to external temperatures during handling at ambient temperatures. Harkema et al. (2017) also emphasised that it is essential that the holes in the cartons should be ideally sized, positioned and the number of holes should guarantee optimum airflow without promoting moisture loss.

7. Conclusion

Most post-harvest treatments mainly aim to enhance product value and delay senescence. For Cape Flora products, there is need for a sound and highly co-ordinated post-harvest system with appropriate protocols in place, in order for the exporters to deliver the quality standards set by their markets. Such a system relies heavily on the efficiency of the postharvest chain, starting at the farmer to the retailer. Post-harvest quality manipulation relies on respiration and water loss control, chemical treatments, along with ethylene and pathogen control. Ideally, temperature control and CA- or MA-related practices all aim to control respiration and delay ethylene production and senescence. Development of new storage

protocols may also lead to reduced incidences of postharvest disorders, pests and diseases, which decrease flower quality. The potential economic and physiological benefits of MA technologies are lucrative, yet currently it is not implemented on a large scale as storage conditions do not yet yield consistently acceptable results. The commercial application of CA and MA technologies during sea freight is yet to be implemented, due to lack of information on the optimum storage conditions for South African indigenous flowers in addition to a considerable cost factor. Fortunately, the increased use of sea freight has prompted investigation into the optimal ventilation conditions during cold storage and transportation of Cape Flora products. However, the use of such techniques is likely to remain low until their commercial success has been proven across a wide range of products.

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PAPER 2: SUITABILITY OF CLOSED VENTILATION AND AUTOMATIC VENTILATION FOR SEA FREIGHT OF *LEUCADENDRON* CUT FLOWER STEMS

Abstract

Technological advances in sea freight have led to the development of automatic ventilation technology. This new technology utilises information on gas dynamics, lower O_2 limit and CO_2 toxicity limits of products. Lack of such information is delaying the implementation of such technologies to optimise long-term sea freight shipping of *Leucadendron* ('Safari Sunset', 'Discolor' and 'Rosette') cut flowers. Furthermore, there are conflicting views on the suitability of sea freight for *Proteaceae* cut flowers that are shipped from South Africa (SA) to Europe. Poor product quality has been attributed to the conventional fixed, open-air exchange ventilation system (AirEx) and the interaction of the lengthy shipping period with low storage temperature of 1 °C, as typically used during shipment. The aim of the study was to test the feasibility of closed ventilation and automatic ventilation technology for sea freight shipping of *Leucadendron* cut flower- and foliage products. The experiments involved storage and evaluation of a range of *Leucadendron* products in a closed ventilation system, the determination of the lower O_2 limit (LOL) of each product and to determine the effect of storage at low O_2 and high CO_2 concentrations during storage. The LOL was determined using the HarvestWatch™ dynamic controlled atmosphere (DCA) system, which is equipped with a chlorophyll fluorescence sensor. The CO_2 toxicity tolerance was evaluated at 15 % CO_2 atmospheric concentration, which was based on gas dynamics results from preliminary closed ventilation experiments. Moisture loss, visual quality, carbohydrate content, total phenolics and lipid peroxidation were evaluated following treatment. The LOL values of the products ranged from 0.09-0.33 % O_2. The respiration rate at 5 °C ranged from 23.68-48.07 mL $CO_2.kg^{-1}.h^{-1}$ in the 2015 season and from 15.11-25.99 mL $CO_2.kg^{-1}.h^{-1}$ in the 2016 season. Q_{10} values for the 5 to 15 °C temperature range were between 1.49 and 3.84. Storage in high CO_2 resulted in damage when the CO_2 concentration was maintained at 15 % throughout the entire storage period. However, high CO_2 caused little or no symptoms of toxicity when CO_2 was steadily increased from 5 % to 15 % over a three-week period. The closed ventilation resulted in lower moisture loss, better visual quality and extended longevity compared to flowers exposed to AirEx. Products stored under low O_2 conditions and those exposed to high CO_2 storage conditions displayed comparable visual quality to that of products

exposed to AirEx ventilation. The monosaccharides and oligosaccharides were the most abundant sugars, compared to polysaccharides and starch. Phenolics were variable among products and treatments and MDA content was only variable between products.

Key words: Automatic ventilation; CO_2 toxicity tolerance; Closed ventilation shipping; Lower O_2 limit; *Proteaceae*

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1. Introduction

Next to *Protea*, *Leucospermum* and *Banksia*, the genus *Leucadendron* is internationally considered as one of the commercially more important cut flower groups within the *Proteaceae* family (Gerber and Hoffman, 2014; Leonhardt and Criley, 1999; Philosoph-Hadas et al., 2010). *Leucadendron* plants are dioecious and the pistillate (female) plants produce attractive woody conical, fruit-bearing flower heads whilst the staminate (male) plants bear a less showy, non-woody inflorescence (Rebelo and Paterson-Jones, 2001). Male *Leucadendron* plants are not usually cultivated as ornamental products, except for 'Discolor', that displays a bright red flower head when in bud, which later changes to sunshine yellow, as the pollen appears (Leonhardt and Criley, 1999). Despite the differences in colour or flower head size and shape, all *Leucadendron* plants produce good cut foliage, thus their common use as bouquet fillers (Leonhardt and Criley, 1999; Matthews, 2002). The market for SA potted *Proteaceae* ornamental products is mainly restricted to domestic markets due to lack of protocols for long-term cold storage, which are mandatory for sea freight to distant export markets (Hoffman and Du Plessis, 2013). On the contrary, the SA *Proteaceae* cut flower industry is export oriented with Europe (44 %), the United Kingdom (20 %) and Middle (26 %) to Far East (5 %) as the main markets (Cape Flora SA, 2017).

In the recent past, the ornamental industry has experienced a shift from the traditionally preferred air freight to sea freight as an alternative as the high air freight charges for cut flowers are a major factor influencing profitability especially for bulky cut flowers (Ekman et al., 2008; Philosoph-Hadas et al., 2007). Reid and Jiang (2005) also highlighted that air transport of cut flowers frequently results in quality loss that results from poor temperature control and sub-optimal humidity during freight. Additionally, there is lower greenhouse gas emission in sea freight since it produces up to 95 % less carbon per tonne of product shipped, which is an important consideration for some markets (Ekman et al 2008; Lennane, 2013). Reefers used during sea freight are now more insulated, reliable and better controlled than before, due to advances in reefer technology (Ekman et al., 2008).

Suspicions exist that the current ventilation rate of 15 000 L fresh air.h^{-1} in AirEx reefers used for *Proteaceae* transport is possibly responsible for overventilation since the gas dynamics and ventilation requirements of *Proteaceae* products are largely unknown. Furthermore, recent advances in the sea freight industry have seen the development of automatic ventilation technology, which has the potential to be the future standard ventilation technology for sea freight. The technology limits ventilation to near toxic gas levels, unlike the conventional fixed ventilation rate. Automatic ventilation also creates a form of modified atmosphere (MA) within the reefers since O_2 levels drop and CO_2 levels rise above the normal atmospheric concentrations.

The use of DCA technology relies on knowledge of the LOL, which is the O_2 level at which cell metabolism shifts from aerobic to anaerobic (Gasser et al., 2010; Wright et al., 2012). In DCA storage, the O_2 concentration applied varies based on the product response during storage. Physiological responses of the produce are monitored by measuring chlorophyll fluorescence or the respiration coefficient through sensors linked to the DCA system where O_2 is automatically adjusted when the O_2 concentration approaches near-LOL levels (Prange et al., 2003; Prange et al., 2002; Weber et al., 2015; Wright et al., 2015).

Although controlled atmosphere (CA), modified atmosphere packaging (MAP) and closed ventilation have been successful in fruit storage, the technologies are yet to be commercially incorporated into the cut flower industry (Rani and Singh, 2014). Modified atmosphere (passive and active) technologies have the benefit of lowering respiration rate, reducing oxidative stress, reducing decay, lowering susceptibility to chilling injury (CI), decreasing ethylene sensitivity and retarding tissue senescence (Halevy and Mayak, 1981; Hodges et al., 2004a; Kader, 2004; Meir et al., 1995; Nowak and Rudnicki, 1990; Philosoph-Hadas et al., 2007; Wang, 1990). Goliáš and Kobza (2002) reported that storage of carnations at low levels of 0.6–0.8 % O_2 was physiologically harmless to the flowers. However, they noted that the flowers stored in low O_2 atmosphere had slower acetaldehyde and ethanol oxidation rates compared to those in regular atmosphere (RA). Philosoph-Hadas et al. (2010) also noted that high CO_2 (5% CO_2) storage, combined with sucrose or glucose pulsing, enabled prolonged sea freight of *Leucadendron* 'Safari Sunset' and reduced leaf blackening during vase life, compared to RA.

Despite this, Hodges and Toivonen (2008) warned that MAP practices for fresh-cut fruits and vegetables frequently result in anaerobic conditions and/or high CO_2 levels, which compromise product quality through the generation of ethanol, acetaldehyde, off-flavours and odours. Although several benefits of MA have been reported, there are risks as well. The use

of techniques like MAP is largely dependent on the accuracy with which respiration rate (Kader, 2002) and LOL and CO_2 toxicity limit during storage can be predicted. According to Voisine et al. (1993) and Hodges et al. (2004b) high CO_2 stress may lead to oxidative stress, loss of antioxidant activity and membrane damage. Oxidative stress occurs when reactive oxygen species (ROS) production exceeds the capability of the cell to maintain redox homeostasis, for example under abiotic stress. Excessive ROS promotes membrane lipid peroxidation and destroys membrane structure, proteins, and nucleic acids, causes solute leakage and generates toxic aldehydes and ketones (Wilhelmová et al., 2006; Xie et al., 2008; Wu et al., 2012; Rogers, 2012).

Lipid peroxidation results from either enzymatic oxidation of unsaturated fatty acids by lipoxygenase (LOX) or by non-enzymatic oxidation due to ROS build up (Apel and Hirt, 2004). The extended periods of cold storage associated with sea freight may also lead to chilling-induced lipid peroxidation in membranes (Goliáš and Kobza, 2002), especially when coupled with low O_2 and/or high CO_2 stress. Goliáš and Kobza (2002) observed that carnations stored in ultra-low O_2 for 19 days showed signs of injury after three days of vase life evaluation, whereas those stored in RA had no signs of damage. Furthermore, lipid peroxidation is linked to low O_2 injury, which is commonly expressed after the exposure of the commodity to ambient air (Blokhina et al., 1999). In turn, loss of membrane and organelle integrity can promote ROS production through uncontrolled electron transfer reactions and production of polyunsaturated fatty acids (Purvis, 1997; Rogers, 2012; Hodges et al., 2004b).

Phenolics are a large group of aromatic, flavonoid compounds which are known for their antioxidant properties (Hodges et al., 2004b; Lutz et al., 2015; Taiz et al., 2015). High phenolic content in plant tissue is associated with a greater ability to withstand stress conditions as it assists in preventing excessive ROS accumulation (Singh et al., 2009) as may typically occur under conditions where CI will manifest (Christie et al., 1994). Several researchers (Henríquez et al., 2010; Lutz et al., 2011; Baiano, 2014) indicated that the composition of phenolics in fresh produce varies greatly and is determined by genotype, tissue type, growing conditions, production practices, postharvest processing and storage. Leaves of *Proteaceae* plants contain large amounts of phenolic compounds, unstable O-glycoside esters and aglycones (Perold et al., 1973; Philosoph-Hadas et al., 2010). It is therefore likely that a high phenolic content may be beneficial during sea freight. On the other hand, polyphenol oxidase or peroxidase can convert phenols to polyphenols associated with several disorders in fruit and vegetables (Hodges et al. 2004a).

To minimise quality loss, there is need to develop storage protocols for low O_2 and/or high CO_2 atmospheres. Currently, the information on lower O_2 limits and CO_2 toxicity tolerance limits of any *Leucadendron* cut flower products is unknown. Considering the automated and low energy cost direction in which sea freight shipping is moving, lack of this information is a major disadvantage and barrier to adopting this technology within the South African Cape Flora industry. The aim of this study was to obtain a better understanding of the physiological parameters that will influence the storability and vase life of *Leucadendron* as an ornamental product. The objectives were thus to first measure respiration rate at 5 and 15 °C and monitor the gas dynamics in a closed ventilation system, in order to evaluate the feasibility of closed ventilation long-term cold storage technology for *Leucadendron* products. The other objective was to determine the lower O_2 limits as well as the CO_2 toxicity tolerance for a range of commercially important *Leucadendron* products.

2. Materials and methods

2.1 Product variety and source

Various export grade *Leucadendron* cut flower products were sourced from Floralae, Paarl (33°43'06.1"S 19°04'05.5"E), Tussenberge, Napier (34°29'10.3"S 19°44'10.7"E), Berghoff, Porterville (32°57'15.1"S 19°03'14.2"E) and Fynbloem, Riviersonderend (34°08'30.3"S 19°49'11.7"E) in the Western Cape, SA. The choice of products assessed was dependent on product availability (Table 1) and its ranking as pertaining to its significance to the export market (Fig. 1). The products that were evaluated included; 'Safari Sunset' (*L. salignum x L. laureolum*), 'Discolor' (*L. discolor*), 'Rosette' (*L. laureolum x L. elimense*) and late season 'Rosette' referred to as 'Rosette Conebush' in this study. The flowers were harvested mid-season and shipped to the Cape Town International airport where they were held at 4 ^{o}C and collected within 24 h of harvesting. The experiments were conducted using ARC Infruitec-Nietvoorbij Stellenbosch cold rooms and Stellenbosch University Horticultural Science Department cold room and laboratory facilities.

2.2 Respiration rate

At arrival at the Department of Horticultural Science of Stellenbosch University, stems were held for 24 h in a cold room at 5 and 15 ^{o}C (± 0.5 ^{o}C). Five replicates were used per temperature per product and each replicate consisted of 10 stems. The respiration analysis method by Jooste (2012) and Smit (2013) was used to measure CO_2 gas concentration in $mL.g^{-1}.h^{-1}$ by means of gas chromatography (GC). For analysis, 10 mL of headspace gas was drawn

from gas tight 4.75 L containers after 90 min of gas exchange. The composition of the gas sample was analysed on a gas chromatograph (6890N, Agilent Technologies, California, USA) fitted with a thermal conductivity detector (TCD), a Porapak Q section (Porapaq Q/Heysep Q, 80-100 mesh, Hayes Separations) and a Mole Sieve 5 A section (80-1000mesh, Alltech Associates) column. Helium (He) was used as a carrier gas and a 1 mL sample was manually injected for analysis, with the oven temperature maintained at 80°C. Respiration rates and temperature coefficients (Q_{10}) of the cut flowers were calculated using the formulas below;

$$\text{Respiration rate (mL.kg}^{-1}\text{.h}^{-1}\text{)} = \left(\frac{\%\ CO_2\ \text{produced}}{100}\right)X\left(\frac{\text{Volume free air (mL)}}{\text{Product weight (kg)}}\right)X\left(\frac{1}{\text{Time (h)}}\right)$$

$$Q_{10} = \left(\frac{\text{Respiration rate T2}}{\text{Respiration rate T1}}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

Where T_1 is 5 °C and T_2 is 15 °C.

2.3 Closed ventilation system gas dynamics

Gas dynamics of a closed ventilation system was simulated in a Janny MT module™ that consists of a rigid plastic box with a 610 L capacity and Tiempo cap lid cover and that was kept in a cold room set at 1 °C (±0.5 °C). In both the 2015 and 2016 seasons, after the flower boxes with their product were placed in the Janny MT box, approximately 45-55 % free air remained inside. In the 2015 season, the CO_2 and O_2 composition of the Janny MT boxes was monitored using a HarvestWatch™ DCA system (AAFC and SAtlantic Inc., Nova Scotia, Canada). For the 2016 season, a hand held gas analyser (ICA, 250, Dual Analyser, Kent, U.K.) was used to allow more accurate simulation of the commercial shipping conditions as this method allowed for less air removal and atmosphere alteration within the system. O_2 and CO_2 concentration was measured on day 0, 4, 8, 12, 16 and 21 of long-term storage. The Janny MT boxes contained mixed loads of *Proteaceae*, which is a common practice under commercial shipping conditions.

In the 2015 season gas dynamics were monitored in an unventilated Janny MT box which contained 35.52 kg of 'Safari Sunset' and another which contained 43.76 kg of mixed *Proteaceae* that included *Leucadendron* (5.6 kg 'Rosette Conebush'), *Leucospermum* (2.15 kg 'Jelena', 3.21 kg 'Goldie', 2.29 kg 'Bright Eyes' and 2.56 kg 'Fire Fly') and *Protea* (9.33 kg 'Barbigera', 7.36 kg 'Grandiceps' and 11.26 kg 'Red Rex'). For the 2016 season, a hand held gas analyser was used to manually monitor the gas dynamics within the closed ventilation system, which contained 63.33 kg of *Leucadendron* products (15.05 kg 'Discolor', 15.78 kg

'Safari Sunset' and 32.5 kg 'Rosette'). A similar manually sampled closed ventilation setup with a total mixed load of 43.31 kg consisted of *Leucadendron* (9.63 kg 'Rosette Conebush') and *Leucospermum* (9.90 kg 'Soleil', 6.43 kg 'Succession', 12.27 kg 'Veld Fire' and 5.08 kg 'Rigoletto'). Products in AirEx were used as the control, where a consistent ventilation rate of 15000 L fresh air.h^{-1} was maintained inside the cold room throughout the storage period and the control products were loosely covered with plastics to reduce airflow and simulate the conditions in a fully loaded reefer.

2.4 Determination of lower O_2 limit

A HarvestWatch™ DCA monitoring system equipped with a fluorescence interactive response monitor (FIRM) sensor was used to determine the LOL of each product according to the method by Prange et al. (2003), but with adjustments. The LOL was determined by lowering the O_2 concentration in a sealed Janny MT box over a 48 h period until a clear stress peak was observed on a chlorophyll fluorescence spectrum. Temperature was set at 1 °C (±0.5 °C) during 21 d of storage. Products in low O_2 were stored at 2 % O_2 during the 2015 season. Storage at increments above the LOL (LOL+0.4 % O_2) was added as DCA storage treatment in the 2016 season.

2.5 Determination of high CO_2 tolerance

The HarvestWatch™ DCA system was used to control the CO_2 concentration in a sealed Janny MT box. In the 2015 season, products under high CO_2 treatment were stored in 5 % CO_2 for 1 week, where after it was ramped to 10 % CO_2 for the following week and finally elevated to 15 % CO_2 the 3rd week of storage (5-10-15 % CO_2). Storage in 15 % CO_2 for 21 d (15 % CO_2) was included as an additional treatment in the 2016 season and the O_2 concentration was maintained at 12 % in both seasons. Temperature was maintained at set at 1 °C (±0.5 °C) during storage.

2.6 Mass loss

A scale (EEW-5000, Everyweigher, UWE, Cape Town, South Africa), accurate to ±0.1 g, was used to measure mass difference as an estimation of moisture loss prior to and immediately following storage. Ten replicate bunches consisting of 10 stems per treatment per product were used for moisture less determinations. All the products were stored for 21 d at 1 °C, both in the low O_2 and high CO_2 trials.

2.7 Colour determination of flowering head and leaves

The colour of leaves and flower heads was determined using a Chroma meter (CR400, Konica Minolta, Tokyo, Japan). The Chroma meter was calibrated using a standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203) prior to colour measurement and readings were taken in the CIE lightness (L*), chroma (C*), hue angle (h°) colour space. Red, yellow, green and blue colour have hue angles of 0 °, 90 °, 180 ° and 270 ° respectively (X-rite, 2007). Colour was measured before storage and immediately after storage, on 10 replicates per treatment. Hue angle was recorded on three outer positions of the flower heads, and again on the upper side of five involucral- and five mature foliage leaves on each shoot.

2.8 Visual quality evaluation

Visual quality was evaluated in tap water at room temperature (20 °C ±2 °C) for 14 d after storage, with 10 replicates consisting of 10 stems each per treatment. A visual quality score was recorded on day 0, 1, 3, 5, 7, 10 and 14 of vase life. The visual quality scale ranged from 0 to 4 and it was interpreted as follows: 4 - Export grade quality, 3 - Good quality, 2 - Low quality with significant damage, 1 - Poor quality with severe damage and 0 – Product to be discarded and considered totally unacceptable.

2.9 Soluble carbohydrates

Leaves were detached from 10 replicate stems upon termination of a 21 d cold storage period, frozen in liquid N_2 and stored at -80 °C until they were freeze dried and milled. Sugars in the milled leaves were extracted and analysed according to the phenol-sulphuric acid assay of Rohwer (2013). Soluble monomers and oligosaccharide sugars were extracted from 0.1 g of powdered samples using three successive washes in 80 % ethanol. The remaining pellet was washed three times (24 h per wash) in de-ionised water to facilitate the extraction of the polysaccharides. Finally, the pellet was heated in sodium acetate and amyloglucosidase (AMG) buffer solution (pH=4.8) for 24 h to extract and hydrolyse starch to glucose monomers. The temperature was maintained at 80 °C using digital heating blocks and extraction analyses were done in triplicate. Carbohydrate content in 10 µL of extract was determined spectrophotometrically (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia) at 480 nm using a standard curve derived from a serial dilution of a glucose standard (0.1 $mg.mL^{-1}$) and it was expressed as glucose equivalents.

2.10 Lipid peroxidation

Lipid peroxidation was quantified according to Hodges et al. (1999), with slight modifications. Briefly, 1.0 g fresh weight (FW) milled leaf samples from 10 replicates were homogenized in 20 mL 80 % ethanol for each storage treatment. The probe was rinsed in 5 mL of 80 % ethanol, which was pooled with the initial extraction solution. The solution was centrifuged for 10 min at 3000 g at 4 °C. A 1 mL sample from the supernatant was mixed with 1 mL thiobarbituric acid (TBA) solution and 10 µl of 1 % butylated hydroxytoluene (BHT) solution. The solution was vortexed and heated for 25 min at 95 °C. The heated solution was cooled on ice and centrifuged at 3000 g for 10 min. Absorbance was read at 532 nm and 600 nm using a UV–Visible Spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia). The blank contained 1 mL 80 % ethanol, 10 µl BHT and 1 mL TBA. The (malondialdehyde) MDA content was calculated using the formula below:

MDA equivalents ($nmol.mL^{-1}.g^{-1}$ FW) =

$$\left(\frac{\text{Volume of extraction solution (mL)}}{\text{Volume of supernatant (mL)}}\right) \text{x} \left(\frac{1}{\text{Sample mass (g)}}\right) \text{x} \left(\frac{\text{Abs 532 nm} - \text{Abs 600 nm}}{157\,000}\right) \text{x } 10^6$$

2.11 Total phenolic content

Leaves were detached from 10 replicate stems immediately after the cold storage period, frozen in liquid nitrogen and stored at -80 °C until they were freeze dried and milled. The phenolic compounds were extracted and analysed according to the method of Rohwer (2016). All the extractions and analyses were done in triplicate. Milled leaf samples of 0.1 g dry weight (DW) were washed in 80 % ethanol for 20 min at 80 °C. For each wash, 5 mL of ethanol was used and the extracts were pooled to produce a 15 mL of extract. A 50 µL sample from the pooled extract was mixed with 450 µL of folin-ciocalteu (FC) reagent and vortexed. After 5 min, 500 µL of 5.6 % Na_2CO_3 was added to the solution and vortexed, and absorbance was measured on the spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia) at 750 nm after 90 min, with gallic acid (GA) used as a standard. The total phenolics in the leaves were expressed as gallic acid equivalents (GAE).

3. Statistical analyses

A complete randomized design was used and analysis of variance (ANOVA) and repeated analysis of variance (RANOVA) was performed on the data using Statistica (Version 13.2, Statsoft Inc., USA). Means were separated by LSD test ($P \leq 0.05$).

4. Results

4.1 Closed ventilation system gas dynamics

4.1.1 Gas dynamics in closed ventilation and respiration rate

The 2015 season gas dynamics for the HarvestWatch™ monitored closed ventilation system with 35.515 kg of 'Safari Sunset' revealed that the O_2 level dropped from 20.43 % to 8.36 % and the CO_2 concentration rose from 0.05 % to 10.36 % (Fig. 2A). Another trial was conducted with a 43.76 kg mixed load of *Leucadendron, Leucospermum* and *Protea* products. The O_2 level dropped from 20.92 % to 8.42 % and the CO_2 rose from 0.06 % to 10.54 % (Fig. 2B). According to 2016 season results, in a manually (hand held gas analyser) monitored closed ventilation system (Fig. 3A), the atmospheric O_2 concentration dropped from 20.8 % to 0.4 % and CO_2 concentration rose from 0.4 % to 22.4 %. The closed ventilation system contained 63.33 kg of *Leucadendron* products.

In a similar manually sampled closed ventilation system with a 43.31 kg mixed load of *Leucadendron* and *Leucospermum* products (Fig. 3B) the initial O_2 concentration was 20.5 % and CO_2 concentration was 0.4 %. The gas concentrations at the end of storage were 1.8 % and 17.4 % for O_2 and CO_2, respectively. The respiration rate at 5 °C ranged from 23.68-48.07 mL $CO_2.kg^{-1}.h^{-1}$ in the 2015 season and between 15.11-25.99 mL $CO_2.kg^{-1}.h^{-1}$ in the 2016 season. In the 2015 season, the Q_{10} values of the products ranged from 1.49 for 'Safari Sunset' to 3.38 for 'Rosette Conebush' between 5 °C and 15 °C (Table 1). In the 2016 season, the Q_{10} values ranged from 2.34 for 'Rosette Conebush' to 3.84 for 'Safari Sunset'. The respiration rates in the 2015 season showed that the products ranged from high to very high respiration at 5 °C. However, in the 2016 season the respiration rates were generally moderate to high at the same temperature.

4.1.2 Mass loss

A non-significant ($p=0.484$) interaction between the product and treatment in the 2015 season was obtained (Table 2). Treatment effect on mass loss was significant ($p<0.0001$) as

product stored in AirEx had higher mass loss than the product stored in closed ventilation. Product effect on mass loss was also significant as mass loss of 'Rosette Conebush' stems was higher than that of 'Safari Sunset'. In the 2016 season, the product and treatment interaction was again insignificant (p=0.420). In this instance, treatment also had a significant influence on mass loss where product stored in closed ventilation had a lower mean mass loss as compared to product in AirEx. Mass loss between the various *Leucadendron* products did not differ significantly from each other.

4.1.3 Colour determination of flowering head and mature leaves

The storage and treatment interaction (p=0.148), and also the treatment (p=0.420) or storage (p=0.314) had an insignificant effect on flowering head hue values in the 2015 season. Similarly, the storage and treatment interaction (p=0.157) as well as the treatment effect (p=0.177) again insignificantly affected hue angle values of mature leaves in the same season (Table 3). Interaction between product, treatment, leaf type and storage had an insignificant (p=0.163) effect on leaf hue angle in the 2016 season (Table 4). However, interaction between storage, product and leaf type was significant (p=0.004). Before and after storage hue readings for only 'Safari Sunset' involucral and mature foliage leaves showed significant differences. Mean hue angle of mature leaves was higher after storage (118.3 ° before storage and 110.5 ° after storage), which shows a change from red to yellow, while that of involucral leaves was lower after storage (74.0 ° before storage and 67.8 ° after storage), indicating an increase in red colour. Exposure to either AirEx or closed ventilation as storage treatment did not affect the hue value of either the involucral bracts or the mature foliage as indicators of product quality in any of the seasons evaluated.

4.1.4 Visual quality: Flowering head

There was no significant (p=0.187) interaction between Time, product and treatment on the visual quality in the 2015 season, but a significant interaction between Time and product was evident (Fig. 4). Day 1 results showed insignificant quality differences between 'Rosette Conebush' and 'Safari Sunset' product, but by day 7, 'Rosette Conebush' displayed better quality than 'Safari Sunset', even though both products were above the lowest acceptable retail quality. For the 2016 season, interaction between Time, product and treatment on visual quality was insignificant (p=0.315), whereas the Time and product interaction was significant (Fig. 5). The mean visual quality indices for 'Safari Sunset', 'Rosette' and 'Rosette Conebush' were not significantly different on day 1 of vase life evaluation, but all differed significantly from that

for ‘Discolor’. By day 7, ‘Rosette Conebush’ had the highest visual quality score, whilst ‘Discolor’ continued the trend with displaying the lowest quality. Yet, quality scores for all the products remained above the lowest acceptable retail quality on day 7. Storage in either AirEx or under closed ventilation conditions did not affect the visual quality of the flowering head or cone, for any of the products evaluated, irrespective of the season when the evaluation was conducted.

4.1.5 Visual quality: Mature foliage leaves

The interaction between Time, product and treatment was non-significant ($p=0.879$) on visual quality of mature foliage leaves, but the interaction between Time and product ($p=0.016$) was significant in the 2015 season (Fig. 6). No significant differences in visual quality of mature leaves of ‘Rosette Conebush’ and ‘Safari Sunset’ could be detected on day 1. However, differences in visual quality were significant on day 7. Yet, the quality of the products remained above the minimum acceptable standard, although ‘Safari Sunset’ had a slightly higher quality score than that of ‘Rosette Conebush’. According to the 2016 season data, interaction between Time, product and treatment was non-significant ($p=0.539$) on visual quality, where a significant ($p<0.0001$) product and Time interaction was recorded (Fig. 7). Non-significant differences in visual quality were recorded for all products on day 1 and day 7 and the visual quality score remained near export grade level for all the products, except for ‘Discolor’ that displayed lower quality from day 7 onwards. However, storage treatment did not affect the mature foliage quality significantly, in either of the seasons evaluated.

4.1.6 Soluble carbohydrates

Monosaccharide plus oligosaccharide content of ‘Rosette Conebush’ was not significantly ($p=0.733$) affected by storage either in AirEx or under closed ventilation in the 2015 season (Table 5). For the 2016 season, the interaction between product, carbohydrate type and treatment was non-significant ($p=0.106$). However, a significant interaction between carbohydrate type and treatment was obtained ($p<0.0001$). Products stored in AirEx had higher monosaccharide plus oligosaccharide content than those in closed ventilation. Polysaccharide content was higher in products kept in closed ventilation as compared to products stored in AirEx. Differences in starch content between the storage treatments were non-significant. Furthermore, the interaction between product and carbohydrate type had a significant effect ($p<0.0001$) on carbohydrate content in the 2016 season. ‘Rosette’ had the highest concentration of both monosaccharides plus oligosaccharides, with ‘Discolor’ displaying the lowest

monosaccharide plus oligosaccharide content. Starch content significantly differed among the products and it was highest in 'Rosette', followed by 'Discolor' and then 'Safari Sunset', which reported the lowest value.

4.2 High CO_2 atmosphere storage

4.2.1 Mass loss

In the 2015 season treatment significantly affected mass loss ($p<0.0001$) as mass loss was higher in product stored in AirEx as compared to product subjected to the 5-10-15 % CO_2 regime (Table 6). In the 2016 season when a wider range of products was evaluated, a significant interaction between product and treatment was recorded. For this trial, results revealed storage of 'Safari Sunset' in 15 % CO_2 to result in higher mass loss than AirEx and 5-10-15 % CO_2. Storage of 'Discolor' in AirEx treatment led to greater moisture loss as compared to 5-10-15 % CO_2 and 15 % CO_2, whilst 'Rosette' and 'Rosette Conebush' showed insignificant differences in mass loss among the treatments.

4.2.2 Colour determination of flowering head and mature foliage leaves

A significant interaction ($p=0.049$) for hue angle between storage and treatment with respect to the flower head was obtained in the 2016 season (Table 7). 'Rosette Conebush' products stored in 5-10-15 % CO_2 and 15 % CO_2 product had non-significant cone hue differences when comparing hue angle values before and after storage (Table 7). Contrary to this, storage under AirEx resulted in a significantly lower flower head hue angle reading after storage (48.4 °**)** as compared to prior storage values (67.2 °). This shows an increase in red colour, which is typical of the 'Rosette Conebush' flowering heads. No significant interaction was noted between storage and treatment ($p=0.520$) affecting the hue angle of mature leaves in the 2016 season. Similarly, the various CO_2 atmosphere to which products were exposed during storage also did not have a significant effect ($p=0.429$) on hue angle of mature leaves (Table 7).

When the foliage quality was considered, no significant ($p=0.983$) interaction between storage, treatment and leaf type on leaf hue angle was obtained in the 2015 season. Leaf hue angle was, however, significantly affected ($p=0.012$) by the interaction between treatment and leaf type. The hue angle of mature leaves of product treated in AirEx and 5-10-15 % CO_2 was not affected, with significant differences only noted on involucral leaves (Table 8). Storage significantly affected ($p<0.0001$) the hue angle in the 2015 season, as the hue was lowered by long-term cold storage (115.6 ° before storage and 115.0 ° after storage).

In the 2016 season, leaf hue angle was significantly (p=0.048) affected by the interaction between product, treatment, leaf type and storage. Involucral and mature leaves of 'Discolor' and 'Rosette' had non-significant differences between before and after storage hue angle readings. The involucral leaves of 'Safari Sunset' stored in AirEx and 15 % CO_2 had significantly higher hue angles after storage as compared to before storage, which shows a change from red to yellow colour. Contrary to this, involucral leaves of 'Safari Sunset' stored in 5-10-15 % CO_2 had lower after storage mean hue angle as compared to before storage, which indicates an increase in red colour (Table 9). Before and after storage hue angles of mature leaves of 'Safari Sunset' were not affected by any storage treatment.

4.2.3 Visual quality: Flowering head

No interaction between Time and treatment (p=0.931) on visual quality of flower heads of 'Rosette' emerged in the 2015 season (Fig. 8). Time had significant effect (p<0.0001), but the CO_2 treatments did not significantly affect the visual quality (p=0.331). The quality score remained above the minimum acceptable level throughout the evaluation period.

In the 2016 season, significant interaction between Time, product and treatment (p<0.0001) was noted with respect to the visual quality of the flower head. Quality ratings for flower heads of 'Safari Sunset' stored in AirEx and 5-10-15 % CO_2 were similar throughout the evaluation period. However, 'Safari Sunset' stems stored in 15 % CO_2 had the lowest flower quality and the quality score was below the minimum acceptable level on day 7 (Fig. 9A). Quality grading of 'Discolor' flower heads on day 1 of vase life showed that product kept in AirEx had a significantly higher quality score than product kept either in 5-10-15 % CO_2 or 15 % CO_2 (Fig. 9B). On day 7, all treatments had quality scores below the minimum acceptable quality, with differences among treatments being non-significant. Quality of 'Rosette' did not differ significantly among the treatments on the first day of vase life (Fig. 9C). However, by Day 7 of evaluation, 'Rosette' stored in AirEx and 5-10-15 % CO_2 was still not significant, whereas product stored in 15 % CO_2 had significantly lower quality. Quality of 'Rosette Conebush' flower heads did not differ significantly among the storage treatments and remained similar throughout the evaluation period (Fig. 9D).

4.2.4 Visual quality: Mature foliage leaves

No interaction between Time and treatment (p=0.931) was reported for the visual quality of mature leaves during the 2015 season. Data analysis of mature leaves of 'Rosette'

showed that quality indices for the treatments were not significantly different over the 14 d evaluation period (Fig.10).

In the 2016 season a significant interaction between Time, product and treatment ($p<0.0001$) affected quality. Quality of stems of 'Safari Sunset' (Fig. 11A) and 'Discolor' (Fig. 11B) treated in AirEx and 5-10-15 % CO_2 was significantly higher than that of products stored in 15 % CO_2 on the first day of vase life evaluation up to day 7 of evaluation. Both 'Safari Sunset' and 'Discolor' stems stored in 15 % CO_2 had mean quality scores lower below 2.5 on day 7 of evaluation. For 'Rosette', quality differences between the treatments were insignificant on the first day of 'Rosette' vase life evaluation (Fig. 11C). Differences in visual quality were only noted mid-way through the vase life evaluation period, where product stored in 15 % CO_2 presented with a slightly lower quality, yet quality means for all treatments remained above the lowest acceptable retail quality throughout the evaluation period. 'Rosette Conebush' evaluations revealed no differences among treatments, with all products, irrespective of treatment, able to maintain a high quality throughout the evaluation period (Fig. 11D).

4.2.5 Soluble carbohydrates

Carbohydrate content analysis of 'Rosette' stems for the 2015 season showed that there was significant ($p<0.0001$) treatment effect (Table 10). Monosaccharides plus oligosaccharides were more abundant in product stored in 5-10-15 % CO_2 as compared to product in AirEx. In the 2016 season, interaction between product, carbohydrate type and treatment was non-significant ($p=0.576$). However, product and carbohydrate type interaction had a significant effect ($p<0.0001$) on carbohydrate content. Monosaccharide plus oligosaccharide content was highest in 'Rosette' stems, whilst having the lowest values in 'Discolor' and 'Safari Sunset' stems. Polysaccharide content presented with non-significant differences in all products analysed. Starch content was highest in 'Rosette' and lowest in 'Rosette Conebush'. Carbohydrate content was also influenced ($p<0.0001$) by treatment in the 2016 season. Total carbohydrate content was significantly higher for product stored in 15 % CO_2, whereas products stored in AirEx and 5-10-15 % CO_2 did not differ significantly in total carbohydrate content.

4.2.6 Lipid peroxidation

Treatment was not significant ($p=0.272$) in the 2015 season (Table 11) for 'Rosette' stems. For the 2016 season, product and treatment interaction ($p=0.949$) and treatment

(p=0.064) were not significant, whereas the product effect proved significant (p<0.0001). 'Rosette Conebush' had the highest amount of MDA, whilst 'Discolor' had significantly lower values compared to the all the other product types, with non-significant differences in MDA values between 'Rosette' and 'Safari Sunset'.

4.3 Low O_2 atmosphere storage

4.3.1 Mass loss

A significant difference (p<0.0001) for 'Rosette Conebush' was reported in the 2015 season as mass loss was higher when stored in 2 % O_2 compared to AirEx (Table 12). In 2016, a product and treatment interaction significantly (p=0.028) affected mass loss. Generally, no significant mass loss difference was noted within a product type stored in AirEx, DCA and 2 % O_2. A trend however was noted, where DCA storage generally resulted in lower mass loss than storage in AirEx and in 2 % O_2.

4.3.2 Colour determination of flowering head and leaves

In the 2015 season, storage and treatment had a significant (p=0.016) interaction on flower head hue values (Table 13). Flower heads of 'Rosette Conebush' stems stored in AirEx had significantly lower after storage mean hue angle (27.6 °**)** as compared to before storage (56.6 °**)**. This shows an increase in typical red colour of the 'Rosette Conebush' flower heads. However, there were non-significant differences in hue angle of flower heads of product stored in 2 % O_2 in the 2015 season. Storage and treatment interaction (p=0.567), storage effect (p=0.710) and treatment effect (p=0.679) did not have a significant effect on hue angle of flower heads in the 2016 season. Thus, differences in before and after storage hue angle means for products stored in AirEx, DCA and 2 % O_2 systems were non-significant. Results for leaf hue angle changes revealed that the interaction between storage and treatment in the 2015 and 2016 seasons was not significant (p=0.054 and p=0.151). Similarly, treatment and storage individually also did not have a significant effect on hue angle of mature leaves of 'Rosette Conebush' in both the seasons (Table 14).

In the 2016 season, no interaction between storage, product, treatment and leaf type was detected (p=0.716), however, the interaction between storage, product and leaf type proved significant (0.042). Although the hue angle of mature leaves of 'Safari Sunset' did not differ significantly with storage, the post storage hue of involucral leaves was significantly higher (86.3 °**)** than the pre-storage hue (83.2 °), which shows an increase in leaf redness. The hue

angle of mature and involucral leaves of both 'Discolor' and 'Rosette Conebush' products, unlike that of 'Safari Sunset', consistently did not differ during storage.

4.3.3 Visual quality: Flower head

In the 2015 season, a significant interaction ($p<0.0001$) between Time and treatment emerged for the quality of flower heads of 'Rosette Conebush' (Fig. 12). Mean visual quality indices for product stored in AirEx were considerably higher throughout the evaluation period as compared to indices for product stored in 2 % O_2. The quality of product stored in 2 % O_2 was below the minimum level for retail by day 7. Interaction between Time, product and treatment had significant ($p<0.0001$) effect on quality in the 2016 season. On day 1 and day 7, 'Safari Sunset' had no significant differences among the mean quality indices of product kept in AirEx, DCA and 2 % O_2 (Fig. 13A).

Results for 'Rosette' indicated no significant differences between the treatments on day 1 of vase life (Fig. 13B). However, by day 7 of vase life, 'Rosette' stored in AirEx had superior quality followed by product stored in 2 % O_2 and DCA, in that order. By comparison, 'Discolor' already had significant quality differences among the treatments by day 1 of vase life (Fig. 13C). Visual quality means for product stored in DCA and 2 % O_2 were not significantly different, however they both differed significantly from the quality score of product kept in AirEx, which was below the minimum acceptable quality. Quality scores for all the treatments were below the 2.5 level benchmark on day 7. Visual quality of 'Rosette Conebush' product was comparable among all treatments, throughout vase life (Fig. 13D).

4.3.4 Visual quality: Mature leaves

Effect of interaction between Time and treatment had significant ($p<0.0001$) influence on quality in the 2015 season for 'Rosette Conebush' (Fig. 14). Visual quality indices on the first day of vase life showed that there was no significant difference between AirEx and 2 % O_2 treatments. However, day 7 results indicate that storage of 'Rosette Conebush' in 2 % O_2 resulted in significantly lower quality than storage in AirEx.

Time and treatment interaction had a significant ($p<0.0001$) influence on quality in the 2016 season. 'Safari Sunset', 'Discolor', 'Rosette' and 'Rosette Conebush' products did not show any significant differences among the treatments. All products were able to retain export grade quality from day 1 up to day 7 of vase life evaluation (Figs. 15A, B, C and D), with only the quality of 'Discolor', which was observed to significantly drop after day 10 of vase life.

4.3.5 Soluble carbohydrates

Treatment did not have a significant (p=0.713) influence on carbohydrate content in the 2015 season (Table 15). Monosaccharides plus oligosaccharides contents of 'Rosette Conebush' stored in AirEx and 2 % O_2 differed non-significantly from each other. In the 2016 season, product, carbohydrate type and treatment interaction was non-significant (p=0.109) for extractable carbohydrate in the leaves. However, a significant interaction between product and treatment (p=0.012) on total carbohydrate content was reported. Total carbohydrate content did not vary significantly among the storage treatments in 'Discolor', 'Rosette' and 'Safari Sunset' products. However, storage of 'Rosette Conebush' in DCA resulted in higher total carbohydrate content as compared to the other treatments. Product and carbohydrate type interaction also had significant effect (p<0.0001) on carbohydrate content in the 2016 season. Monosaccharide plus oligosaccharide content differed significantly among the products, being the highest in 'Rosette' followed by 'Rosette Conebush', 'Discolor' and 'Safari Sunset', in that order. Polysaccharide and starch content did not differ significantly among products.

4.3.6 Total phenolic content

Total phenolic content of 'Rosette Conebush' was significantly (p=0.004) affected by the storage regime in the 2015 season (Table 16). Phenolic content of 'Rosette Conebush' stored in AirEx was higher than that of product kept in 2 % O_2. In the 2016 season, a significant product and treatment interaction was observed (p=0.036). Differences in total phenolics were minor between AirEx, DCA and 2 % O_2 storage treatments in 'Discolor', 'Safari Sunset' and 'Rosette Conebush'. However, treatment means for 'Rosette' varied significantly from each other for phenolics, which were more abundant in stems stored in 2 % O_2, compared to those in AirEx and in DCA.

5. Discussion

5.1 Gas dynamics and respiration rate

During the study, we observed that the HarvestWatch™ system is more suitable for gas composition monitoring of CA rooms than for the smaller Janny MT box system as the volume of gas required for sampled during monitoring of the Janny MT boxes under HarvestWatch™ system resulted in the a low pressure being generated inside the bins. This promoted the influx of fresh atmospheric air into the Janny MT boxes, which altered the composition of the experimental storage atmosphere. The passive MA created inside the closed ventilation system,

as simulated in the Janny MT box, created significant modifications to the gas composition, to levels, which are known to lower the respiration rate, as well as reduce moisture loss due to high humidity of the air surrounding the products (Kader, 2014; Taiz and Zeiger, 2016). However, this method of atmosphere modification is slow and most of the benefits of MA may be lost (Kader, 2014).

In all the experiments, there were no visible signs of low O_2 and/or high CO_2 damage that resulted from the closed ventilation atmosphere, despite the very high CO_2 levels in the Janny MT boxes monitored manually. In this study, in both trials where the Janny MT bins were monitored by means of a hand held gas analyser, both had higher concentrations of CO_2 and lower concentrations of O_2 than those monitored by the HarvestWatch™ DCA system. This could probably be ascribed to a higher volume of gas that was removed during sampling by the HarvestWatch™ system, which created a low pressure system inside the boxes, and thus, resulted in suction of air into the bins, which altered the gas concentration of the system.

The Q_{10} can be used to estimate the respiration rate at 1 °C, however, this method of estimating respiration rate is unreliable since the respiration rate of products in a closed ventilation or MAP system do not follow a linear increase or decrease in response to temperature. Thus, the Q_{10} derived from respiration rates that were measured at 5 and 15 ^{o}C within 48 h of harvesting will vary from the respiration rate at 1 ^{o}C during the three weeks of shipment and after storage. Respiration rate, temperature coefficient and respiratory coefficient are known to vary greatly with temperature, water stress, gas composition during storage and storage duration (Atkin and Tjoelker, 2003; Beaudry et al., 1992; Kader et al., 1989; Lipton, 1990). Additionally, the amount of free air in a reefer used for *Proteaceae* freight (<35 %) is usually lower than 45-55 % free air was achieved inside the Janny MT boxes, which alters product respiration. The O_2 and CO_2 sensors on automatically ventilated reefers are a vital technology as they give more accurate real time gas concentrations and allow close monitoring of reefer atmospheres with little interference during shipping.

5.2 Mass loss

Mass loss was higher in product stored under AirEx as compared to those under closed ventilation. Moisture loss from the leaves depends on the difference in water vapour concentration between the leaf air spaces, the external bulk air and the diffusional resistance of the transpiration pathway (Wills et al., 2007; Taiz et al., 2015). Reducing the ventilation rate lowers RH and temperature fluctuations that arise from injection of fresh atmospheric air. This in turn promotes a thicker boundary layer around stoma and reduces water loss. Constant

injection of warm moist air into the AirEx reefers leads to condensation and formation of ice on the evaporators in the cooling system, which results in frequent defrosting cycles whereby temperature and humidity control is absent. Various anatomical and morphological features also influence the thickness of the boundary layer (Taiz et al., 2015), which possibly explains the differences in moisture loss between 'Safari Sunset' and 'Rosette' in the 2015 season. However, product effect was insignificant in the 2016 season, possibly due to the masking of variability between products due to the evaluation of a wider range of cultivars.

There is no control of RH in an AirEx reefers used for transportation of *Proteaceae* cut flowers (M. Huysamer 2017, personal communication, March 7), yet the optimum RH for storage of Fynbos cut flowers is recommended to be around 95 % (Reid, 2016). Water loss inevitably increases when products are kept below the optimum RH during storage or during retail as low RH increases transpiration rate, which reduces tissue water content (Fahmy and Nakano, 2013; Herppich et al., 1999).

AirEx storage also resulted in higher mass loss than that of products stored in 5-10-15 % CO_2 or 15 % CO_2. Again, this finding may be due to the lower ventilation rate and closing of stoma, which are triggered under high CO_2 conditions, such as in 5-10-15 % CO_2 and 15 % CO_2 systems (Taiz and Zeiger, 2016). However, storage in 15 % CO_2 atmosphere has been shown to elevate mass loss and induce chilling damage in some products, as noted on 'Safari Sunset' in the 2016 season. Hakim et al. (1999) reported that mass loss at low temperature was likely linked to CI progression through loss of membrane integrity, cellular breakdown and loss of epicuticular wax. Additionally, several researchers (Paull et al., 1980; Whitehead and de Swardt, 1982) have suggested that leaf blackening of some *Proteaceae* was a result of water stress.

Storage in AirEx, DCA and 2 % O_2 resulted in comparable moisture loss from the products, although notable differences in mass loss were recorded among products. The difference observed in the 2015 season could have been due to experimental error, for example, placing the stems near the ventilation valves in the Janny MT boxes. Despite the reduced ventilation rate in DCA and 2% O_2, there were non-significant benefits in mass loss reduction with these treatments compared to the conventional AirEx system. This may have been due to the absence of a gas humidifier in the DCA system. As a result, continuous pumping of dry N_2 and O_2 to maintain the set gas concentrations could have led to a steep water vapour gradient between the plant tissue and the surrounding storage atmosphere, and promoted water loss.

5.3 Colour determination of flowering heads and leaves

Storage in closed ventilation compared to AirEx did not significantly affect the hue angle of the flower heads and leaves. Even when 'Safari Sunset' showed significant differences between the before and after storage with regard to the hue readings of involucral and mature leaves, differences in hue angle were visually undetectable and commercially irrelevant. Despite statistical significance that was recorded in hue angle, differences in colour of the flower heads and mature leaves of product stored in AirEx, 5-10-15 % CO_2 and 15 % CO_2 atmosphere was small and visually indistinguishable. Van Doorn and Van Lieburg (1993) outlined that darkness converts the phytochrome to the Pfr form, which can trigger several physiological processes, including leaf colour change. Low light levels have been reported to cause leaf yellowing in some cut flower products like *Chrysanthemum*, and *Alstroemeria* (Reid, 1997). However, de-greening and re-greening of *Leucadendron* products is a complex phenomenon that is linked to pollination (Schmeisser et al., 2010).

Detached plant material stored in low O_2 and/or high CO_2 atmospheres normally undergoes reductions in respiration rate, chlorophyll degradation, oxidase activities and ethylene production (Kader, 1986). However, the involucral and mature leaves of the products were generally unaffected by AirEx, DCA or 2 % O_2 storage. Possible differences in hue angle due to storage treatment effect could have been masked by the low storage temperature, which slows down most of the cellular reactions. On the other hand, involucral leaves of 'Safari Sunset' showed statistically significant change in hue angle. Despite being statistically significant, the changes were too small to detect visually, and therefore, did not affect product quality.

5.4 Visual quality: Flowering heads

Quality differences between products stored in AirEx atmosphere and those stored in closed ventilation atmosphere were negligible. Notable quality differences, however, existed between the various product types. 'Rosette Conebush' displayed better flower head quality and 'Discolor' had the lowest quality as compared to other products. The flower head of 'Discolor', 'Safari Sunset' and 'Rosette' products is comprised of involucral leaves, which enclose the inflorescence, while that of the 'Rosette Conebush' is a fully developed cone. The improved vase life of 'Rosette Conebush' flowering heads is possibly because the mature cone is more resistant to dehydration as compared to the involucral leaves that make up the flowering head in 'Rosette', 'Safari Sunset' and 'Discolor'. The response of the flowering heads the

products to AirEx, 5-10-15 % CO_2 and 15 % CO_2 storage was variable among the different cultivars.

Generally, storage of 'Safari Sunset' and 'Rosette' products in AirEx and 5-10-15 % CO_2 resulted in better flower head quality than the 15 % CO_2 treatment, which caused discolouration and accelerated senescence of involucral leaves. This is possibly due to CI and/or CO_2 damage promoted by the high CO_2 levels, as is the case with flesh browning in apples (Contreras et al., 2014; Watkins and Nock, 2012) and brown stain in lettuce (Kader and Saltveit, 2003). Several authors (López-Gálvez et al., 1996; Watkins et al., 1997) explained that tolerance of horticultural commodities to elevated CO_2 is affected by cultivar, timing of exposure during storage, duration in ambient air before exposure and post-harvest treatments.

The AirEx, DCA and 2 % O_2 treatments were equally effective in 'Rosette Conebush' and 'Discolor' storage since the flower heads responded similarly to the treatments. In the 2015 season, flower heads of products stored in AirEx atmosphere had better visual quality than those of products stored in 2 % O_2. In the 2016 season, products stored in AirEx, DCA and 2 % O_2 had similar flower head quality throughout the evaluation period. Similarly, Devecchi et al. (2003) claimed that storage of 'First Red' cut roses in low O_2 atmosphere did not increase longevity. Flower head quality of 'Safari Sunset' seemed similar in all the treatments, but quality of 'Rosette' flower heads was better in product stored in AirEx atmosphere. Variable results were obtained for 'Discolor' although DCA storage treatment appeared to result in slightly better quality. The differences were likely due to seasonal effect and/or differences in pre-harvest conditions due to different product suppliers. Fanourakis et al. (2013 and Hodges and Toivonen (2008) explained that variability in visual quality of cut flowers may be due to environmental factors, genetic variability, and/or postharvest practices.

5.5 Visual quality: Mature leaves

There were insignificant quality differences between the leaf quality of products stored in closed ventilation and products stored in AirEx atmospheres and only slight differences existed among *Leucadendron* products in the 2015 season. The differences in quality are likely due to the same factors identified for flower heads. Additionally, the products come into season at different times of the year, which affects respiration rates and consequently, longevity. According to Ferreira (1986) and Joyce et al. (1995), developing inflorescences have considerably higher respiration rates as compared to mature inflorescences. Thus, early season products may have shorter vase life as compared to mid- and late season products. Visual quality of 'Discolor' dropped significantly after 7 d of vase life evaluation. This could be due

to the lower monosaccharide plus oligosaccharide levels, maturity at harvest and a higher respiration rate as compared to the other products and/or other reasons. Despite this anomaly, the products generally had similar vase-life leaf quality throughout the evaluation period.

Visual quality of 'Rosette', 'Safari Sunset' and 'Discolor' products was considerably better when the products were stored in AirEx atmosphere and 5-10-15 % CO_2 as compared to 15 % CO_2. Lower visual quality of product stored in 15 % CO_2 may have been a result of CO_2 damage and/or CI. Several researchers (Contreras et al., 2014; Hammer et al., 1990; Tshwenyane et al., 2012) linked elevated CO_2 concentrations to tissue discolouration, which lowers leaf quality. On the other hand, 'Rosette Conebush' tolerated the 15 % CO_2 without significant visual quality loss. This may be due to presence of more mature leaves and flowering heads since 'Rosette Conebush' is harvested late in the year.

Similar to the observations made on flower head quality, AirEx storage of 'Rosette Conebush' resulted in better leaf quality than 2 % O_2 storage in the 2015 season. Leaf quality of 'Safari Sunset', 'Discolor' and 'Rosette Conebush' remained consistently high during the evaluation period. Based on leaf quality, AirEx, DCA and 2 % O_2 storage atmospheres were equally suitable for storage of most *Leucadendron* products. The findings were similar to those made by Devecchi et al. (2003) who reported that longevity of 'First Red' roses was marginally improved by storage in low O_2 (1-6 kPa) as compared to storage in normal air.

5.6 Soluble carbohydrates

The first season's results on 'Rosette Conebush' showed non-significant differences between monosaccharide plus oligosaccharide content of products stored in AirEx atmosphere and in closed ventilation. However, in the 2016 season closed ventilation resulted in higher monosaccharide plus oligosaccharide content than closed ventilation atmosphere. Monosaccharides plus oligosaccharides were most abundant in 'Rosette' and lowest in 'Discolor', although 'Discolor' had a higher starch content than detected in 'Safari Sunset'. These inter-hybrid differences can possibly be ascribed to the different lineage, varying production environments and farm practices, as well as that harvest maturity is reached at different times of the year, which would introduce a season effect in addition to cultivar differences.

Polysaccharides were more abundant in products stored in closed ventilation as compared to products stored in AirEx atmosphere. Senescence causes degradation of complex carbohydrates and may lead to a transient increase in soluble sugars (Sood et al., 2006; Karimi and Asil, 2017). Trusty and Miller (1991) also reported that petal expansion in *Chrysanthemum*

promotes breakdown of polysaccharides such as starch and fructans. However, differences in starch content, between AirEx and closed ventilation storage treatments, were found not to be significant at the 5% confidence level. This study did not identify the specific sugars present, which may have offered more insights into the role of carbohydrate during the various storage conditions.

Findings for the 2015 season showed that monosaccharides plus oligosaccharides were higher in products stored in 5-10-15 % CO_2 as compared to products stored in AirEx atmosphere. However, 2016 season results indicated that total carbohydrate content was significantly higher in products kept in 15 % CO_2 as compared to products stored in AirEx atmosphere and 5-10-15 % CO_2. Monosaccharide plus oligosaccharide content was highest in 'Rosette' as compared to 'Discolor' and 'Safari Sunset' products where significantly lower levels were detected. Non-significant differences in the polysaccharide content of the products were recorded, however starch content was highest in 'Rosette' and lowest in 'Rosette Conebush'. Again, differences in carbohydrate content between cultivars could be ascribed to various factors, as discussed above.

When considering the total carbohydrate content of the various products stored under conditions of AirEx, DCA and 2 % O_2 in the 2015 season, no significant differences were detected. However, in the 2016 season monosaccharide plus oligosaccharide concentration was greatest in 'Rosette' and lowest in 'Safari Sunset', whereas the quantities of polysaccharides and starch were generally comparable among products.

For *Leucadendron*, the monosaccharide plus oligosaccharide content of leaves was much higher compared to the polysaccharide and starch content. Earlier studies revealed that cold storage of cut lilies (Van Doorn and Han, 2011) and cut peony (Walton et al., 2010) resulted in rapid starch and sucrose breakdown in the first two weeks of storage, resulting in the amount of hexose sugars produced surpassing the quantity utilised by respiration. In addition, escalation in reducing sugars in plant tissue is a common defence mechanism against chilling or frost injury. Elevated levels of mono-and oligosaccharides may also be a mechanism to facilitate continued flower opening during storage since accumulation of sugars will reduce petal water potential and promote influx of water to allow cell enlargement and flower opening (Bodelón et al., 2010; Kumar et al., 2008). The starch levels were relatively low, compared to the monosaccharides plus oligosaccharides, and polysaccharides, in all the products that were analysed.

5.6 Lipid peroxidation

Janero (1990) highlighted that the MDA produced during lipid peroxidation is a common and reliable diagnostic score of oxidative damage. The AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments did not significantly influence the amount of MDA in the products, even though there was considerable variability among products. The extent and cellular location of ROS generation varies due to tissue type, species, plant developmental stage, but also due to the nature, period and amount of stress applied (Hodges, 2003).

5.8 Total phenolic content

Varied findings were obtained for the effect of AirEx and low O_2 on total phenolics content available in leaves of *Leucadendron* products. Data for most of the products ('Discolor', 'Safari Sunset' and 'Rosette Conebush') showed that treatments did not significantly contribute to differences in phenolic content of the product as there was an overall lack of significant variation among treatments. However, phenolic content of 'Rosette Conebush' stored in AirEx atmosphere was higher than that of product stored in 2 % O_2. In addition, 'Rosette' stored in 2 % O_2 had a higher concentration of total phenolics, while stems stored under DCA had the lowest phenolic content. Browning and blackening changes in plant tissue are normally linked to phenolic activity (Paull et al., 1980; Whitehead and de Swardt, 1982; Van Doorn, 2001). According to Kader (2004), low O_2 and high CO_2 storage retards chlorophyll loss, and slows down the biosynthesis of anthocyanins and carotenoids, and oxidation of several phenolic compounds. Although Ekman et al. (2008) did not quantify total phenolics, they noted that flower browning (linked to phenolic activity) of *Backhousia myrtifolia* during storage increased significantly when the storage temperature was reduced from 15 to 2 or 0 ^{o}C.

Contrary to this, (Stephens, 2003; Stephens et al., 2001) reported that *Protea* leaf blackening increases under high storage temperatures. The observed differences in phenolic content could have been due to effect of treatment, product and storage temperature and this interaction was beyond the scope of this study since experiments were only done at 1 ^{o}C. High CO_2 atmospheres (5% CO_2) during long-term storage (3 weeks at 2°C) have been reported to reduce leaf blackening, which is linked to phenolics, and desiccation during vase life of 'Safari Sunset' (Philosoph-hadas et al., 2007).

Phenolic compounds are secondary metabolites that are involved in the defence of plants against pathogens, diseases, parasites, and predators and are also involved in several physiological mechanisms. Phenolics have antioxidant activity and perform an important role

in alleviating lipid peroxidation (Sala, 1998). Paliyath et al. (2008) emphasised that phenolics assist the plant in adapting to changing environmental conditions and serve as signalling molecules. The quantity of phenolics produced by a plant is variable among products since it is determined by temperature, ultraviolet light, available nutrition, and genetic factors (Bhattacharyya et al., 2014; Ling and Subramaniam, 2007; Zheng and Wang, 2001). Furthermore, Georgé et al. (2005) reported that polyphenols are usually over-estimated as folin-ciocalteu reagent may also interact with other reducing nonphenolic substances.

6. Conclusions and recommendations

In a closed ventilation system which contained a mixed load of *Proteaceae* products and 45-55 % free air and where the HarvestWatch™ DCA system was used for sampling, the O_2 level fell to about 8.5 % and the CO_2 level rose to approximately 10 %. When a handheld gas analyser was used as an alternative sampling method, O_2 concentrations fell below 2 % whilst CO_2 levels rose to above 17 %. Gas dynamics during storage were variable among the trials and depended on respiration rate of the products, the amount of free air and gas sampling method used. The Q_{10} values were between 1.49 and 3.84 and the respiration rates of the *Leucadendron* products at 5 ℃ ranged from 15.11-48.07 mL $CO_2.kg^{-1}.h^{-1}$ in the 2015 and 2016 seasons.

The AirEx ventilation system resulted in higher moisture loss from the products than under closed ventilation storage. Moisture loss was also higher in AirEx treatment as compared to 5-10-15 % CO_2 treatment in the 2015 season. However, in the 2016 season moisture loss was comparable between the AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments. The 2 % O_2 treatment resulted in higher moisture loss as compared to AirEx storage in the 2015 season, but mass loss was comparable between the AirEx, DCA and 2 % O_2 treatments in the 2016 season.

The AirEx, closed ventilation and low O_2 and high CO_2 treatments had visually non-significant influence on flower head and leaf hue angles and did not affect the visual quality of the products. There were also non-significant differences between the visual quality of flower heads and leaves of products stored in AirEx and those stored under closed ventilation atmosphere. Products stored in AirEx and 5-10-15 % CO_2 treatments had similar flower head visual quality, but exposure to 15 % CO_2 for a three week storage period greatly reduced longevity of some products. The AirEx, 5-10-15 % CO_2 and 15 % CO_2 atmospheres had comparable effects on visual quality of the leaves, although the quality of 'Discolor' and 'Safari

Sunset' was considerably reduced by the 15 % CO_2 treatment. Results for effect of AirEx, DCA and 2 % O_2 on flower head visual quality were inconclusive in some products, and minor quality differences were noted among the treatments in some cases. Nonetheless, AirEx storage produced consistently high visual quality in most products. Storage in AirEx, DCA and 2 % O_2 treatments resulted in similar leaf visual quality, although the longevity of 'Rosette Conebush' leaves was shortened by 2 % O_2 in the 2015 season.

Comparison of AirEx atmosphere and closed ventilation and that of AirEx, DCA and 2 % O_2 treatments showed that treatments led to similar total carbohydrate content. On the other hand, inconsistent results were obtained from comparison of total carbohydrates resulting from storage in AirEx atmosphere, 5-10-15 % CO_2 and 15 % CO_2. Monosaccharides plus oligosaccharides were the most abundant sugars followed by polysaccharides, with starch being the least abundant. The MDA quantities produced due to the impact of AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments were generally similar. While AirEx atmosphere resulted in higher total phenolic content in the 2015 season, the treatment effect was non-significant in the 2016 season, with differences only existing between products.

The study proved that on a small experimental scale, closed ventilation, low O_2 and high CO_2 storage is suitable for use during long-term cold storage of *Leucadendron* products as supported by the better visual quality and longevity. It is important to maintain strict control of O_2 concentration to ensure that the levels remain above the lower O_2 limits of the products, which range from 0.09-0.26 % O_2. As safe cut off limits for use with automatic ventilation shipping, ventilation should be restricted to a minimum limit of 2 % O_2 concentration and maximum limit of 15 % CO_2 concentration. There is need for further research to determine the changes in respiration rate during cold storage in different atmospheres and the extent to which growing season, harvesting maturity, growing conditions, post-harvest practices and shipping temperatures affect the various products.

7. References

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8. Tables and figures

Table 1. Lower O_2 limit (LOL) values, respiration rates, temperature quotients (Q_{10}) and trial dates for *Leucadendron* products in the 2015 and 2016 seasons.

2015 Season

Product	Respiration at 5 °C (mL $CO_2.kg^{-1}.h^{-1}$)	Respiration at 15 °C (mL $CO_2.kg^{-1}.h^{-1}$)	Q_{10}	Estimated respiration rate at 1 °C	LOL (O_2 %)	Respiration rate date	CO_2 toxicity tolerance trial date	Closed ventilation trial date	LOL trial date
'Safari Sunset'	41.41	61.57	1.49	35.30	0.09	15-May	21-May	22-Apr	-
'Discolor'	48.07	91.88	1.91	37.11	0.21	20-May	-	-	-
'Rosette'	40.09	73.75	1.84	31.41	0.24	20-May	09-Jul	-	28-Jul
'Rosette Conebush'	23.68	79.96	3.38	14.55	0.33	14-Nov	-	11-Oct	28-Oct

2016 Season

Product	Respiration at 5 °C (mL $CO_2.kg^{-1}.h^{-1}$)	Respiration at 15 °C (mL $CO_2.kg^{-1}.h^{-1}$)	Q_{10}	Estimated respiration at 1 °C	LOL (O_2 %)	Respiration rate date	CO_2 toxicity tolerance trial date	Closed ventilation trial date	LOL trial date
'Safari Sunset'	15.11	58.04	3.84	8.82	0.26	12-Apr	12-Apr	31-Mar	12-Apr
'Discolor'	15.87	37.48	2.36	11.26	0.26	12-Apr	12-Apr	31-Mar	12-Apr
'Rosette'	-	-	-	-	0.18	-	12-Apr	31-Mar	12-Apr
'Rosette Conebush'	25.99	60.91	2.34	18.50	0.24	03-Sep	01-Sep	09-Sep	02-Sep

Table 2

Mass loss (%) of *Leucadendron* products ('Safari Sunset', 'Discolor' and 'Rosette') stored in AirEx and closed ventilation for 21 d at 1 °C in the 2015 and 2016 seasons.

2015 Season			
Treatment		**Mean mass loss (%)**	
AirEx		6.0	a
Closed ventilation		2.9	b
Product			
'Rosette Conebush'		5.2	a
'Safari Sunset'		3.7	b
Effect	Product	Treatment	Product x Treatment
P value	*0.001*	*<0.0001*	*0.484ns*
2016 Season			
Treatment		**Mean mass loss (%)**	
AirEx		6.3	a
Closed ventilation		2.8	b
Product			
'Rosette'		6.5	ns
'Discolor'		4.5	
'Safari Sunset'		3.9	
'Rosette Conebush'		3.4	
Effect	Product	Treatment	Product x Treatment
P value	*0.219ns*	*0.002*	*0.420ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 3. Colour determination (h°) of flower heads and mature leaves of 'Rosette Conebush' stored in AirEx and closed ventilation for 21 d at 1 °C in the 2015 season**.**

	2015 Season Flower head		
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	49.9	ns
	After	34.9	
Closed ventilation	Before	35.9	
	After	38.7	
Effect	Treatment	Storage	Storage x Treatment
P value	*0.420ns*	*0.314ns*	*0.148ns*
	2015 Season Mature leaves		
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	111.4	ns
	After	119.5	
Closed ventilation	Before	120.1	
	After	119.7	
Effect	Treatment	Storage	Storage x Treatment
P value	*0.177ns*	*0.199ns*	*0.157ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 4

Colour determination (h°) of involucral and mature leaves of *Leucadendron* products ('Safari Sunset', 'Rosette' and 'Discolor') stored in AirEx and under closed ventilation for 21 d at 1 °C in the 2016 season.

2016 Season Involucral and mature leaves			
Product	**Leaf type**	**Storage**	**Mean hue angle (°)**
'Discolor'	Mature	Before	124.7 a
		After	124.6 a
	Involucral	Before	121.9 a
		After	121.6 a
'Rosette'	Mature	Before	122.1 a
		After	122.0 a
	Involucral	Before	121.8 a
		After	121.2 a
'Safari Sunset'	Mature	Before	110.5 b
		After	118.3 a
	Involucral	Before	74.0 b
		After	67.8 c

Effect	***P value***
Product	*<0.0001*
Treatment	*0.498ns*
Leaf type	*<0.0001*
Storage	*0.924ns*
Product x Treatment	*0.619ns*
Treatment x Leaf type	*0.119ns*
Storage x Product	*0.871ns*
Storage x Treatment	*0.250ns*
Product x Leaf type	*<0.0001*
Storage x Leaf type	*0.011*
Product x Treatment x Leaf type	*0.089ns*
Storage x Product x Treatment	*0.495ns*
Storage x Treatment x Leaf type	*0.184ns*
Storage x Product x Leaf type	*0.004*
Storage x Product x Leaf type x Treatment	*0.163ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 5

Soluble carbohydrates in leaves of *Leucadendron* products after 21 d at 1 °C in AirEx and closed ventilation in the 2015 season ('Rosette Conebush') and in the 2016 season ('Safari Sunset', 'Rosette' and 'Discolor').

2015 Season (Monosaccharides + Oligosaccharides)							
Treatment					**Mean glucose mg.g^{-1}**		
AirEx					104.3	ns	
Closed ventilation					106.4		
Effect					Treatment		
P value					*0.733ns*		
2016 Season							
Treatment		**Carbohydrate type**			**Mean glucose mg.g^{-1}**		
AirEx		Monosaccharides + oligosaccharides			119.0	b	
Closed ventilation					130.1	a	
AirEx		Polysaccharides			30.7	d	
Closed ventilation					46.1	c	
AirEx		Starch			26.4	d	
Closed ventilation					34.2	d	
Product							
'Rosette'		Monosaccharides + oligosaccharides			167.7	a	
		Polysaccharides			43.6	d	
		Starch			37.7	de	
'Discolor'		Monosaccharides + oligosaccharides			97.0	c	
		Polysaccharides			37.9	de	
		Starch			28.8	e	
'Safari Sunset'		Monosaccharides + oligosaccharides			108.9	b	
		Polysaccharides			39.5	d	
		Starch			18.6	f	
Effect	Treatment	Carb type	Product	Product x Treatment	Product x Carb type	Carb type x Treatment	Product x Carb type x Treatment
P value	*0.079ns*	*<0.0001*	*<0.0001*	*0.055ns*	*<0.0001*	*<0.0001*	*0.106ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 6

Mass loss (%) of *Leucadendron* products stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 in the 2015 season ('Rosette') and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Safari Sunset', 'Discolor', 'Rosette' and 'Rosette Conebush') in the 2016 season.

2015 Season			
Treatment			**Mean mass loss (%)**
AirEx			8.9 a
5-10-15 % CO_2			5.9 b
Effect			Treatment
P value			*<0.0001*
2016 Season			
Product	**Treatment**		**Mean mass loss (%)**
'Safari Sunset'	AirEx		3.8 c
	5-10-15 % CO_2		2.3 d
	15 % CO_2		12.1 a
'Rosette'	AirEx		3.7 c
	5-10-15 % CO_2		3.2 cd
	15 % CO_2		3.3 cd
'Discolor'	AirEx		5.7 b
	5-10-15 % CO_2		4.0 c
	15 % CO_2		4.3 c
'Rosette Conebush'	AirEx		3.6 c
	5-10-15 % CO_2		3.4 cd
	15 % CO_2		3.2 cd
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 7

Colour determination (h°) of flower heads and mature leaves of 'Rosette Conebush' stored at 1 °C for 21 d in AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments in the 2016 season.

2016 Season Flower head			
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	67.2	a
	After	48.4	b
5-10-15 % CO_2	Before	50.2	b
	After	48.7	b
15 % CO_2	Before	51.3	b
	After	50.9	b
Effect	Treatment	Storage	Storage x Treatment
P value	*0.099ns*	*0.043*	*0.049*

2016 Season Mature leaves			
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	112.7	ns
	After	113.8	
5-10-15 % CO_2	Before	114.8	
	After	114.0	
15 % CO_2	Before	114.8	
	After	114.7	
Effect	Treatment	Storage	Storage x Treatment
P value	*0.429ns*	*0.926ns*	*0.520ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 8

Colour determination (h°) of leaf involucral and mature leaves of *Leucadendron* products stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Rosette') in the 2015 season.

2015 season Involucral and mature leaves - 'Rosette'							
Leaf type		**Treatment**			**Mean hue angle (°)**		
Involucral		AirEx			112.1	b	
		5-10-15 % CO_2			108.3	c	
Mature		AirEx			120.5	a	
		5-10-15 % CO_2			120.5	a	
Storage							
Before					115.6	a	
After					115.0	b	
Effect	Treatment	Leaf type	Storage	Storage x Leaf type	Storage x Treatment	Treatment x Leaf type	Storage x Treatment x Leaf type
P value	*0.014*	*<0.0001*	*<0.0001*	*0.888ns*	*0.313ns*	*0.012*	*0.983ns*

*****Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 9

Colour determination (h°) of involucral and mature leaves of *Leucadendron* products stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 and 15 % CO_2 ('Rosette Conebush', 'Safari Sunset', 'Discolor' and 'Rosette') in the 2016 season.

2016 Season Involucral and mature leaves					
Product	**Treatment**	**Leaf type**	**Storage**	**Mean hue angle (°)**	
'Discolor'	AirEx	Mature	Before	123.2	ns
			After	123.8	
		Involucral	Before	121.7	
			After	121.1	
	5-10-15 % CO_2	Mature	Before	125.0	
			After	125.6	
		Involucral	Before	122.0	
			After	122.2	
	15 % CO_2	Mature	Before	124.1	
			After	124.9	
		Involucral	Before	121.5	
			After	121.4	
'Safari Sunset'	AirEx	Mature	Before	118.0	
			After	118.4	
		Involucral	Before	84.6***	
			After	85.7***	
	5-10-15 % CO_2	Mature	Before	119.2	
			After	119.9	
		Involucral	Before	93.2***	
			After	91.3***	
	15 % CO_2	Mature	Before	119.3	
			After	119.7	
		Involucral	Before	91.6***	
			After	92.4***	
'Rosette'	AirEx	Mature	Before	121.1	
			After	121.3	
		Involucral	Before	119.8	
			After	119.9	
	5-10-15 % CO_2	Mature	Before	120.8	
			After	120.8	
		Involucral	Before	118.8	
			After	118.7	
	15 % CO_2	Mature	Before	120.2	
			After	119.7	
		Involucral	Before	117.1	
			After	117.1	

***Table continued on next page.**

Effect	***P value***
Product	*<0.0001*
Treatment	*0.420ns*
Leaf type	*<0.0001*
Storage	*0.250ns*
Product x Treatment	*0.291ns*
Product x Leaf type	*<0.0001*
Treatment x Leaf type	*0.842ns*
Storage x Product	*0.497ns*
Storage x Treatment	*0.390ns*
Storage x Leaf type	*0.075ns*
Product x Treatment x Leaf type	*0.664ns*
Storage x Product x Treatment	*0.121ns*
Storage x Product x Leaf type	*0.362ns*
Storage x Treatment x Leaf type	*0.193ns*
Storage x Product x Treatment x Leaf type	*0.048*

*Mean separation within main factors was done by LSD (5 %) and means indicated by *** are significantly different from each other and ns shows no significance.

Table 10

Soluble carbohydrates (mg.g^{-1}) in leaves of *Leucadendron* products after storage for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Rosette') in the 2015 season and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Safari Sunset', 'Rosette', 'Discolor' and 'Rosette Conebush') in the 2016 season.

2015 Season (Monosaccharides + oligosaccharides)							
Treatment					**Mean glucose mg.g^{-1}**		
AirEx					82.6	b	
5-10-15 % CO_2					123.9	a	
Effect					Treatment		
P value					*<0.0001*		
2016 Season							
Product	**Carbohydrate type**				**Mean glucose mg.g^{-1}**		
'Rosette'	Monosaccharides + Oligosaccharides				196.0	a	
	Polysaccharides				24.0	def	
	Starch				28.6	d	
'Discolor'	Monosaccharides + Oligosaccharides				101.9	c	
	Polysaccharides				23.2	def	
	Starch				17.6	eg	
'Safari Sunset'	Monosaccharides + Oligosaccharides				92.5	c	
	Polysaccharides				27.2	def	
	Starch				14.2	fgh	
'Rosette Conebush'	Monosaccharides + Oligosaccharides				166.8	b	
	Polysaccharides				9.4	de	
	Starch				6.1	h	
Treatment							
AirEx					53.1	b	
5-10-15 % CO_2					57.9	b	
15 % CO_2					65.9	a	
Effect	Product	Carb type	Treatment	Product x Treatment	Carb type x Treatment	Product x Carb type	Product x Carb type x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*0.325ns*	*0.373ns*	*<0.0001*	*0.576ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 11

Lipid peroxidation (nmol.mL^{-1}.g^{-1}) in *Leucadendron* products stored at 1 °C for 21 d in AirEx and 5-10-15 % CO_2 in the 2015 season ('Rosette') and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Safari Sunset', 'Rosette', 'Discolor' and 'Rosette Conebush') in the 2016 season.

2015 Season			
Treatment		**Mean MDA nmol.mL^{-1}.g^{-1}**	
AirEx		155.6	ns
5-10-15 % CO_2		187.8	
Effect		Treatment	
P value		*0.272ns*	
2016 Season			
Product		**Mean MDA nmol.mL^{-1}.g^{-1}**	
'Rosette Conebush'		182.7	a
'Rosette'		128.3	b
'Safari Sunset'		117.5	b
'Discolor'		99.1	c
Treatment			
AirEx		132.5	ns
5-10-15 % CO_2		141.0	
15 % CO_2		122.2	
Effect	Treatment	Product	Product x Treatment
P value	*0.064ns*	*<0.0001*	*0.949ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance. Lipid peroxidation expressed as malondialdehyde (MDA) content.

Table 12

Mass loss (%) of *Leucadendron* products stored at 1 °C for 21 d in AirEx and 2 % O_2 ('Rosette Conebush') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Safari Sunset', 'Rosette', 'Discolor' and 'Rosette Conebush') in the 2016 season .

2015 Season			
Treatment			**Mean mass loss (%)**
AirEx			6.9 b
2 % O_2			18.2 a
Effect			Treatment
P value			*<0.0001*
2016 Season			
Product	**Treatment**		**Mean mass loss (%)**
'Safari Sunset'	AirEx		5.2 cde
	DCA		5.0 def
	2 % O_2		4.4 dg
'Rosette'	AirEx		6.0 cd
	DCA		6.4 bc
	2 % O_2		5.8 dc
'Discolor'	AirEx		8.7 a
	DCA		6.1 dc
	2 % O_2		7.4 b
'Rosette Conebush'	AirEx		3.8 fg
	DCA		3.2 g
	2 % O_2		3.7 g
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.055ns*	*0.028*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 13

Colour determination (h°) of flower heads of 'Rosette Conebush' stored for 21 d at 1 °C in AirEx and 2 % O_2 in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 and in the 2016 season.

2015 Season			
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	56.6	a
	After	27.6	b
2 % O_2	Before	29.0	b
	After	36.3	b
Effect	Treatment	Storage	Storage x Treatment
P value	*0.200ns*	*0.127ns*	*0.016*
2016 Season			
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	48.3	ns
	After	47.4	
DCA	Before	49.6	
	After	50.9	
2 % O_2	Before	48.1	
	After	48.7	
Effect	Treatment	Storage	Storage x Treatment
P value	*0.679ns*	*0.710ns*	*0.567ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 14

Colour determination (h°) of mature leaves of *Leucadendron* products stored for 21 d at 1 °C in AirEx and 2 % O_2 ('Rosette Conebush') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Safari Sunset', 'Discolor', 'Rosette' and 'Rosette Conebush') in the 2016 season.

2015 Season **Mature leaves- 'Rosette Conebush'**			
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	104.0	ns
	After	120.1	
2 % O_2	Before	119.1	
	After	118.6	
Effect	Treatment	Storage	Storage x Treatment
P value	*0.131ns*	*0.067ns*	*0.054ns*

2016 Season **Mature leaves- 'Rosette Conebush'**			
Treatment	**Storage**	**Mean hue angle (°)**	
AirEx	Before	113.9	ns
	After	113.7	
DCA	Before	115.0	
	After	114.6	
2 % O_2	Before	114.2	
	After	114.4	
Effect	Treatment	Storage	Storage x Treatment
P value	*0.542ns*	*0.262ns*	*0.151ns*

2016 Season **Involucral and mature leaves**				
Product	**Leaf type**	**Storage**	**Mean hue angle (°)**	
'Safari Sunset'	Mature	Before	118.8	b
		After	116.8	b
	Involucral	Before	83.2	d
		After	86.3	c
'Discolor'	Mature	Before	123.5	a
		After	123.4	a
	Involucral	Before	119.6	b
		After	118.4	b
'Rosette'	Mature	Before	120.2	ab
		After	119.9	ab
	Involucral	Before	118.1	b
		After	117.8	b

***Table continued on next page.**

Effect	***P value***
Product	*<0.0001*
Treatment	*0.430ns*
Leaf type	*<0.0001*
Storage	*0.846ns*
Product x Treatment	*0.778ns*
Treatment x Leaf type	*0.925ns*
Storage x Product	*0.587ns*
Storage x Treatment	*0.868ns*
Storage x Leaf type	*0.216ns*
Storage x Product x Treatment	*0.975ns*
Product x Treatment x Leaf type	*0.904ns*
Storage x Treatment x Leaf type	*0.590ns*
Product x Leaf type	*<0.0001*
Storage x Product x Leaf type	*0.042*
Storage x Product x Treatment x Leaf type	*0.716ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 15

Soluble carbohydrates (mg.g^{-1}) in leaves of *Leucadendron* products after storage for 21 d at 1 °C in AirEx and 2 % O_2 ('Rosette Conebush') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Safari Sunset', 'Rosette', 'Discolor' and 'Rosette Conebush') in the and 2016 season.

2015 Season							
(Monosaccharides + oligosaccharides)							
Treatment					**Mean glucose mg.g^{-1}**		
AirEx					209.2	ns	
2 % O_2					203.0		
Effect					Treatment		
P value					*0.713ns*		
2016 Season							
Product	**Treatment**				**Mean glucose mg.g^{-1}**		
'Discolor'	AirEx				143.4	ef	
	DCA				142.6	ef	
	2 % O_2				150.7	ed	
'Rosette'	AirEx				184.5	a	
	DCA				163.7	a	
	2 % O_2				188.4	a	
'Safari Sunset'	AirEx				146.9	gf	
	DCA				147.4	g	
	2 % O_2				139.3	g	
'Rosette Conebush'	AirEx				167.5	c	
	DCA				171.4	b	
	2 % O_2				170.7	cd	
	Carbohydrate type						
'Rosette'	Monosaccharides + Oligosaccharides				191.4	a	
	Polysaccharides				25.4	e	
	Starch				19.3	hf	
'Discolor'	Monosaccharides + Oligosaccharides				108.3	c	
	Polysaccharides				21.7	efg	
	Starch				16.2	hg	
'Safari Sunset'	Monosaccharides + Oligosaccharides				82.6	d	
	Polysaccharides				26.9	e	
	Starch				15.5	h	
'Rosette Conebush'	Monosaccharides + Oligosaccharides				136.7	b	
	Polysaccharides				22.8	ef	
	Starch				18.4	hf	

Effect	Product	Carb type	Treatment	Carb type x Treatment	Product x Carb type	Product x Treatment	Product x Carb type x Treatment
P value	*<0.0001ns*	*<0.0001*	*0.813ns*	*0.350ns*	*<0.0001*	*0.012*	*0.109ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 16

Total phenolic content (nmol.mL^{-1}.g^{-1}) in leaves of *Leucadendron* products after storage for 21 d at 1 °C in AirEx and 2 % O_2 ('Rosette Conebush') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Safari Sunset', 'Rosette', 'Discolor' and 'Rosette Conebush') in the 2016 season .

2015 Season			
Treatment			**Mean GAE nmol.mL^{-1}.g^{-1}**
AirEx			131.9 a
2 % O_2			114.7 b
Effect			Treatment
P value			*0.004*
2016 Season			
Product	**Treatment**		**Mean GAE nmol.mL^{-1}.g^{-1}**
'Discolor'	AirEx		143.4 e
	DCA		142.6 e
	2 % O_2		150.7 ed
'Rosette'	AirEx		184.5 ab
	DCA		163.7 cd
	2 % O_2		188.4 a
'Safari Sunset'	AirEx		146.9 e
	DCA		147.4 e
	2 % O_2		139.3 e
'Rosette Conebush'	AirEx		167.5 c
	DCA		171.4 cb
	2 % O_2		170.7 cb
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.232ns*	*0.036*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance. Phenolic content expressed as gallic acid equivalents (GAE).

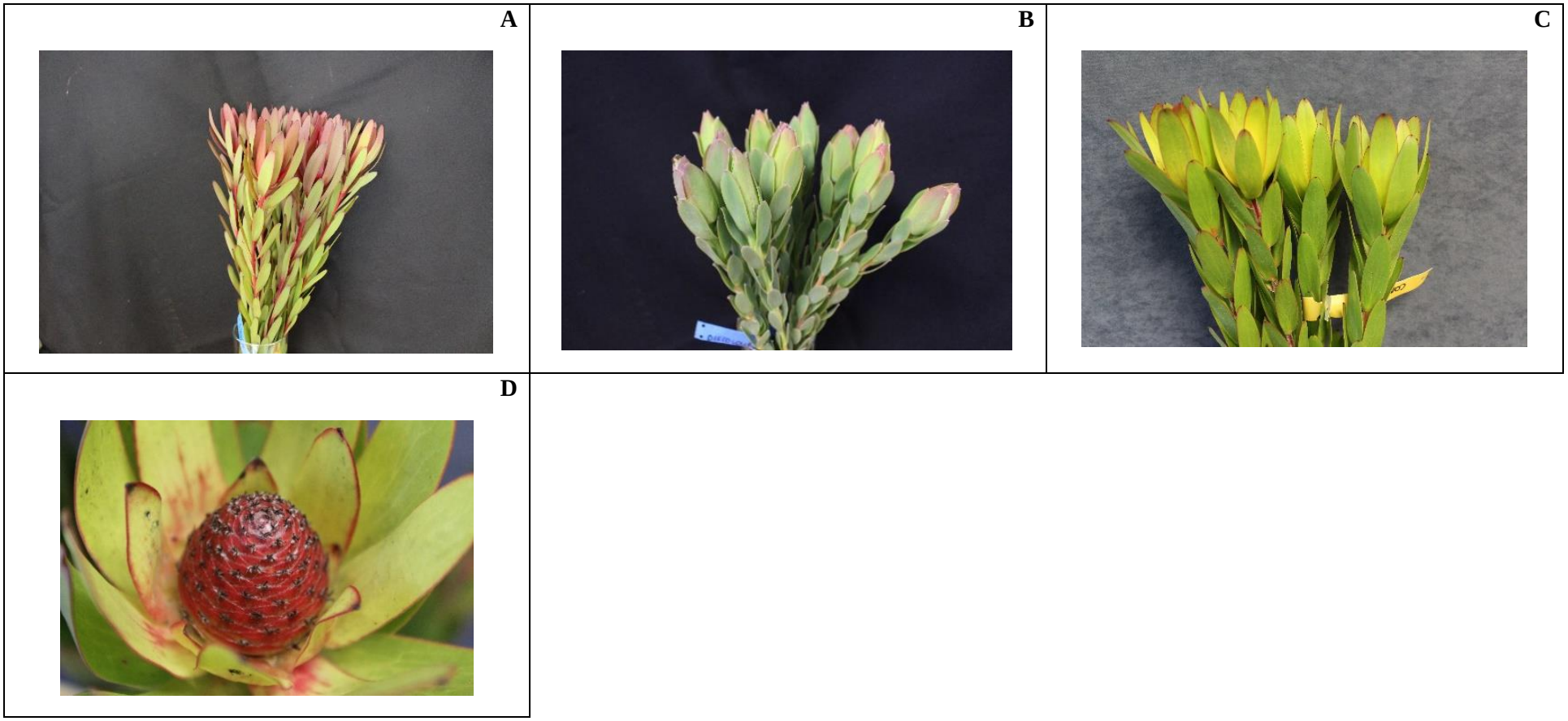


Fig. 1. *Leucadendron* products [‘Safari Sunset’ (A), ‘Discolor’ (B), ‘Rosette’ (C), ‘Rosette Conebush’ (D)] evaluated in the 2015 and 2016 seasons.

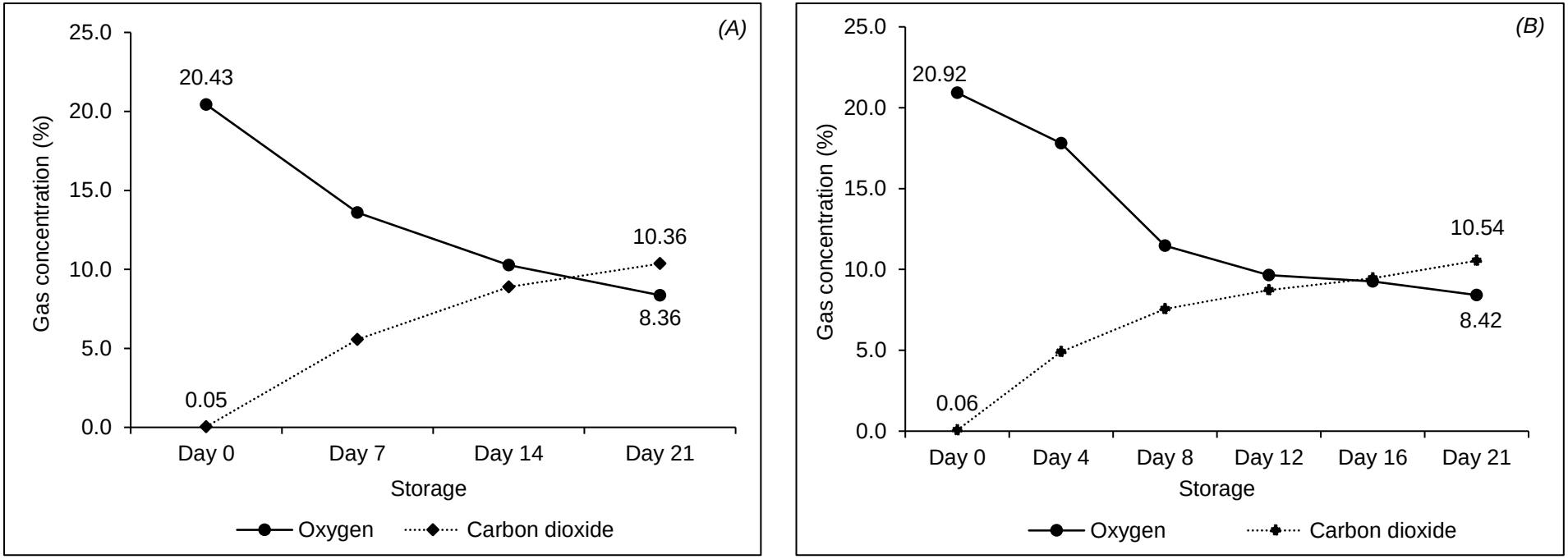


Fig. 2. Gas dynamics at 1 °C within a closed ventilation system as monitored in the 2015 season using the HarvestWatch™ DCA system over a 21-day cold storage period whilst either containing *Leucadendron* ('Safari Sunset' Fig. 2A) or a mixed-load (Fig. 2B) of *Leucadendron* ('Rosette Conebush'), *Leucospermum* ('Jelena', 'Goldie, 'Bright Eyes' and 'Fire Fly') and *Protea* (Barbigera, 'Grandiceps' and 'Red Rex') products at 1 °C.

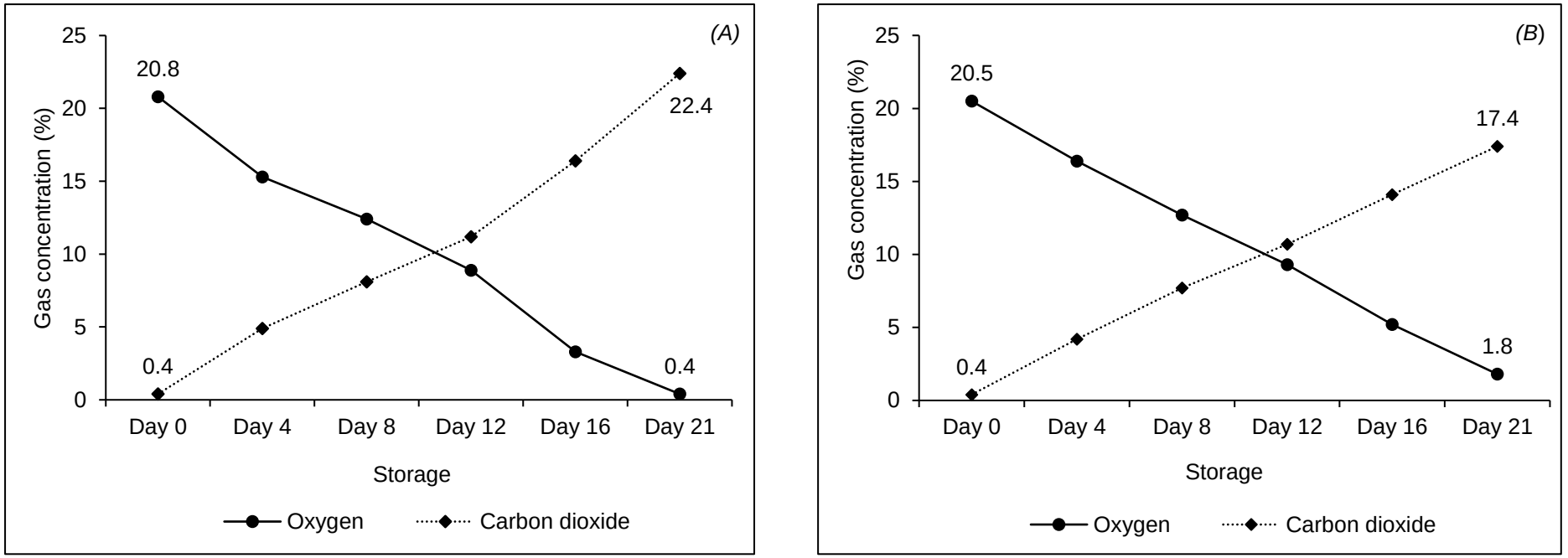
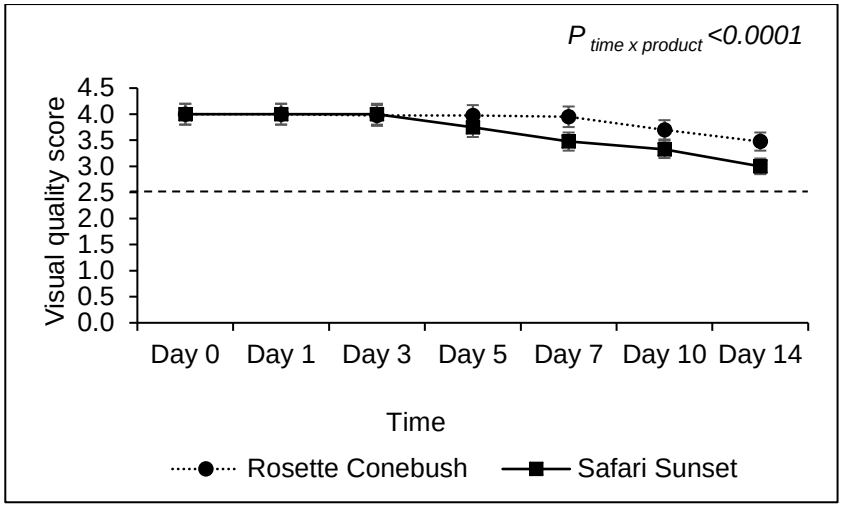
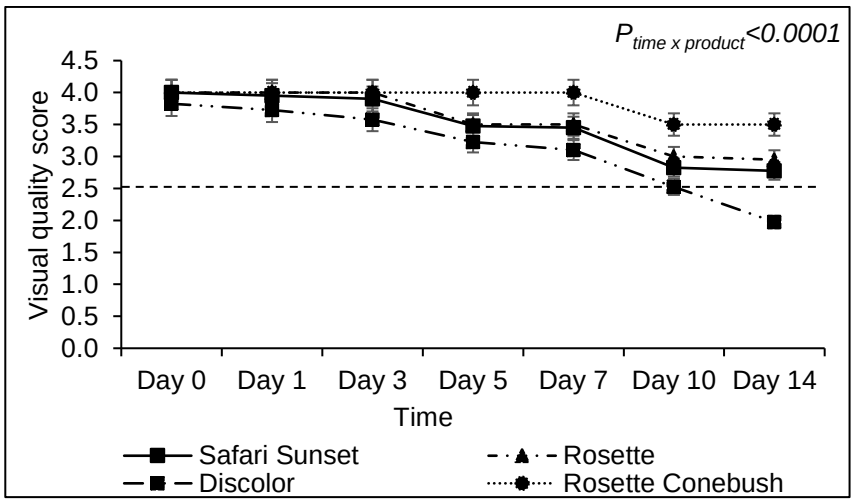


Fig. 3. Gas dynamics at 1 °C within a closed ventilation system as monitored during the 2016 season by means of a hand held gas analyser whilst either containing *Leucadendron* ('Discolor', 'Safari Sunset' and 'Rosette') products (Fig. 3A) or a mixed-load of *Leucadendron* ('Rosette Conebush') and *Leucospermum* ('Soleil', 'Succession', 'Veld Fire' and 'Rigoletto') products (Fig. 3B).



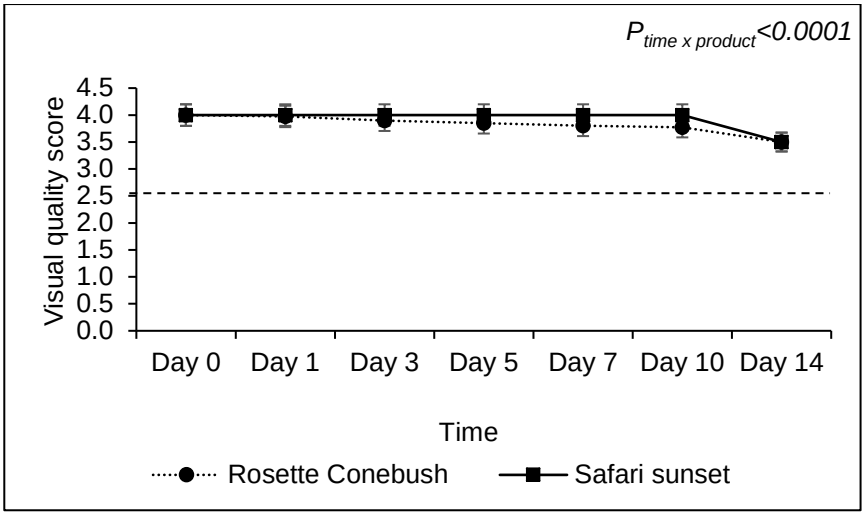
Effect	Treatment	Product	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*0.604ns*	*<0.0001*	*<0.0001*	*0.944ns*	*0.841ns*	*<0.0001*	*0.857ns*

Fig. 4. Visual quality of flower heads of *Leucadendron* products ('Rosette Conebush' and 'Safari Sunset') after 21 d of cold storage at 1 °C in AirEx and closed ventilation in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



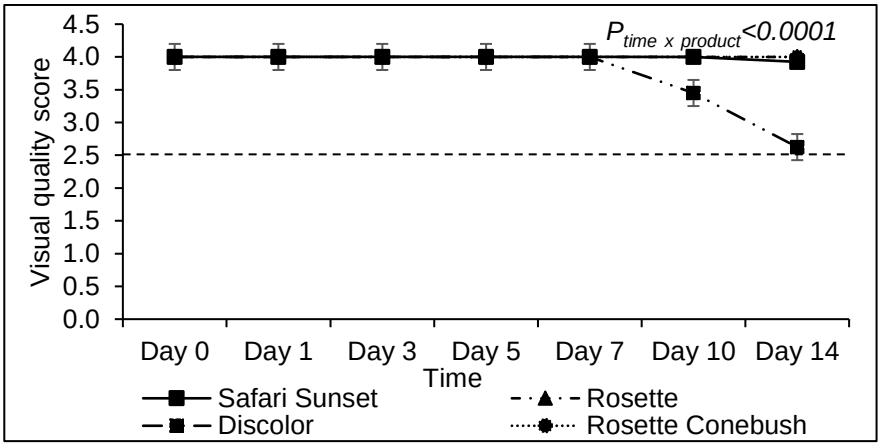
Effect	Treatment	Product	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*0.778ns*	*<0.0001*	*<0.0001*	*0.18ns*	*0.991ns*	*<0.0001*	*0.315ns*

Fig. 5. Visual quality of flower heads of *Leucadendron* products ('Safari Sunset', 'Rosette', 'Discolor' and 'Rosette Conebush') after cold storage for 21 d at 1 °C in AirEx and closed ventilation in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



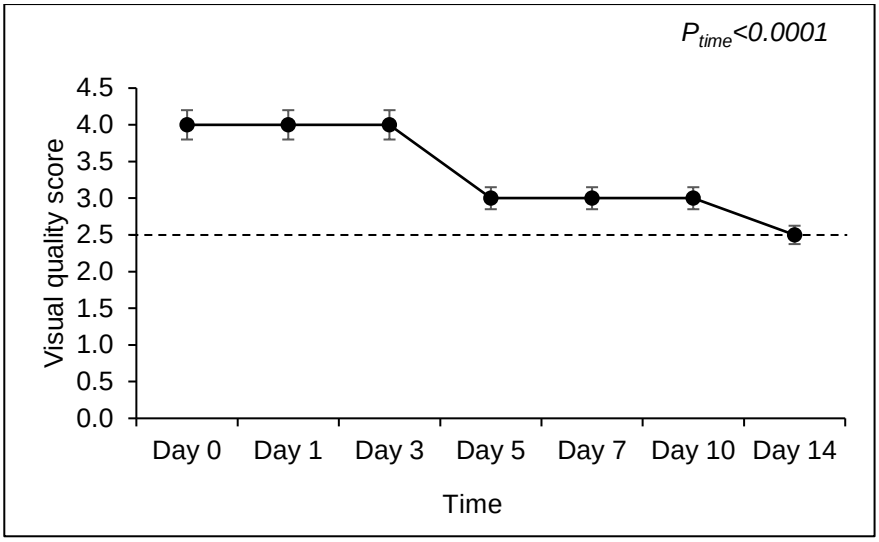
Effect	Product	Treatment	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*0.18ns*	*0.699ns*	*<0.0001*	*0.699ns*	*0.879ns*	*0.016*	*0.879ns*

Fig. 6. Visual leaf quality of *Leucadendron* products ('Safari Sunset' and 'Rosette Conebush') after 21 d of cold storage at 1 °C in AirEx and closed ventilation in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



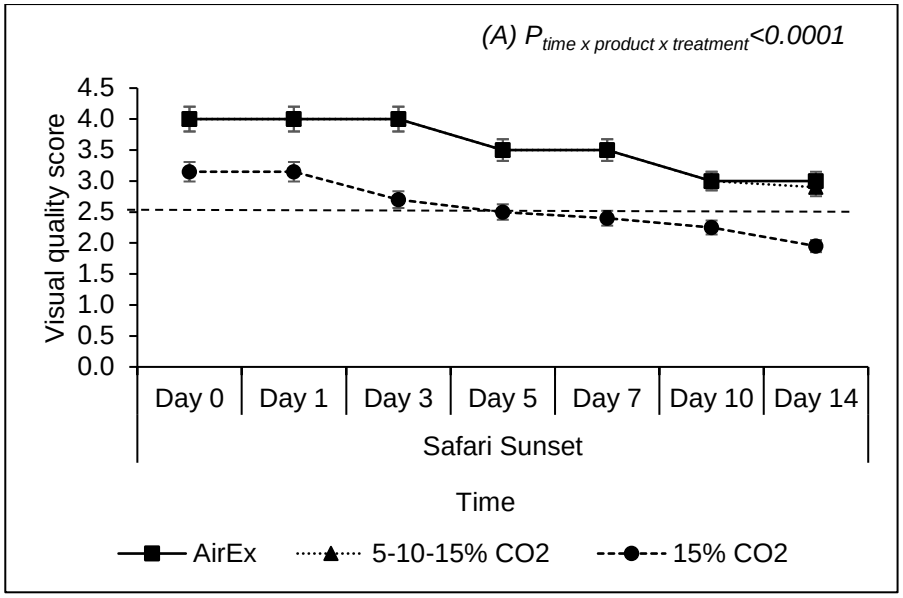
Effect	Product	Treatment	Product x Treatment	Time	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*<0.0001*	*0.164ns*	*0.366ns*	*<0.0001*	*0.090ns*	*<0.0001*	*0.539ns*

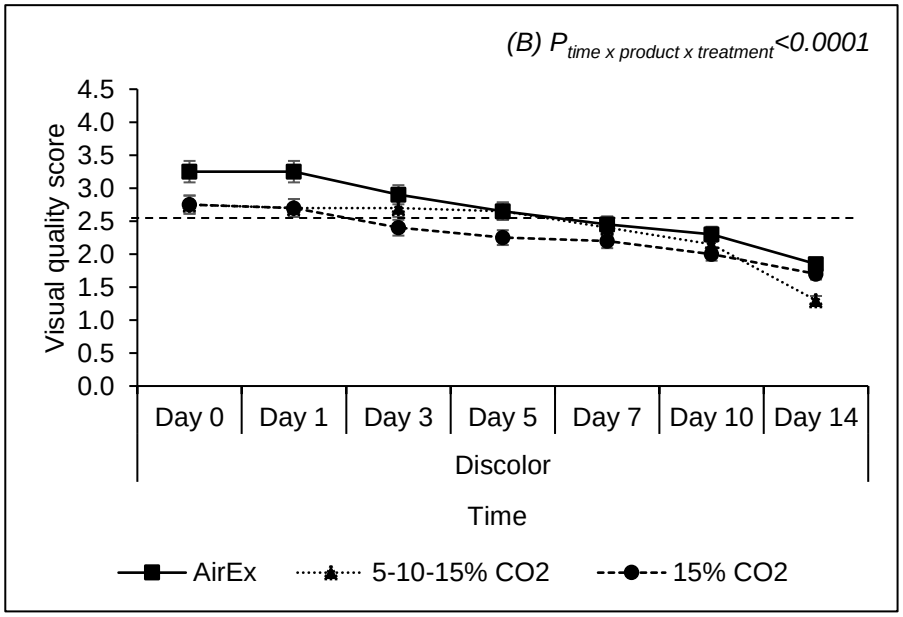
Fig. 7 Visual quality of flower heads of *Leucadendron* products ('Safari Sunset', 'Rosette', 'Discolor' and 'Rosette Conebush') after cold storage for 21 d at 1 °C in AirEx and closed ventilation in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

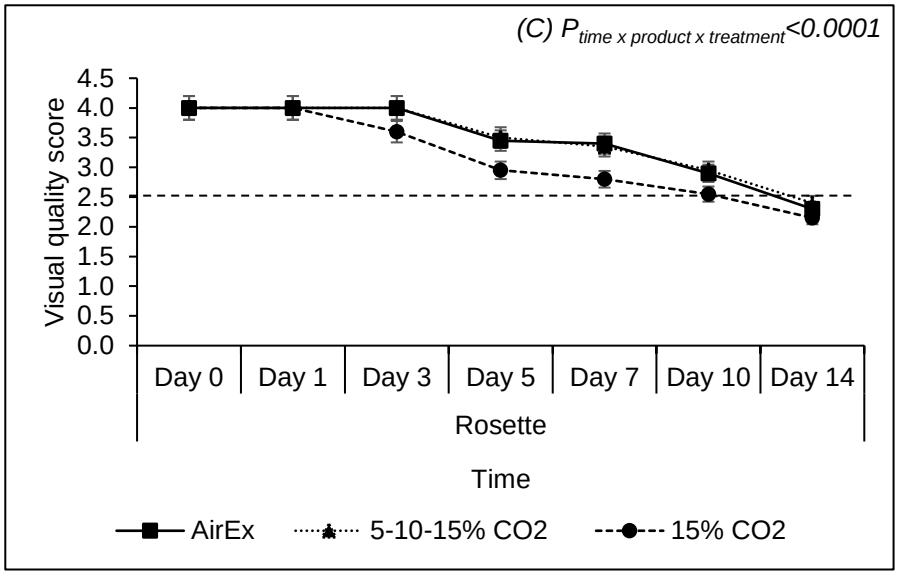


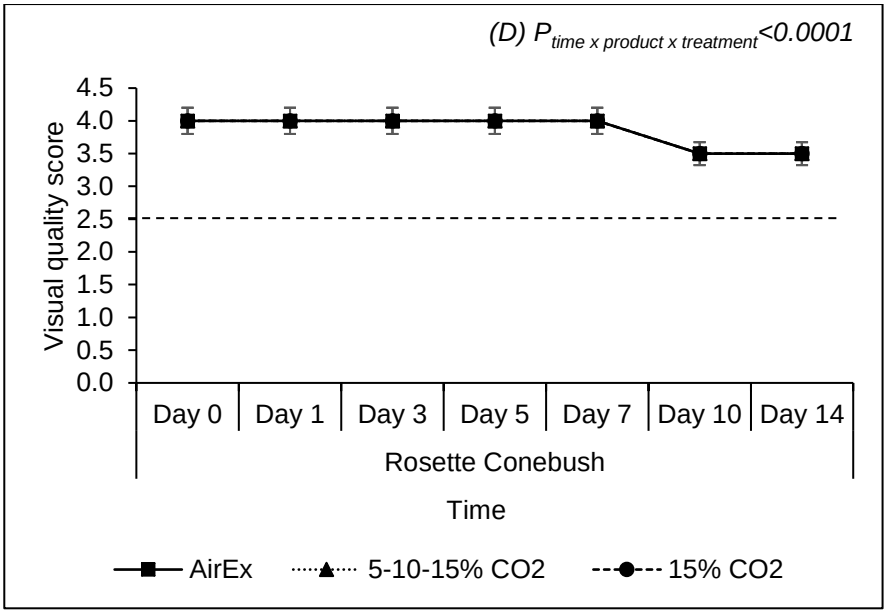
Effect	Treatment	Time	Time x Treatment
P value	*0.331ns*	*<0.0001*	*0.931ns*

Fig 8. Visual quality of 'Rosette' flower heads following storage in AirEx and in 5-10-15 % CO_2 for 21 d at 1 °C in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



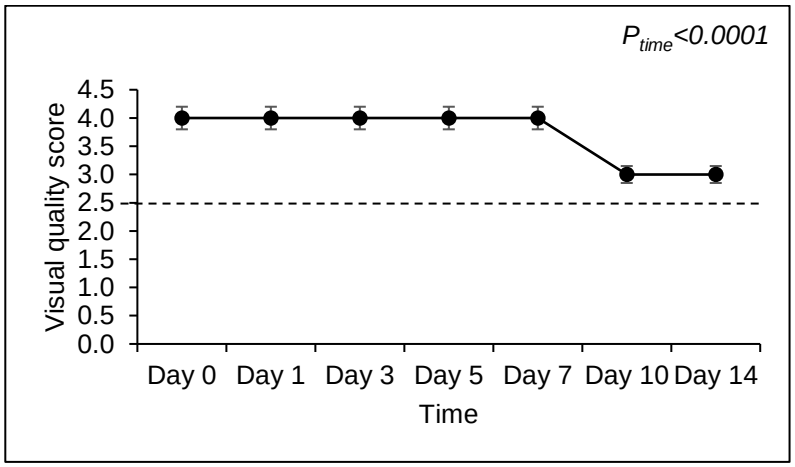






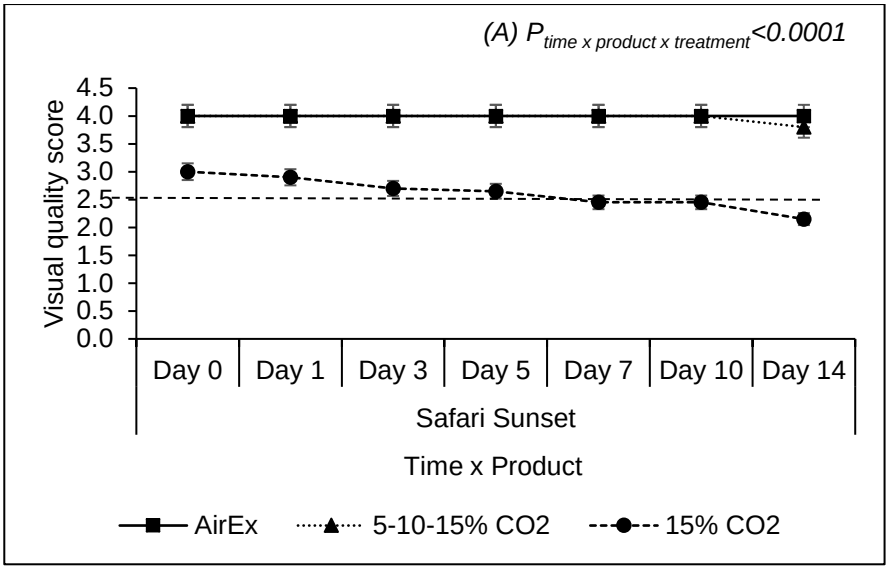
Effect	Product	Treatment	Product x Treatment	Time	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

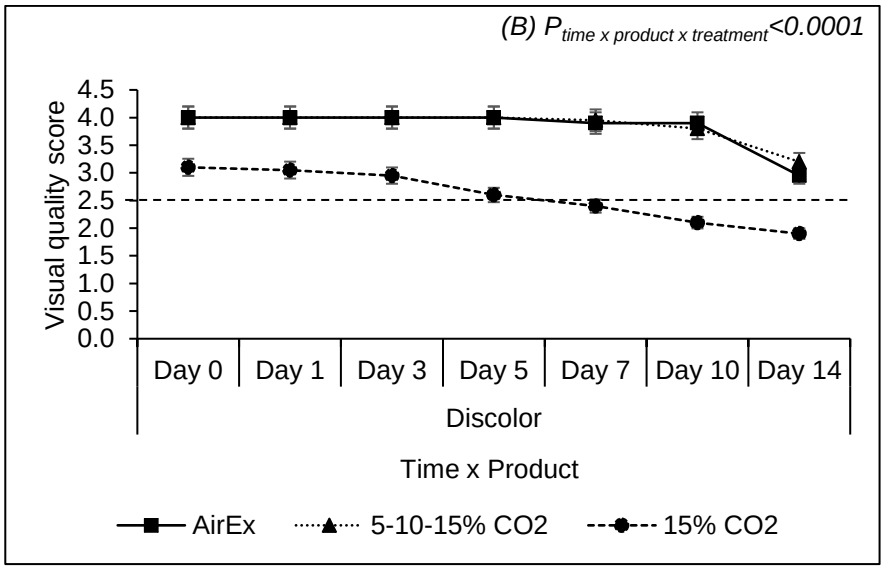
Fig. 9. Visual quality of flower heads of *Leucadendron* products [‘Safari Sunset’ (A), ‘Rosette’ (B), ‘Discolor’ (C) and ‘Rosette Conebush’ (D)] after AirEx, 5-10-15 % CO_2 and 15 % CO_2 storage for 21 d at 1 °C in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

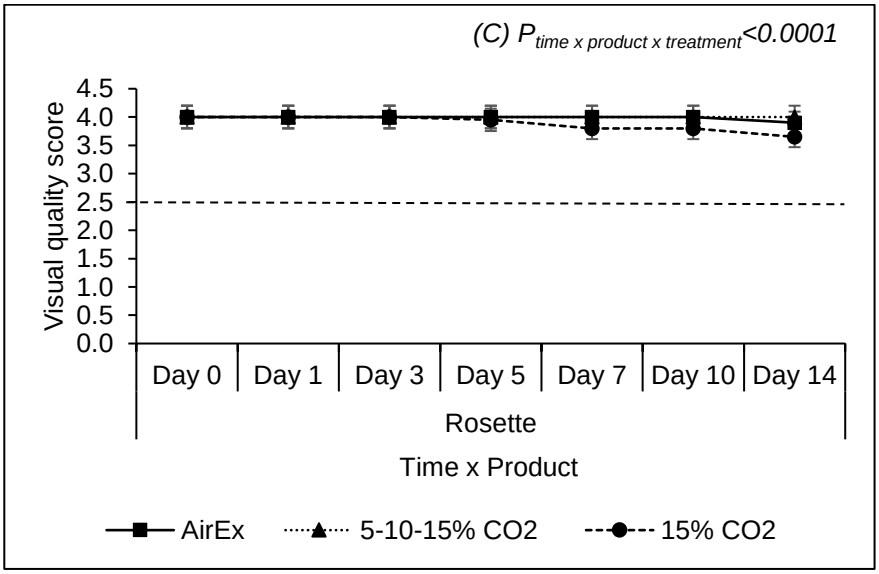


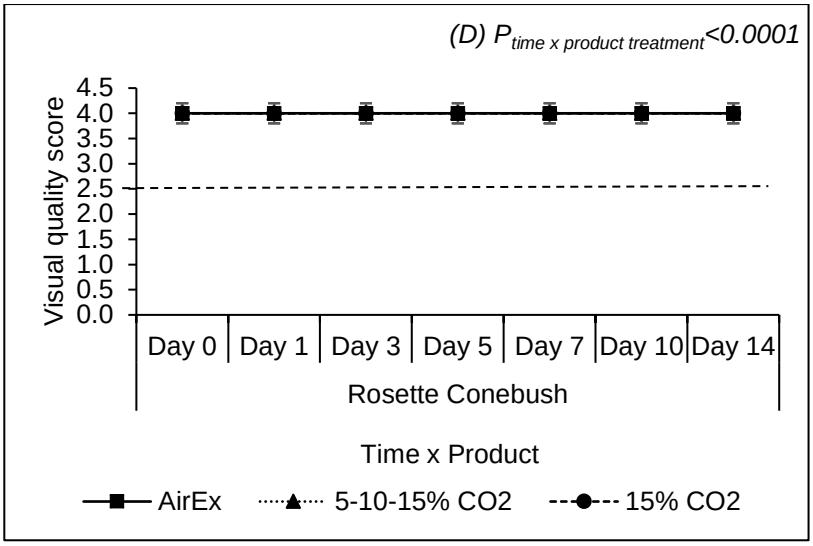
Effect	Treatment	Time	Time x Treatment
P value	*0.331ns*	*<0.0001*	*0.931ns*

Fig. 10. Visual quality of mature leaves of ‘Rosette’ stored in AirEx and 5-10-15 % CO_2 for 21 d at 1 °C in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



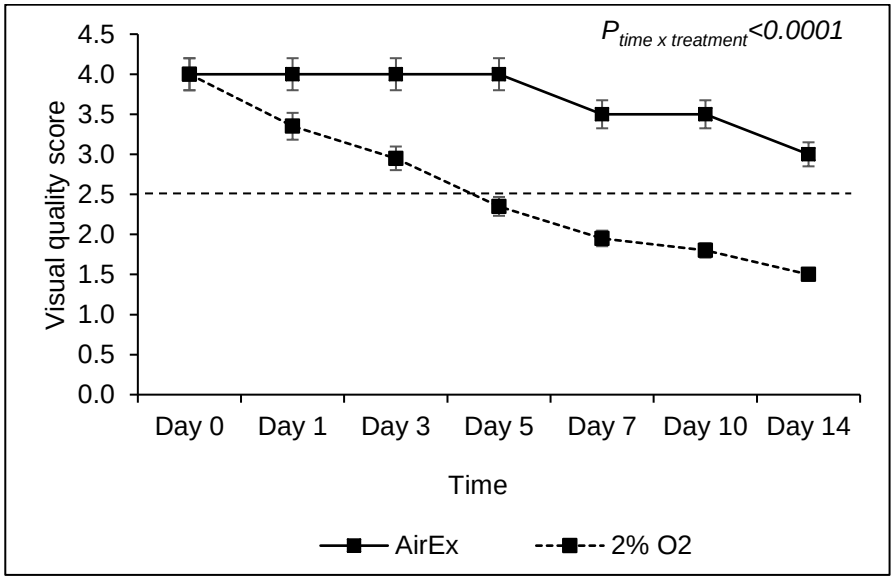






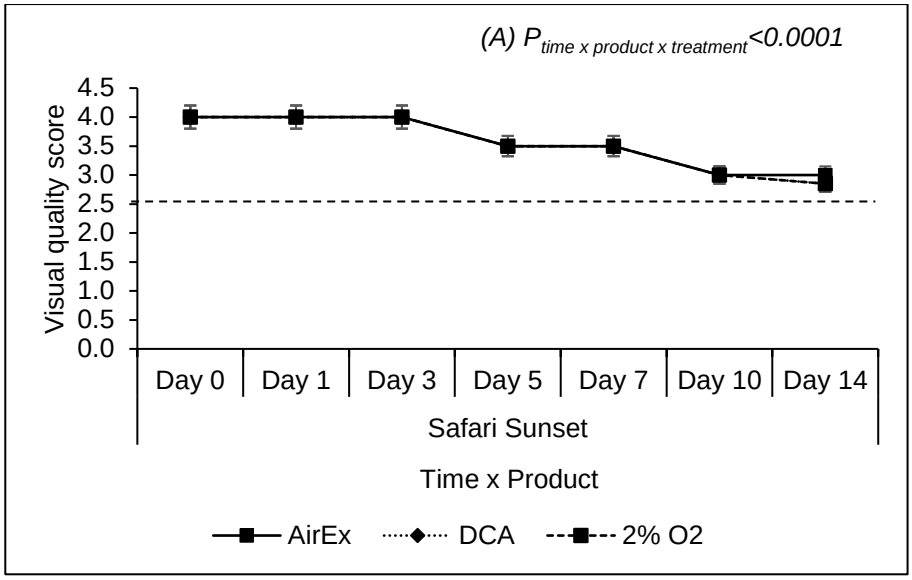
Effect	Product	Treatment	Product x Treatment	Time	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

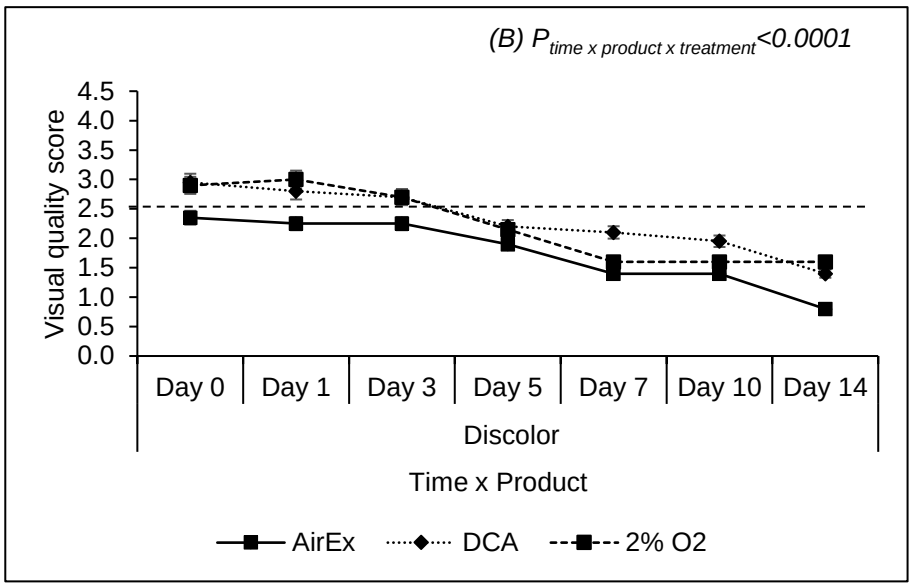
Fig. 11. Visual quality of mature leaves of *Leucadendron* products ['Safari Sunset' (A), 'Rosette' (B), 'Discolor' (C) and 'Rosette Conebush' (D)] after 21 d at 1 °C in AirEx, 5-10-15 % CO_2 and 15 % CO_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

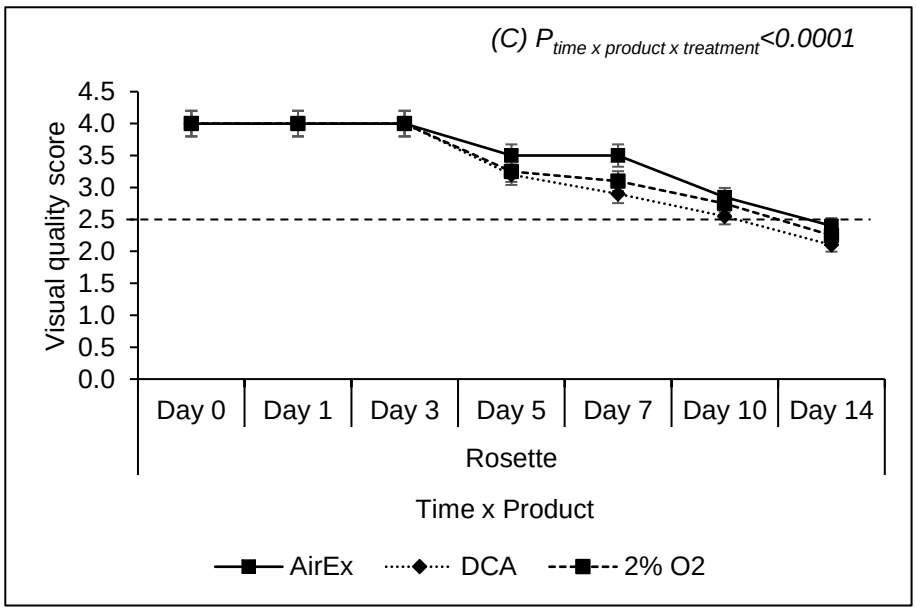


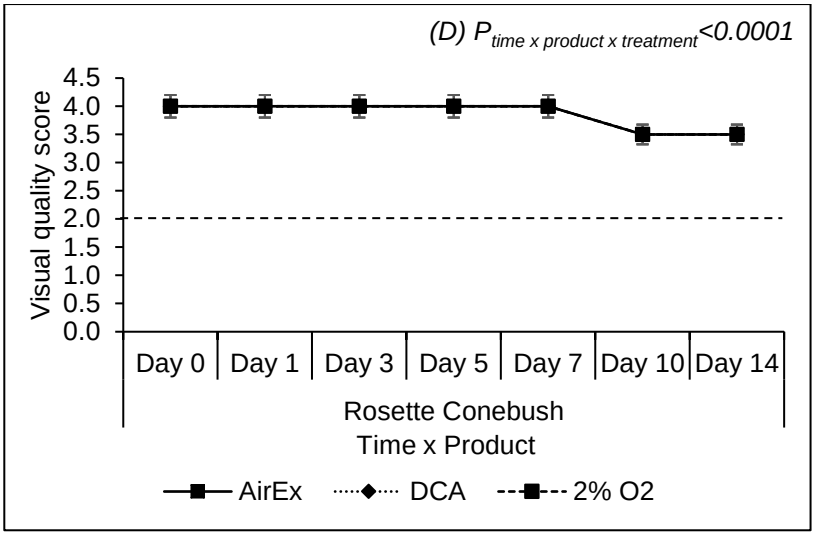
Effect	Treatment	Time	Time x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*

Fig. 12. Visual quality of flower heads of 'Rosette Conebush' following 21 d of cold storage at 1 °C in AirEx and 2 % O_2 in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



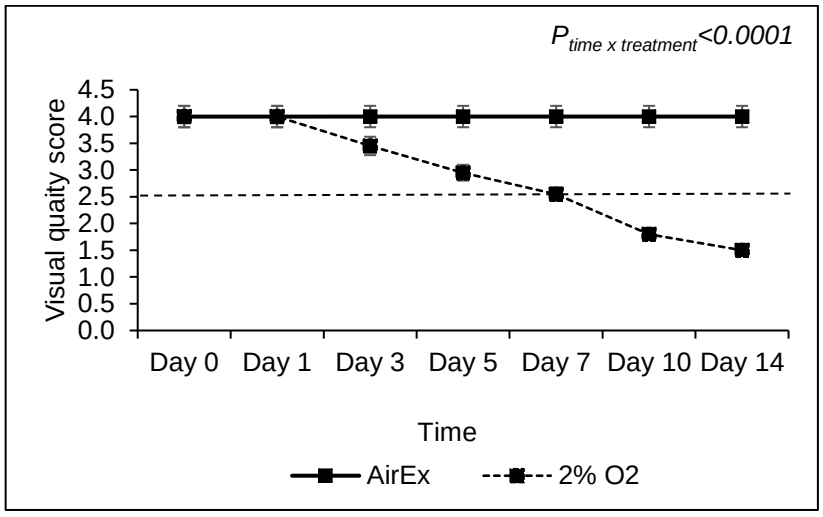






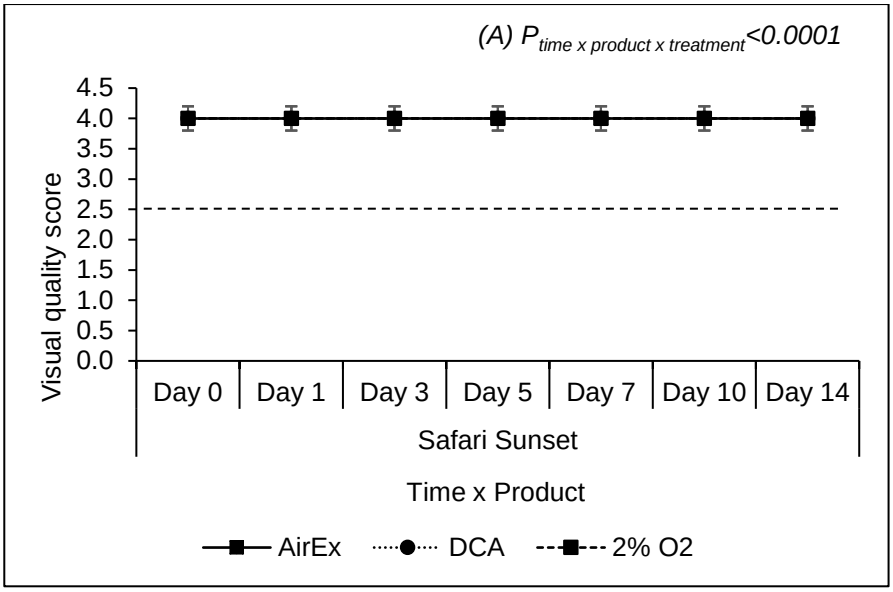
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.180ns*	*<0.0001*	*<0.0001*	*<0.0001*	*0.021*	*<0.0001*

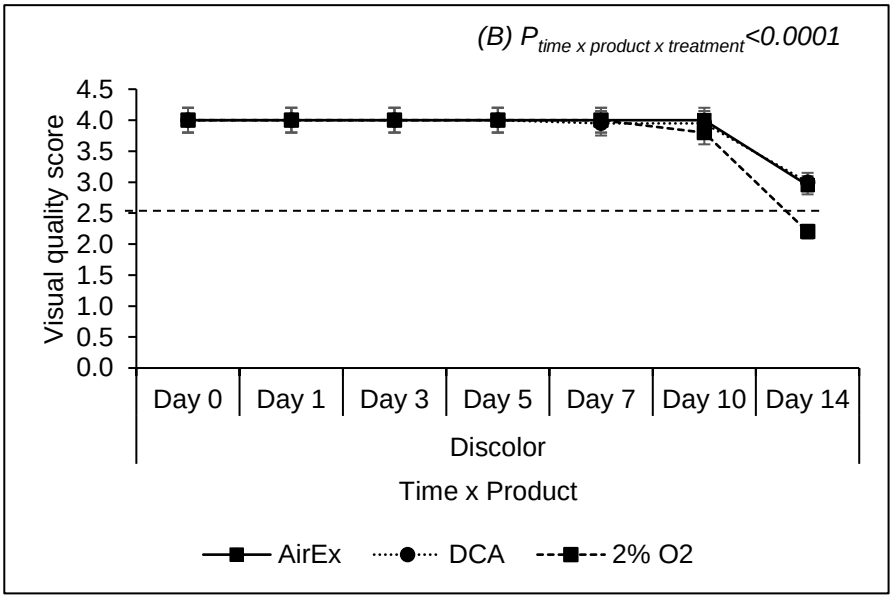
Fig. 13. Visual quality of flower heads quality of *Leucadendron* products ['Safari Sunset' (A), 'Discolor' (B), 'Rosette' (C) and 'Rosette Conebush' (D)] following 21 d of cold storage at 1 °C in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

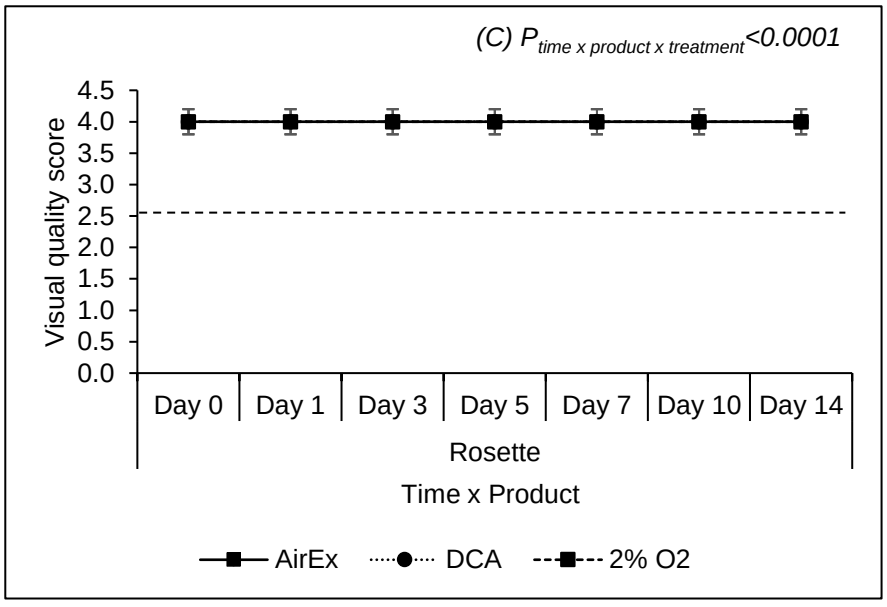


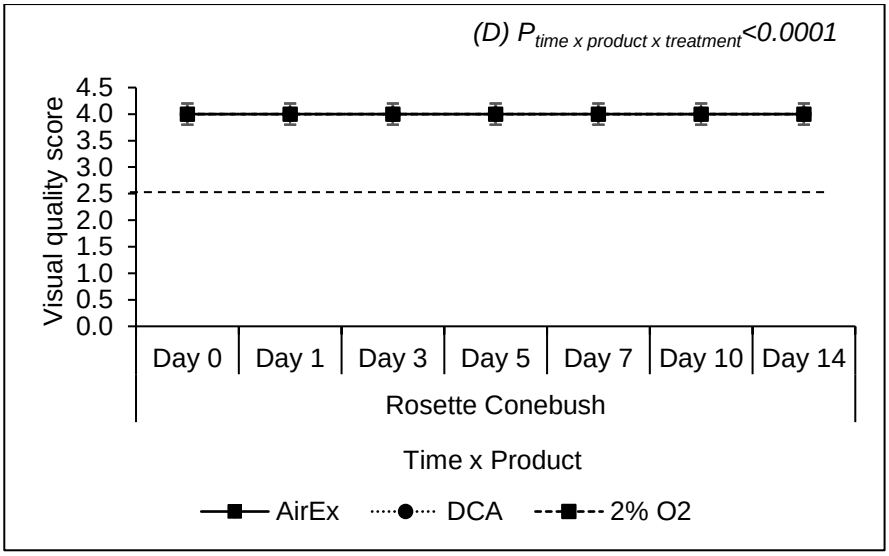
Effect	Treatment	Time	Time x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*

Fig. 14. Visual quality of mature leaves of 'Rosette Conebush' following storage in AirEx and 2 % O_2 for 21 d at 1 °C in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.









Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.006*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

Fig. 15. Visual quality of mature leaves of *Leucadendron* products [‘Safari Sunset’ (A), ‘Rosette’ (B), ‘Discolor’ (C) and ‘Rosette Conebush’ (D)] after storage in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 for 21 d at 1 °C in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

PAPER 3: FEASIBILITY OF CLOSED VENTILATION AND AUTOMATIC VENTILATION FOR SEA FREIGHT OF *LEUCOSPERMUM* CUT FLOWER STEMS

Abstract

Sea freight as a transport option is increasingly being used in export of *Proteaceae* cut flowers from South Africa (SA) to Europe, despite criticism on the suitability of this mode of transport for Cape Flora, along with often conflicting reports on the quality of products on arrival at their destination. The fixed, open air exchange ventilation system (AirEx), lengthy cold storage period and low storage temperatures of 1-2 °C used commercially have all been blamed for quality losses encountered during sea transport. Recent developments in sea freight technology have seen the invention of the automatic ventilation technology. This new technology relies on information on lower O_2 limit (LOL) and CO_2 toxicity limits of products to optimise sea freight shipping conditions. The use of automatic ventilation technologies during sea freight of *Leucospermum* cut flowers is currently not accessible as this information is unknown. This study thus aimed to evaluate the suitability of the closed ventilation (passive modified atmosphere) transport versus automatic ventilation during sea freight shipment of *Leucospermum* cut flower products from South Africa to The Netherlands. The trials involved storage in closed ventilation, determining the lower O_2 limit of the products, storage at low O_2 concentrations and evaluating the toxicity tolerance in high CO_2. The LOL was determined using a HarvestWatch™ dynamic controlled atmosphere (DCA) system attached to chlorophyll fluorescence sensors. DCA and 2 % O_2 were used to evaluate effects of allowing O_2 levels to drop to near LOL levels. The CO_2 toxicity tolerance was evaluated at 15 % CO_2 atmospheric concentration, which was based on gas dynamics data from preliminary closed ventilation experiments. Evaluations were done on moisture loss, hue angle, visual quality, carbohydrate content, total phenolics, respiration rate and lipid peroxidation. The LOL values for the products ranged from 0.08-0.41 % O_2. The respiration rate was between 21.66-45.44 mL $CO_2.kg^{-1}.h^{-1}$ in the 2015 season and between 19.06-34.52 mL $CO_2.kg^{-1}.h^{-1}$ in the 2016 season. The Q_{10} was between 2.45 and 4.60 for the 5 to 15 °C temperature range. The closed ventilation resulted in lower moisture loss and better visual quality compared to AirEx ventilation. Changes in hue angle were visually undetectable regardless of storage treatment. Mixed results were obtained for low O_2 and high CO_2 storage, which when unfavourable, then resulted in products with visual quality that was comparable to AirEx.

Monosaccharide plus oligosaccharide were more abundant than polysaccharides and starch and malondialdehyde (MDA), as well as phenolic content, generally differed among products.

Key words: Automatic ventilation; Closed ventilation shipping; CO_2 toxicity tolerance; Lower O_2 limit; *Proteaceae*

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1. Introduction

The key economically important Cape Flora and specifically *Proteaceae* cut flower products under cultivation in SA include; *Banksia, Leucadendron, Leucospermum* and *Protea*. Gerber and Hoffman (2014) reported the *Leucospermum* genus to make up 14% of the total export output. Inflorescences of *Leucospermum* occur singular or in clusters, with the separate florets comprising of a perianth made up of fused segments (Leonhardt and Criley, 1999; Venkata Rao, 1971). The perianth of mature florets reflexes to expose the pollen presenter, displaying a striking ribbon-like feature, which collectively provide the mature inflorescence a resemblance to a pincushion, thus earning these striking flowers the common name 'pincushion protea'. *Leucospermum* are sold both as cut flower stems and potted plants in the ornamental industry. Hoffman and Du Plessis (2013) claimed that almost all potted *Leucospermum* produced in SA is destined for the domestic market, as overseas markets are excluded due to the lack of suitable protocols for long-term cold storage as is required for sea freight, which is the only viable transport option for these bulky products. On the contrary, for *Proteaceae* cut flower stems the major destination is Europe, which makes up more than 85% of the market share.

Over the last decade, the SA *Proteaceae* industry has embarked on a shift from air- to sea freight, similar to other countries like Israel, Ecuador and Colombia. This is mainly driven by an unpredictable allocation of airfreight space together with high air freight rates charged for cut flowers (Philosoph-Hadas et al., 2007). However, the extended cold storage period required during sea-freight shipping may potentially pose quality problems, as air-freighted *Leucospermum* cut flowers will reach the European market within 2-3 d, whereas sea-freighted products require storage at temperatures as low as 1 °C over an extended period of up to 21 d. Low O_2 and high CO_2 atmospheres, as examples of modified atmospheres (MA), have been offered as possible alternatives to conventional regular atmosphere (RA) to alleviate disorders and retard senescence during cold storage (Wang, 1990; Saltveit, 2003). Benefits of MA are well documented and include that of decreased respiration, reduced oxidative stress, lower incidence of decay, improved tolerance to chilling injury (CI), decreased ethylene sensitivity and delayed tissue senescence (Halevy and Mayak, 1981; Hodges et al., 2004a; Kader, 2014; Meir et al., 1995; Philosoph-Hadas

et al., 2007). These findings concur with those made more recently for ornamental products by Aros et al. (2017) who reported MAP to extend storage and vase life of cut tulip flowers.

While passive and active MA storage have been highly effective in storage of fruit and vegetables, there has been no consistent and widely accepted commercially adopted MA protocols for ornamentals (Rani and Singh, 2014; Reid, 2016), despite the potential to maintain quality. As a caution, Hodges and Toivonen (2008) emphasised that modified atmosphere packaging (MAP) technologies for fresh-cut fruits and vegetables have often promoted anaerobic conditions and/or toxic CO_2 levels, with detrimental results to product quality. CO_2 injury will occur if CO_2 concentrations exceed tolerable levels, which may develop under conditions that promote anaerobic respiration. Furthermore, anaerobic respiration often leads to the production of ethanol, acetaldehyde, off-flavours and odours. Yet, despite this potential negative impact on product quality, successful long-term storage of cut flowers has been achieved through the implementation of low O_2 (Goliáš and Kobza, 2002) and high CO_2 atmospheric conditions (Philosoph-Hadas et al., 2010).

Beaudry and Gran (1993) stated that the lower O_2 limits of commodities are determined by temperature, nature of the product and cultivar or an interaction of these factors. Such LOL values have been reported to range from 0.15 % to 5 %. Reid (1997) listed the ranges of non-damaging O_2 and CO_2 concentrations for several ornamentals, but regrettably no data were documented on *Leucospermum*. Oxygen concentrations below the LOL are likely to trigger anaerobic fermentation, promote accumulation of acetaldehydes, ethanol, ethyl acetate and/or lactate and support ethylene synthesis (Kader, 1995; Kader and Saltveit, 2003). These atmospheric conditions may also result in the accumulation of ROS, which favour membrane lipid peroxidation and destroy membrane-, protein- and nucleic acid structures, cause solute leakage and generate toxic aldehydes and ketones (Rogers, 2012; Taiz et al., 2015; Wilhelmová et al., 2006; Wu et al., 2012; Xie et al., 2008).

Ethane and MDA levels are reliable estimates for the extent of lipid peroxidation in plant cells, by indicating the antioxidant status (Winston, 1990). The leaves of *Proteaceae* plants contain significant amounts of phenolics (Philosoph-Hadas et al., 2010), which are known to have antioxidant properties that assist to alleviate ROS effects (Hodges et al., 2004b; Lutz et al., 2015; Singh et al., 2009; Taiz et al., 2015). Thus, high phenolic content may be beneficial during long-term sea freight, which lasts about 21 d for products shipped from SA to Europe. However, the risk exists of encountering tissue discolouration as the polyphenol oxidase (PPO) enzyme can convert phenols to polyphenols under favourable conditions, which will negatively impact on product quality.

The AirEx ventilation in reefer containers used for transport of *Proteaceae* products is set by convention at a fixed rate of 15 000 $L.h^{-1}$. This ventilation regime is possibly responsible for over-ventilation and elevated moisture loss as it is based on the ventilation requirements of ethylene-sensitive fruit. Invention of new automatic ventilation technology for reefer transit now permits for greater control of ventilation, allowing atmospheric levels to approach near toxic levels instead of relying on a fixed ventilation rate, irrespective of respiration rates and product characteristics. Automatic ventilation technology is similar to DCA storage, where the O_2 level is adjusted according to product response to near-critical gas concentrations. The LOL is the O_2 level at which cell metabolism turns anaerobic. Gasser et al. (2010) and Wright et al. (2012) explained that the DCA technique involves maintaining the O_2 level near, but just above this limit. Unlike conventional CA approaches where gas concentrations and ventilation rates are fixed, DCA systems detect product stress through ethanol or chlorophyll fluorescence sensors, whereafter O_2 concentration is adjusted accordingly (Gasser et al., 2003; Prange et al., 2002; Wright et al., 2010). Of importance to note is that Mditshwa (2015) reported that fruit stored in DCA have higher ascorbic acid, total phenolics and antioxidant pools compared to fruit stored in regular atmosphere. Thus, DCA technology may actively assist to reduce oxidative stress, which would greatly benefit fresh produce stored under these conditions.

The gas exchange dynamics and ventilation requirements of Cape Flora cut flowers shipped from SA are largely unknown and have been a major limiting factor to the implementation of automatic ventilation as an alternative shipping technology. In addition, the suitability of current sea freight regimes for *Proteaceae* cut flowers that are shipped from SA to Europe is under question due to inconsistencies in product quality upon arrival at their markets. The low storage temperature, AirEx ventilation and lengthy cold storage period have been identified as possible causes of quality loss during sea freight to long distance markets.

The aim of this study was thus to investigate alternative storage techniques for *Leucospermum* stems, as important Cape flora cut flower export products, in order to optimise sea freight conditions and align with advances in reefer ventilation technology. The objectives of this study were firstly to measure respiration rate at 5 and 15 °C and monitor the gas dynamics in a closed ventilation system and evaluate the feasibility of long-term closed ventilation cold storage shipping technology for *Leucospermum* products. A next objective was to determine the lower O_2 limits for these products along with a final objective to determine the CO_2 toxicity tolerance limit for *Leucospermum* in order to recommend a safe atmospheric range for *Leucospermum* stems under cold storage and transport to export markets.

2. Materials and methods

2.1 Product variety and source

Various *Leucospermum* cut flower products were sourced from Tussenberge, Napier (34°29'10.3"S 19°44'10.7"E), Berghoff, Porterville (32°57'15.1"S 19°03'14.2"E), Floralae, Paarl (33°43'06.1"S 19°04'05.5"E) and Fynbloem, Riviersonderend (34°08'30.3"S 19°49'11.7"E) in the Western Cape, South Africa. Export grade flowers were used for this study and the selection of products evaluated was based on product availability (Table 1) and importance to the export market. Products that were analysed in the 2015 and 2016 seasons (Fig. 1) include the following selections and/or hybrids; 'Jelena' (*L. cordifolium* x *L. cuneiforme*), 'Goldie' (*L. cuneiforme*), 'Bright Eyes' (*L. cordifolium*), 'Fire Fly' (*L. cordifolium*), 'Soleil' (*L. glabrum* x *L. cordifolium*), 'Succession' (*L. cordifolium* x *L. lineare*), 'Rigoletto' (*L. cordifolium* x *L. glabrum*), 'Veld Fire' (*L. glabrum* x L. *conocarpodendron*), 'Super Gold' (*L. cordifolium* x *L. patersonii*) and 'Crimson' (*L. cordifolium*). The flowers were harvested and transported to the Cape Town International airport where they were held at 4 °C and collected within 24 h of harvesting. The experiments were conducted using ARC Infruitec-Nietvoorbij Stellenbosch cold rooms and Stellenbosch University Horticultural Science Department cold room and laboratory facilities.

2.2 Respiration rate

The flowers were stored for 24 h in cold rooms set at 5 °C and at 15 °C (±0.5 °C) upon arrival at the Department of Horticultural Science of Stellenbosch University. Five replicates were used per temperature per product and each replicate had three stems. The respiration analysis method by Jooste (2012) and Smit (2013) was used to measure CO_2. A 10 mL sample of headspace gas was drawn from gas tight containers after allowing gas exchange for 90 min. The CO_2 content of the sample was measured on a gas chromatograph (6890N, Agilent Technologies, California, USA) fitted with a thermal conductivity detector (TCD) and a Porapak Q section (Porapaq Q/Heysep Q, 80-100 mesh, Hayes Separations) and a Mole Sieve 5 A section (80-1000 mesh, Alltech Associates) column and the oven temperature was set at 80 °C. The GC uses helium as a carrier gas and 1 mL of headspace gas was manually injected for analysis. Respiration rates and temperature coefficients (Q_{10}) of the cut flowers were calculated using the formulas below:

$$\text{Respiration rate (mL.kg}^{-1}\text{.h}^{-1}\text{)} = \left(\frac{\%\ CO_2\ \text{produced}}{100}\right) x \left(\frac{\text{Volume free air (mL)}}{\text{Product weight (kg)}}\right) x \left(\frac{1}{\text{Time (h)}}\right)$$

$$Q_{10} = \left(\frac{\text{Respiration rate T2}}{\text{Respiration rate T1}}\right)^{\left(\frac{10}{T2-T1}\right)}$$

Where T_1 is 5 °C and T_2 is 15 °C.

2.3 Closed ventilation gas dynamics

Several *Proteaceae* products were stored in a Janny MT module™ (Janny MT, France) that consists of a rigid plastic box with a 610 L capacity and Tiempo cap lid cover at 1 °C (±0.5 °C) for 21 d to simulate sea shipping conditions during sea freight. The Janny MT boxes contained mixed loads of *Proteaceae*, which is a common practice under commercial shipping conditions. An estimated 45-55 % free air was attained inside the closed ventilation system in both the 2015 and 2016 seasons. Products in AirEx were used as the control, where a consistent ventilation rate of 15000 L fresh air.h^{-1} was maintained inside the cold room for 21 d. The boxes with control stems were loosely covered with plastics to reduce air flow and simulate the conditions in a fully loaded reefer. In the 2015 season, gas dynamics were monitored in an unventilated Janny MT box which contained 43.76 kg of mixed *Proteaceae* that included *Leucadendron* (5.6 kg 'Rosette Conebush'), *Leucospermum* (2.15 kg 'Jelena', 3.21 kg 'Goldie', 2.29 kg 'Bright Eyes' and 2.56 kg 'Fire Fly') and *Protea* (9.33 kg 'Barbigera', 7.36 kg 'Grandiceps' and 11.26 kg 'Red Rex') using a DCA monitoring system (AAFC and SAtlantic Inc., Nova Scotia, Canada). In the 2016 season, a hand held gas analyser (ICA, 250, Dual Analyser, Kent, U.K.) was used to improve simulation of the shipping conditions by reducing air removal from the system. The manually monitored Janny MT box contained a total mixed load of 43.31 kg which consisted of *Leucadendron* (9.63 kg 'Rosette Conebush') and *Leucospermum* (9.90 kg 'Soleil', 6.43 kg 'Succession', 12.27 kg 'Veld Fire' and 5.08 kg 'Rigoletto'). The O_2 and CO_2 concentrations were recorded on day 0, 4, 8, 12, 16 and 21 during storage.

2.4 Determination of lower O_2 limit

A HarvestWatch™ DCA (AAFC and SAtlantic Inc., Nova Scotia, Canada) system equipped with a fluorescence interactive response monitor (FIRM) sensor was used to determine the LOL values of the products as stated by Prange et al. (2003), with minor adjustments. The LOL was identified by lowering the O_2 concentration over a 48 h period until a clear stress peak was observed on a chlorophyll fluorescence spectrum. Products in low O_2 were stored in 2 % O_2 during the 2015 season. Storage near the LOL (LOL + 0.4 % O_2) was added as DCA treatment in the 2016 season, with CO_2 maintained at 0.04 % in both seasons. The temperature was maintained at 1 °C (±0.5 °C) for 21 d in all the treatments.

2.5 Determination of high CO_2 tolerance

The HarvestWatch™ DCA system was used to maintain the high CO_2 atmospheric composition with the Janny MT box. Storage temperature was at set at 1 °C (±0.5 °C) during the 21 d of simulated shipping. Products in high CO_2 systems were stored at 5 % CO_2 for the first week, where after the concentration was ramped to 10 % CO_2 in the second week and elevated again to 15 % CO_2 in the third week, in order to attain a 5-10-15 % CO_2 regime in the 2015 season. In the 2016 season, storage in a fixed 15 % CO_2 for the entire 21 d duration was included as an additional treatment, whilst O_2 was maintained at 12 % in both seasons.

2.6 Mass loss

Mass loss was determined by obtaining the difference in mass prior to and immediately following storage. The scale (EEW-5000, Everyweigher, UWE, Cape Town, South Africa) was accurate to 0.1 g. Ten replicates were evaluated per treatment per product. All products were stored for 21 d at 1 °C (±0.5 °C) in all the trials.

2.7 Colour determination of leaves

Colour readings were taken on ten replicates per treatment, before and immediately after storage. Hue angle readings were taken from the upper side of five leaves on each shoot using a chroma meter (CR400, Konica Minolta, Tokyo, Japan). Calibration was done using a standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203) prior to colour measurement and readings were taken in the CIE lightness (L*), chroma (C*), hue angle (h°) colour space. Red, yellow, green and blue colour have hue angles of 0 °, 90 °, 180 ° and 270 ° respectively (X-rite, 2007). The colour of inflorescences was not determined on inflorescences since the complex structure does not allow accurate measurements.

2.8 Visual quality evaluation

Visual quality was evaluated for 14 d after storage using 10 replicates per treatment per product. All products were kept at room temperature (20 °C ±2 °C) in tap water and visual quality scores for inflorescences and leaves were recorded on day 0, 1, 3, 5, 7, 10 and 14 of vase life. A visual quality scale ranging from 0 to 4 was interpreted as follows: 4 - Export grade quality, 3 - Good quality, 2 - Low quality with considerable damage, 1 - Poor quality with severe damage and 0 - Product to be discarded and considered totally unacceptable.

2.9 Soluble carbohydrates

Leaves were removed from ten replicate stems after 21 d of cold storage, frozen in liquid N_2 and stored at -80 °C until freeze-dried and milled. Carbohydrates were extracted and analysed according to the phenol-sulphuric acid method of Rohwer (2013), with slight modifications. Soluble monomers and oligosaccharides were extracted from 0.1 g samples using three consecutive washes in 80 % ethanol. The pellet was washed three times (24 h per wash) in de-ionised water to extract the water-soluble polysaccharide fraction. To extract and hydrolyse the starch fraction, the pellet was heated in sodium acetate and amyloglucosidase (AMG) buffer solution (pH=4.8) for 24 h. The temperature was maintained at 80 °C using digital heating blocks. All the extractions and analyses were done in triplicate. The amount of sugars in 10 µL of extract was determined spectrophotometrically at 480 nm on a UV-Visible spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia). A glucose standard curve derived from a serial dilution of a glucose standard (0.1 $mg.mL^{-1}$) was used to estimate the carbohydrate content and sugars were expressed as glucose equivalents.

2.10 Lipid peroxidation

Lipid peroxidation was measured according to Hodges et al. (1999), with slight adjustments. Fresh leaves were removed from ten replicate stems, milled in liquid N_2 and stored at -80 °C, until extraction and analysis was carried out. In brief, 1.0 g fresh weight (FW) was homogenized in 20 mL 80 % ethanol for each storage treatment. The probe was rinsed in 5 mL of 80 % ethanol, which was pooled with the initial extraction solution. The solution was then centrifuged for 10 min at 3000 g at 4 °C. A 1 mL sample from the supernatant was mixed with 1 mL thiobarbituric acid (TBA) solution and 10 µL of 1 % butylated hydroxytoluene (BHT) solution. The solution was vortexed and heated for 25 min at 95 °C. The heated solution was cooled on ice and centrifuged at 3000 g for 10 min. Absorbance was measured in glass cuvettes at 532 nm and 600 nm using a UV–Visible Spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia). The blank contained 1 mL 80 % ethanol, 10 µL BHT and 1 mL TBA. The MDA content was calculated using the formula below:

MDA equivalents ($nmol.mL^{-1}.g^{-1}$ FW) =

$$\left(\frac{\text{Volume of extraction solution (mL)}}{\text{Volume of supernatant (mL)}}\right) \text{x} \left(\frac{1}{\text{Sample mass (g)}}\right) \text{x} \left(\frac{\text{Abs 532 nm} - \text{Abs 600 nm}}{157\,000}\right) \text{x } 10^6$$

2.11 Total phenolic content

Leaves were obtained from ten replicate stems immediately after cold storage, placed in liquid N_2 and stored at -80 °C until being freeze dried and milled. Total phenolic content was measured according to the method of Rohwer (2016), with slight modifications. Briefly, 0.1 g DW of milled leaves were washed three consecutive times in 5ml 80 % ethanol for 20 min at 80 °C, whereafter extracts were pooled to yield 15 mL of extract. A 50 µL sample from the extract was added to 450 µL of folin-ciocalteu (FC) reagent and left to stand for five minutes. The solution was then mixed with 500 µL of 5.6 % Na_2CO_3, vortexed and allowed to react for 90 min. All the extractions and analyses were done in triplicate. Absorbance was read on a spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia) at 750 nm and a standard curve derived from a gallic acid (GA) standard was used to estimate the phenolic content. The total phenolic content in the leaves was expressed as gallic acid equivalents (GAE).

3. Statistical analyses

A complete randomized design was used and analysis of variance (ANOVA) and repeated analysis of variance (RANOVA) was performed on the data using Statistica (Statistica 13.2, Statsoft Inc., USA). Means were separated by LSD test ($P \leq 0.05$).

4. Results

4.1 Closed ventilation trial

4.1.1 Gas dynamics in closed ventilation and respiration rate

Gas dynamics results for the 2015 season showed that in a closed ventilation system containing a 43.76 kg mixed load of *Leucadendron*, *Leucospermum* and *Protea* products that was monitored using the HarvestWatch™ system, the O_2 concentration declined from 20.92 % to 8.42 %, whilst the CO_2 concentration rose from 0.06 % to 10.54 % during 21 d of storage (Fig. 2A). In the 2016 season, a hand held gas analyser was used to monitor a closed ventilation system with a 43.31 kg mixed load of *Leucadendron* and *Leucospermum* products and in this system the O_2 level fell from 20.5 % to 1.8 %, whereas the CO_2 concentration rose from 0.04 % to 17.4 % (Fig. 2B).

In the 2015 and 2016 seasons the respiration rates of the *Leucospermum* products at 5 °C were between 21.66-45.44 mL $CO_2.kg^{-1}.h^{-1}$ and 19.06-34.52 mL $CO_2.kg^{-1}.h^{-1}$ respectively. In the 2015 season, the Q_{10} values of the products ranged from 2.45 for ‘Crimson’ to 4.60 for ‘Bright

Eyes' for the 5 °C and 15 °C temperature range (Table 1). In the 2016 season, the Q_{10} values ranged from 2.61 for 'Veld Fire' to 3.86 for 'Rigoletto'.

4.1.2 Mass loss

No significant (p=0.526) interaction between the product and treatment was obtained in the 2015 season (Table 2). Treatment effect was, however, significant (p=0.003) as products stored in AirEx displayed considerably higher mass loss than products stored in closed ventilation. Product effect was also significant (p=0.011) where 'Bright Eyes' had a significantly higher mean mass loss compared to 'Jelena', 'Goldie' and 'Fire Fly' which did not differ significantly from each other. In the 2016 season, again no significant interaction (p=0.393) between product and treatment was found. As in 2015, treatment had a significant effect (p<0.0001), with mass loss being significantly higher for products stored in AirEx compared to those stored under closed ventilation.

4.1.3 Colour determination of leaves

No significant interaction between storage, product and treatment was observed (p=0.976) in the 2015 season. All interactions between main factors were non-significant except for a highly significant (p<0.0001) product effect (Table 3).

4.1.4 Visual quality: Inflorescences

A significant (p=0.014) interaction between time, product and treatment was obtained for the 2015 season. The quality of inflorescences of 'Jelena', 'Goldie' and 'Fire Fly' stored in AirEx and closed ventilation was similar on day 1. However, after 7 d of vase life, product stored in closed ventilation had better quality than product stored in AirEx (Fig. 3A, 3B, 3C and 3D). Quality of product stored in AirEx generally reached the minimum acceptable quality for retail before 7 d, whereas products stored in closed ventilation were still able to maintain quality above the 2.5 visual score level. However, 'Fire Fly' products were unsaleable by day 5, regardless of treatment (Fig 3D).

In the 2016 season the factors time, product and treatment produced a significant interaction (p=0.002). There were insignificant differences between 'Veld Fire' stored in AirEx and closed ventilation on day 1. After 7 d, product stored under closed ventilation had superior quality to those kept in AirEx (Fig. 4A). 'Rigoletto', 'Succession' and 'Soleil' (Fig. 4B) had insignificant quality differences between treatments on day 1 and day 7. In general, closed ventilation was more beneficial for 'Rigoletto' from day 5 onwards, whereas the benefits of closed

ventilation storage only became evident in ‘Succession’ after day 7. Quality of ‘Soleil’ stored in closed ventilation and in AirEx was more or less comparable, although storage under closed ventilation resulted in slightly better quality. However, ‘Succession’ had the best inflorescence quality after 7 d of storage, whilst ‘Rigoletto’ displayed the most severe quality loss.

4.1.5 Visual quality: Leaves

A significant interaction ($p=0.010$) between time, product and treatment was observed for the 2015 season. Leaf quality differences between the treatments were insignificant for all the products on day 1. Results on day 7 of vase life revealed that ‘Goldie’ stored in AirEx had a slightly better quality score than stems stored in closed ventilation, although this trend was reversed towards the end of vase life, when products stored under closed ventilation remained saleable for longer (Fig. 5A). Quality differences between treatments remained insignificant throughout the vase life period for ‘Jelena’ (Fig. 5B). For ‘Bright Eyes’ (Fig. 5C) and ‘Fire Fly’ (Fig. 5D) storage treatment effect was noted after the third day of vase life, whereafter stems that were stored in closed ventilation generally achieved a higher quality than stems stored under AirEx. However, the quality of ‘Fire Fly’ stems declined to below the minimum acceptable standard for both treatments after 5 d of vase life.

In the 2016 season no significant interaction ($p=0.190$) between time, product and treatment was obtained. Leaf quality was significantly influenced ($p<0.0001$) by an interaction between time and treatment. Differences in quality of products stored in AirEx and closed ventilation were non-significant on the first day of vase life, however quality loss occurred more rapidly in products stored in AirEx than stems stored in closed ventilation, which could retain a saleable product throughout the entire vase life period (Fig. 6A). A significant interaction was found between time and product for leaf quality ($p<0.0001$). Comparable quality of ‘Veld Fire’, ‘Succession’ and ‘Soleil’ leaves were observed on the first day of vase life (Fig. 6B). Leaf quality of these products were all superior to that of ‘Rigoletto’, where the quality of the leaves rendered the stems not saleable after the third day of vase life. ‘Succession’, to be followed closely by ‘Veld Fire’ displayed the best leaf quality throughout vase life, whilst ‘Rigoletto’ displayed a low tolerance for cold storage, already early on during the vase life evaluation.

4.1.6 Soluble carbohydrates

Carbohydrate content was significantly ($p<0.0001$) affected by an interaction between product and treatment in the 2015 season (Table 4). Monosaccharide plus oligosaccharide content

did not differ significantly between the AirEx and closed ventilation treatment in 'Bright Eyes' and 'Fire Fly'. However, storing 'Jelena' in AirEx resulted in significantly higher monosaccharide plus oligosaccharide concentrations compared to closed ventilation. Contrary to this, storage of 'Goldie' in closed ventilation resulted in considerably higher carbohydrate content than storage in AirEx.

4.2 High CO2 toxicity tolerance trial

4.2.1 Mass loss

No significant interaction between product and treatment ($p=0.969$) for mass loss in stems occurred in the 2015 season (Table 5). Treatment effect was also not significant ($p=0.127$), with only the product effect ($p=0.004$) that emerged as significant as 'Crimson' had higher moisture loss than recorded for 'Super Gold' stems.

In the 2016 season, product and treatment again did not produce a significant interaction ($p=0.240$) with regard to mass loss. Both treatment ($p<0.0001$) and product ($p<0.0001$) had a significant effect on moisture loss. Mass loss was far greater in product stored in AirEx compared to product stored in 5-10-15 % CO_2 or 15 % CO_2. A comparison of mass loss among cultivars identified 'Rigoletto' to be most susceptible to highest moisture loss, whilst 'Succession' had the lowest mass loss of all the products evaluated (Table 5).

4.2.2 Colour determination of leaves

A significant ($p=0.035$) interaction between storage, product and treatment emerged for the 2015 season (Table 6). Significant differences in hue angle were not observed due to either storage regimes, as significant differences in hue angle only existed between the various cultivars.

In the 2016 season however, a non-significant interaction between storage, product and treatment was achieved at the 5% confidence level ($p=0.055$). A significant interaction between storage duration and product was recorded, where the post-storage leaf hue angle of 'Rigoletto' and 'Soleil' products declined with storage, but 'Veld Fire' was unaffected by long-term cold storage (Table 6).

4.2.3 Visual quality: Inflorescence

Inflorescence quality was significantly ($p<0.0001$) affected by an interaction between time, product and treatment in the 2015 season. No significant differences in quality of 'Super Gold' stems stored either in AirEx or 5-10-15 % CO_2 were observed on the first day of vase life.

However, after 7 days of vase life the product treated with 5-10-15 % CO_2 displayed better quality than product stored in AirEx (Fig. 7A). 'Crimson' stems stored in AirEx and 5-10-15 % CO_2 both maintained export grade quality on the first day of vase life evaluation. However, by day 7 of vase life product stored in AirEx exhibited superior quality to that of stems stored in under elevated CO_2 levels (Fig. 7B).

Similar to the 2015 season, stem quality was significantly ($p=0.009$) affected by an interaction between time, product and treatment in the 2016 season. Quality of 'Rigoletto', 'Succession', 'Veld Fire' and 'Soleil' products stored in AirEx, 5-10-15 % CO_2 and 15 % CO_2 was comparable on the first day of vase life evaluation. Quality scoring on day 7 revealed that 5-10-15 % CO_2 and 15 % CO_2 storage regimes resulted in superior inflorescence quality in 'Rigoletto' compared to AirEx stored stems (Fig. 8A). 'Succession' (Fig. 8B) and 'Soleil' (Fig. 8D) stems, however, were not significantly affected by any of the storage regimes, whereas 'Veld Fire' stems stored in 5-10-15 % CO_2 had superior quality after 7 d, compared to the other treatments (Fig. 8C).

4.2.4 Visual quality: Leaves

A significant interaction ($p<0.0001$) between time, product and treatment for visual quality was reported for the 2015 season. 'Super Gold' stems stored in AirEx and 5-10-15 % CO_2 had similar quality on the first day of vase life. However, by day 7 of vase life, the mean quality score for 5-10-15 % CO_2 treated stems was higher than that of stems stored under AirEx (Fig. 9A). For 'Crimson', quality differences between the treatments were also not significant on day 1 of vase life (Fig. 9B), however stems stored in AirEx had significantly better quality by day 7 of vase life compared to stems stored under the high CO_2 regime.

Interaction between time, product and treatment significantly ($p<0.0001$) affected leaf quality in the 2016 season. Results for 'Rigoletto' (Fig. 10A) and 'Succession' (Fig. 10C) showed no significant differences in mean quality scores for stems held within AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments on day 1 of vase life, however all quality scores declined to below 2.5 by day 7 of vase life. Similarly, no significant differences in quality of 'Veld Fire' (Fig. 10B) and 'Soleil' (Fig. 10D) stored in AirEx, 5-10-15 % CO_2 and 15 % CO_2 were reported on the first day of vase life. However, storage of 'Veld Fire' in AirEx resulted in an unsaleable product by day 7 of vase life. To the contrary, 'Soleil' stored in 15 % CO_2 had the best leaf quality compared to the other treatments.

4.2.5 Soluble carbohydrates

No significant ($p=0.151$) interaction between product and treatment for carbohydrates was achieved in the 2015 season (Table 7). Nevertheless, monosaccharide plus oligosaccharide content were significantly influenced by the product effect as 'Crimson' stems recorded a much higher concentration of monosaccharide plus oligosaccharide than was determined for 'Super Gold' stems.

In the 2016 season a significant ($p<0.0001$) interaction between product, carbohydrate type and treatment was observed for total carbohydrate content (Fig. 11). Monosaccharide plus oligosaccharide content differences between 'Veld Fire' treated with 5-10-15 % CO_2 and 15 % CO_2 were minor. Furthermore, differences between AirEx and 15 % CO_2 treatment means were also not significant. Storage of 'Soleil' in AirEx and 15 % CO_2 resulted in higher monosaccharide plus oligosaccharide concentration than storage within the 5-10-15 % CO_2 regime. The data revealed that monosaccharide plus oligosaccharide content was considerably higher when 'Succession' stems were stored in 15 % CO_2, compared to the other treatments.

Results for 'Rigoletto' indicated that monosaccharide plus oligosaccharide was more abundant in product treated with AirEx and 5-10-15 % CO_2. Polysaccharide and starch content were not significantly affected by the treatments in 'Veld Fire', 'Soleil' or 'Succession' stems. However, polysaccharides were more abundant in 'Rigoletto' stored in 15 % CO_2 compared to product stored in AirEx or 5-10-15 % CO_2. Starch content of 'Rigoletto' was comparable among the treatments.

4.2.6 Lipid peroxidation

Product and treatment interaction did not significantly ($p=0.280$) influence the MDA content in the 2015 season (Table 8). Lipid peroxidation, however, was significantly ($p<0.0001$) affected by treatment and product ($p=0.001$). There was a much higher MDA content in stems stored in AirEx compared to that of stems stored in the 5-10-15 % CO_2 regime. Product comparison showed that MDA was also more abundant in 'Super Gold' compared to 'Crimson'.

Product and treatment interaction ($p=0.241$) and treatment effect ($p=0.291$) did not significantly affect lipid peroxidation in the 2016 season (Table 8). However, product produced a significant ($p<0.0001$) effect as 'Soleil' had higher MDA values than that recorded for 'Veld Fire' and 'Succession'.

4.3 Lower O_2 limit trial

4.3.1 Mass loss

Product and treatment interaction significantly ($p<0.0001$) affected mass loss in the 2015 season (Table 9). No significant moisture loss differences between the storage treatments were recorded for 'Succession', 'Soleil', 'Veld Fire' and 'Goldie'. Storage of 'Bright Eyes' in AirEx caused elevated moisture loss compared to storage of stems in 2 % O_2. However, data for 'Fire Fly' and 'Jelena' revealed the contrary in that mass loss was greater for product stored in 2 % O_2 compared to stems in AirEx.

In the 2016 season, no significant interaction between product and treatment was observed ($p=0.311$). Treatment, however, did have a significant ($p=0.021$) effect as the DCA treatment resulted in elevated mass loss compared to AirEx and 2 % O_2 treatments. The moisture loss results for stems stored under AirEx, DCA and 2 % CO_2 treatments were generally variable between the seasons, among treatments and among products (Table 9).

4.3.2 Colour determination of leaves

According to the 2015 hue angle results, no significant ($p=0.988$) interaction between storage, product and treatment was achieved (Table 10). Yet, an interaction between storage and product significantly ($p=0.006$) affected leaf colour. Storage did not cause significant hue angle difference in the leaves of 'Veld Fire', 'Bright Eyes', 'Goldie' and 'Jelena'. To the contrary, leaves of 'Soleil' and 'Fire Fly' stems had lower hue angle values after storage (122.7 ° and 124.9 °), compared to before storage (120.8 ° and 123.6 °). This shows a change of leaf colour from green to yellow.

During the 2016 season, a significant interaction between storage, product and treatment was obtained ($p=0.011$) (Table 10). Initial hue angle values for products under AirEx (129.2 °), DCA (128.8 °) and 2 % O_2 (129.6 °) were higher than the readings after storage (127.2 °, 122.8 ° and 125.9 ° respectively), which indicates change of colour from green to yellow.

4.3.3 Visual quality: Inflorescences

No significant interaction ($p=0.264$) for time, treatment and product was reported for in the 2015 season (Fig. 12). However, an interaction between product and time had significant influence ($p<0.0001$) on quality. Visual quality scores for the first day of vase life indicated only minor differences between 'Succession', 'Soleil', Veld Fire and 'Goldie', whilst 'Bright Eyes', 'Jelena', 'Fire Fly' and 'Succession' also maintained comparable quality. Furthermore,

differences between quality of 'Soleil', Veld Fire and 'Fire Fly' were also minor. However, by day 7 of vase life, 'Succession' and 'Soleil' recorded the highest quality ratings whilst 'Jelena', 'Goldie' and 'Fire Fly' had undergone considerable quality loss.

In the 2016 season, interaction between time, treatment and product significantly ($p<0.0001$) affected inflorescence quality. The stems exposed to the various treatments were similar on the first day of vase life, however by day 7, 'Veld Fire' stored in DCA had the highest quality, whereas quality scores for products stored in AirEx and 2 % O_2 recorded below 2.5 (Fig. 13A). To the contrary, by day 7 of vase life, storage of 'Succession' stems in DCA and 2 % O_2 resulted in better quality than that of stems stored in AirEx (Fig. 13C). On the same day the quality of 'Soleil' was, however, similar among the treatments (Fig. 13B). 'Rigoletto' stems stored in 2 % O_2 were still saleable on day 7, but stems stored in AirEx and DCA obtained quality scores of below 2.5 (Fig. 13D).

4.3.4 Visual quality: Leaves

Visual quality was not significantly ($p=0.707$) influenced by an interaction between time, treatment and product in the 2015 season (Fig. 14). However, a significant interaction ($p<0.0001$) between time and product was achieved. Stems of 'Succession', 'Jelena', 'Bright Eyes' 'Goldie' and 'Veld Fire' did not differ significantly on the first day of vase life, whereas 'Fire Fly' and 'Soleil' had considerably lower quality than the other products. By day 7, 'Succession', 'Veld Fire' and 'Bright Eyes' had the highest quality scores whereas 'Soleil', 'Goldie' and 'Fire Fly' scored scores of below 2.5.

In the 2016 season data showed that there was significant ($p=0.042$) interaction between vase life, product and treatment. Quality scores were similar among the treatments in 'Veld Fire' (Fig. 15A), 'Soleil' (Fig. 15B) and 'Succession' (Fig. 15C) products for the first day of vase life whereas the quality of 'Rigoletto' stems varied among the treatments (Fig. 15D). Comparison between cultivars stored in AirEx, DCA and 2 % O_2 by day 7 of vase life showed that 'Veld Fire' and 'Soleil' produced similar quality among the treatments, on day 7, whereas all the 'Succession' and 'Rigoletto' stems, irrespective of treatments, recorded scores below 2.5.

4.3.5 Soluble carbohydrates

Carbohydrate content was significantly ($p=0.009$) affected by product and treatment interaction in the 2015 season (Table 11). Treatments did not significantly influence carbohydrate levels in any of the 'Fire Fly', 'Bright Eyes' 'Goldie', 'Jelena' and 'Veld Fire' stems. However, monosaccharide plus oligosaccharide content of 'Soleil' product was significantly higher in

product stored in AirEx compared to 2 % O_2. Contrary to this, 'Succession' stored in 2 % O_2 had higher monosaccharide plus oligosaccharide content than the product stored in AirEx.

No significant ($p=0.119$) product, carbohydrate type and treatment interaction contributed to the observed differences in the 2016 season. Interactions between carbohydrate type and treatment ($p<0.0001$) and between product and carbohydrate type ($p<0.0001$) were however significant (Table 12). Carbohydrate content significantly differed among the treatments, where stems exposed to DCA treatment generally favoured a higher monosaccharide plus oligosaccharide content. Evaluation of polysaccharide content and starch content showed that these carbohydrates were generally unaffected by treatments. The highest monosaccharide plus oligosaccharide levels were recorded in 'Succession', which was followed by 'Soleil', whereas significantly lower values were measured in 'Veld Fire' compared to the other two cultivars. Polysaccharide content was higher in 'Rigoletto' and 'Soleil' compared to 'Veld Fire' and 'Succession'. No significant differences in starch content were detected among cultivars.

4.3.6 Total phenolic content

No significant interaction between product and treatment ($p=0.968$) was observed for the 2015 season (Table 13). Conversely, product effect was significant ($p<0.0001$) as phenolics were most abundant in 'Goldie', whilst 'Bright Eyes' and 'Veld Fire' recorded phenolics in the upper range, with 'Soleil' in the middle range and 'Jelena' and 'Succession' in the lower range, whereas significantly lower values was recorded in 'Fire Fly' compared to any of the other cultivars. In the 2016 season phenolic content was significantly ($p<0.001$) affected by product and treatment interaction (Table 13). Phenolic content among the treatments were comparable for 'Succession', 'Veld Fire' and 'Soleil'. However, phenolic content was considerably higher in 'Rigoletto' stored in DCA, whereas a much lower content was measured in stems stored in AirEx.

5. Discussion

5.1 Gas dynamics in closed ventilation and respiration rate

Using a hand held gas analyser to monitor the atmosphere inside the Janny MT boxes resulted in higher CO_2 and lower O_2 concentration than when using the HarvestWatch™ system. We observed that the HarvestWatch™ system is more suitable for gas composition monitoring of CA rooms than for the smaller Janny MT box system as the volume of gas required for sampling during monitoring of the Janny MT boxes under the HarvestWatch™ system resulted in a low pressure being generated inside the bins. This promoted the influx of normal atmospheric air into

the Janny MT boxes, which distorted the composition of the experimental storage atmosphere. Although the Janny MT boxes are generally considered airtight, extreme differences in pressure between the storage atmosphere inside the Janny MT box and that of the exterior atmosphere will promote movement of outside atmospheric air towards the low pressure system.

The mass, volume and respiration rates of the products along with the gas exchange between the closed ventilation system and the external atmospheric air affected the recorded air composition of a closed ventilation system. The closed ventilation creates a passive MA, which promotes lower respiration and increased RH, thereby decreasing moisture loss due to a smaller vapour pressure deficit between the plant tissue and the air surrounding the products (Wills et al., 2007). Although a 45-55 % free air content was achieved inside the closed ventilation Janny MT boxes, the amount of free air is much lower than this in a reefer used for sea freight (< 35 % free air). Therefore, different results will likely be obtained in a commercial trial, as the O_2 depletion and CO_2 accumulation will occur faster than in a Janny MT box, which has more free air and smaller temperature fluctuations. On the contrary, air leakage in reefers may result in distortion of the gas composition and delays in O_2 depletion and CO_2 accumulation compared to Janny MT boxes, which are more airtight than reefers.

The respiration rates for both seasons indicated that *Leucospermum* stems are high respiring products at 5 ^oC. Gas dynamics of the closed ventilation system are dependent on the respiration rate of the products and the amount and proportion of products in the system. Although the respiration rates that were determined at 5 ^oC and 15 ^oC, within 48 h of harvesting, and will likely vary during shipping storage at 1 ^oC, Q_{10} values were used to estimate the respiration rate at 1 ^oC. The main restriction of this method in its accuracy in predicting O_2 and CO_2 levels lies in its inability to accommodate changes in respiration rate, Q_{10} and respiratory coefficient, which result from changes in temperature, gas composition and storage duration (Beaudry et al., 1992; Kader et al., 1989; Lipton, 1990). The O_2 and CO_2 sensors that monitor gas concentrations in automatically ventilated reefers during sea freight shipping are thus, vital in providing real time gas concentrations. This further demonstrates the importance of advances in digitalisation and automation of sea freight shipping and the need to adopt new technologies

5.2 Mass loss

Mass loss from the plant tissue is affected by the water vapour gradient between the plant tissue and the external bulk air, and also the diffusional resistance to transpiration (Taiz et al., 2015). Products that were stored in AirEx generally lost more moisture compared to products

stored in closed ventilation. Thicker boundary layers, as would occur in the absence or with a reduction of air movement, such as under closed ventilation and in new advanced reefers with variable fan speed, result in lower moisture loss from fresh produce. The optimum RH for storage of Cape Flora ornamental product is considered to be around 95 %. There is lack of temperature control during defrosting cycles and no control of RH inside the reefers used for sea freight of *Proteaceae* products from SA and as a result, RH fluctuates greatly due to constant ventilation (M. Huysamer 2016, personal communication, 7 March). Storage of products below their optimum RH during transport or retail increases moisture loss from plant tissue (Bergquist et al., 2006; Herppich et al. 1999).

Despite the minor differences observed between the treatments in the first season, the second season's findings revealed that stems exposed to AirEx experienced greater mass loss than those kept either in 5-10-15 % CO_2 or 15 % CO_2. During constant ventilation in the AirEx system the water vapour gradient between the plant tissue and the storage atmosphere was possibly promoted by the consistent replacement of air. Additionally, this may be due to the lower ventilation rate and closing of stoma, which occurs under high CO_2 storage (Taiz and Zeigler, 2016). There were notable differences between products in both seasons, which are likely due to variability in product physiology, harvesting maturity, and/or growing conditions. Mass loss differences between AirEx and 2 % O_2 within the various cultivars were not significant in either of the two seasons. However, DCA treatment promoted mass loss in the products. The placement of the product may have been accidentally close to the ventilation valves, which would also have promoted the observed moisture loss with DCA technology.

5.3 Colour determination of leaves

The AirEx and closed ventilation treatments did not significantly affect leaf hue angle of the various products in either of the two seasons. Likewise, there was no visually detectable change in leaf hue angle between product stored in AirEx, 5-10-15 % CO_2 and 15 % CO_2, with hue differences that were only detected among products. Nevertheless, some products such as 'Rigoletto' and 'Soleil' recorded slight decreases in hue angle due to storage, which could be attributed to the yellowing of leaves commonly associated with dark storage (Kader, 1986). Darkness converts the phytochrome to the Pfr form, which causes colour changes in chlorophyll containing tissue (Van Doorn and Van Lieburg, 1993). Hoffman et al. (2015) demonstrated that use of light emitting diode (LED) lights (25 $\mu mol.m^{-1}.s^{-1}$) significantly reduced CI and leaf yellowing and reduced quality loss in *Leucospermum* potted plants during long-term storage at 0

°C. However, differences in leaf colour were visually indistinguishable in our study, and thus, had no effect on quality.

Storage in AirEx, DCA and 2 % O_2 treatments did not have a significant impact on leaf hue angle. While some products displayed lower hue angle readings after storage, compared to prior to storage, most products in general had non-significant hue angle changes. Changes in leaf colour were visually undetectable, except in cases where there was evidence of chilling damage on the leaves. Chilling injury was unlikely caused by treatment, as it is a common physiological disorder associated with *Leucospermum* as a result of extended exposure to the low storage temperature (Jing et al., 2009; Lafuente et al., 2005; Raison and Orr, 1990), in combination with harvesting maturity and/or growing conditions (Gonzalez-Aguilar et al., 2000; Hordijk, 2013; Lindhout, 2007).

5.4 Visual quality: Inflorescences

The closed ventilation treatment led to better inflorescence quality in the 2015 season as products stored in AirEx were generally unsaleable by day 7 of vase life evaluation. However, differences between inflorescence quality of product stored in AirEx and closed ventilation were not significant in the 2016 season, although a general trend implicated that the quality of stems stored in closed ventilation were also marginally better. Bishop et al. (2007) reported that closed ventilation storage of roses and carnations improved longevity by slowing respiration rate and reducing water loss from the flowers by assisting in maintaining a stable RH around the product. The stems exposed to AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments resulted in comparable quality generally in ‘Succession’, ‘Veld Fire’ and ‘Soleil’ during evaluation. However, storage in AirEx significantly lowered the inflorescence quality of ‘Super Gold’ and ‘Rigoletto’. Contrastingly, ‘Crimson’ stems stored in AirEx displayed better quality compared to stems stored in 5-10-15 % CO_2.

Differences in responses of cultivars to treatments are likely due to different tolerances to post harvest stresses that arise from variability in genetics, cultivation conditions, harvesting maturity, post-harvest handling and genetics, among other factors. Results of the first season showed little difference in stems stored either in AirEx or DCA. However, data from the second season revealed that although the inflorescence quality was initially similar, it became variable with the progression of vase life, depending on both the product and the particular treatment. The response to the storage treatments varied considerably among the products so that no specific trend in response to AirEx, DCA and 2 % O_2 could be identified.

5.5 Visual quality: Leaves

Storage in closed ventilation resulted in better leaf quality than that of stems stored under AirEx in both seasons. The findings concur with reports by Aros et al. (2017) and Bishop et al. (2007) who claimed that closed ventilation and MAP improved longevity of carnations and tulips. Further comparison of the products showed that the leaf quality of 'Succession' remained consistently high during vase life, whilst 'Rigoletto' stems distinctly senesced at a faster rate than those of other products evaluated. Again, differences between products can be ascribed to a range of factors, which include genetics, production area, cultivation conditions, harvesting conditions and harvesting maturity.

Conflicting findings were noted from the high CO_2 study. Some products such as 'Crimson', 'Rigoletto' and 'Succession' had better leaf quality when stored in AirEx. On the other hand, high leaf quality was favoured by the 5-10-15 % CO_2 or 15 % CO_2 storage regime in products such as 'Super Gold', 'Veld Fire' and 'Soleil'. In general, quality loss was less severe in products stored in AirEx. This implies that 5-10-15 % CO_2 and 15 % CO_2 treatment may have increased the severity to chilling damage, which was observed on some products. Of interest is that Girardi et al. (2005), together with Lee (2014) and Levin et al. (1995) noticed that a high CO_2 treatment prevented a decline in antioxidant activity and total phenolics content, thus causing a reduction of CI in fruit. However, despite a definite role for postharvest storage conditions to reduce CI, Schirra and Mulas (1995) reminded that the effectiveness of postharvest treatments in reducing chilling damage is also influenced by pre-harvest factors.

Results of the first season revealed that the AirEx, DCA and 2 % O_2 treatments generally had no significant effect on leaf quality. However, the effectiveness of the storage atmosphere in delaying leaf senescence seemed product dependent and the response to storage treatments was slightly variable. Products such as 'Veld Fire' and 'Soleil' were not significantly affected by storage treatments, while other products experienced an extended vase life due to 2 % O_2 ('Succession') and AirEx ('Rigoletto') storage regimes. Van Doorn and Han (2011) identified genetics, cultivation practices and storage conditions as the main factors that determine cut flower vase life longevity.

5.6 Soluble carbohydrates

Mixed results were obtained from the analysis of monosaccharide plus oligosaccharide content in *Leucospermum* products stored in AirEx and closed ventilation. Some products such as 'Bright Eyes' and 'Fire Fly' were not significantly different between the treatments, while storage of other products such as 'Jelena' resulted in higher monosaccharide plus oligosaccharide content

when stored in AirEx. On the other hand, 'Goldie' stored in closed ventilation had higher monosaccharide plus oligosaccharide content than when it was stored in AirEx. The AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments also impacted differently on the carbohydrate content among the products. In general, the findings for effect of storage treatment on monosaccharide plus oligosaccharide content of the products were inconclusive, although it was evident that the storage treatments did not have a significant effect on either polysaccharide content or starch content of all *Leucospermum* products.

The presence of monosaccharide plus oligosaccharide, polysaccharides and starch was marginally affected by the AirEx, DCA and 2 % O_2 treatments. 'Soleil' stored in AirEx had the highest monosaccharide plus oligosaccharide content compared to the other treatments where the 2 % O_2 treatment had a similar effect on monosaccharide plus oligosaccharide content of 'Succession'. Monosaccharide plus oligosaccharide content was more abundant compared to polysaccharide and oligosaccharide content. Long-term cold storage of cut lilies (Van Doorn and Han, 2011) and cut peony (Walton et al., 2010) has been reported to cause rapid starch and sucrose breakdown. Rise in reducing sugar content in plant tissue is a common defence mechanism against chilling or frost injury, which is a norm during long-term cold storage of cut flowers. Reducing sugar accumulation also aids in reduction of petal water potential and stimulates water inflow to permit cell enlargement and flower opening (Bodelón et al., 2010; Kumar et al., 2008). In general, starch levels were relatively low, compared to monosaccharide plus oligosaccharide, and polysaccharides, throughout the product range.

5.7 Lipid peroxidation

Although AirEx resulted in elevated MDA levels compared to 5-10-15 % CO_2 in 2015, the AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments produced inconsistent results between seasons. The amount of MDA formed during lipid peroxidation is often used as a sensitive diagnostic index of oxidative damage (Janero, 1990) and a higher MDA content in products stored in AirEx could be indicative of moisture stress since the products stored in AirEx also had higher moisture loss. However, results from the MDA analysis done in this study can not be used to explain product differences since stress tolerance is also affected by variation in growing conditions, harvesting maturity and differences between species.

5.8 Total phenolic content

Differences in leaf phenolic content appeared to be product dependent, whilst the storage regimes of AirEx, DCA and 2 % O_2 did not significantly influence total phenolic content in both

seasons. The quantity of phenolics produced by a plant is variable among products since it is determined by several pre-harvest and post-harvest factors (Bhattacharyya et al., 2014; Ling and Subramaniam, 2007; Manach et al., 2004; Zheng and Wang, 2001). Occurrence of high phenolic activity is an indication of high antioxidant activity, which should provide protection against metabolic stress. A detailed review on the production and role of phenolic compounds in response to biotic and abiotic plant stress was published by Taiz and Zeiger (2016). Studies by Rivero et al. (2001) also demonstrated that heat stress in tomatoes and cold stress in watermelons induced the production of phenolics.

6. Conclusions and recommendations

The O_2 concentration within a mixed load of *Proteaceae* products with 45-55 % free space dropped to about 8.5 % and the CO_2 concentration rose approximately 10 % in a closed ventilation setup monitored with HarvestWatch™ DCA system. In a similar experimental design, though monitored with a hand held gas analyser, the O_2 concentration fell to below 2 % whereas the CO_2 level rose above 17 %. Thus, gas dynamics during storage were quite variable among the trials as it was likely determined by a combination of the respiration rate of the products in storage, the amount of free air and the choice of gas sampling method. The amount of free air in a reefer used for the commercial long-term freight of *Proteaceae* is usually lower than 35 %. *Leucospermum* are high respiring products and proper maintenance of the cold chain is vital during sea shipping. The respiration rate of *Leucospermum* products 5 °C varied between 19.06-45.44 mL $CO_2.kg^{-1}.h^{-1}$. Storage in closed ventilation greatly decreased moisture loss compared to storage in AirEx, whilst moisture loss was comparable between AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments, despite inconsistencies between the seasons. The moisture loss results for stems exposed to AirEx, DCA and 2 % CO_2 treatments were generally inconsistent between seasons, among treatments and among products. The leaf hue angle appeared to be insignificantly affected by AirEx, low O_2 or high CO_2 treatments. Changes in hue angle were likely due to storage in darkness and differences among products. Storage in closed ventilation resulted in superior inflorescence and leaf longevity compared to storage in AirEx. The visual quality of products stored in AirEx, low O_2 and high CO_2 was comparable and treatment effect was not significantly different although product responses to the treatments were variable both within and between seasons. Furthermore, mixed findings were obtained from comparison of AirEx and high CO_2 since effect of storage atmosphere on visual quality was variable among products. Despite treatment effect being non-significant in the first season, leaf visual quality between the AirEx and low O_2 was comparable in the 2016

season. The monosaccharide plus oligosaccharide quantities in the product stored in AirEx were similar to those in products stored in low O_2 and in high CO_2 in the first season. Monosaccharide plus oligosaccharide were the most abundant sugars followed by polysaccharides, and starch was the least abundant in the leaves. The differences in amount of polysaccharides and starch were minor, between AirEx and low O_2 treatments, and also between AirEx and high CO_2 treatments. Storage in AirEx and 15 % CO_2 resulted in higher MDA content than storage in 5-10-15 % CO_2, which possibly means that lipid peroxidation was lowest under the latter treatment. Differences in total phenols in the leaves were likely due to product variability since differences among treatments were predominantly minor. These findings demonstrated that low O_2, closed ventilation and high CO_2 storage of *Leucospermum* products is feasible for use during long-term sea freight shipping. However, CA techniques are expensive and require strict monitoring of O_2 concentration to maintain its level above the lower O_2 limits of the products, which varied from 0.08-0.41 % O_2. As recommendations for automatic ventilation shipping, the minimum O_2 limit may be set at 2 % and the maximum CO_2 concentration may be set at 15 %. There is need to conduct small scale commercial trials to verify the results.

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8. Tables and figures

Table 1

Lower O_2 limit (LOL) values, respiration rates, temperature quotients (Q_{10}) and trial dates for *Leucospermum* products in the 2015 and 2016 seasons.

2015 Season									
Product	**Respiration at 5 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Respiration at 15 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Q_{10}**	**Estimated respiration at 1 °C**	**LOL (O_2 %)**	**Respiration rate date**	**CO_2 toxicity tolerance trial date**	**Closed ventilation trial date**	**LOL trial Date**
'Jelena'	40.14	124.56	3.10	25.53	0.33	20-Nov	-	11-Oct	30-Oct
'Fire Fly'	45.44	133.85	2.95	29.48	0.08	20-Nov	-	11-Oct	30-Oct
'Bright Eyes'	28.63	131.62	4.60	15.55	0.41	20-Nov	-	11-Oct	30-Oct
'Goldie'	26.95	108.74	4.04	15.42	0.25	20-Nov	-	11-Oct	30-Oct
'Super Gold'	21.66	53.18	2.46	15.11	-	08-Nov	14-Oct	-	-
'Crimson'	29.25	71.67	2.45	20.44	-	08-Oct	14-Oct	-	-
'Soleil'	28.35	95.54	3.37	17.44	0.08	08-Oct	-	-	13-Sep
'Succession'	39.53	123.78	3.13	25.04	0.25	08-Oct	-	-	13-Sep
'Veld Fire'	31.18	79.87	2.56	21.41	0.25	08-Oct	-	-	13-Sep
2016 Season									
Product	**Respiration at 5 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Respiration at 15 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Q_{10}**	**Estimated respiration at 1 °C**	**LOL (O_2 %)**	**Respiration rate date**	**CO_2 toxicity tolerance trial date**	**Closed ventilation trial date**	**LOL trial date**
'Succession'	34.52	101.83	2.95	22.39	-	03-Sep	01-Sep	09-Sep	02-Sep
'Veld Fire'	22.44	58.67	2.61	15.29	0.32	04-Sep	01-Sep	09-Sep	02-Sep
'Soleil'	19.06	55.36	2.90	12.45	0.16	04-Sep	01-Sep	09-Sep	02-Sep
'Rigoletto'	27.68	106.88	3.86	16.13	0.32	03-Sep	01-Sep	09-Sep	02-Sep

Table 2

Mass loss (%) in *Leucospermum* stems stored in AirEx and closed ventilation for 21 d at 1 °C in the 2015 season ('Bright Eyes', 'Jelena', 'Goldie' and 'Fire Fly') and the 2016 season ('Soleil', 'Succession', 'Veld Fire' and 'Rigoletto').

2015 Season			
Product			**Mean mass loss (%)**
'Bright Eyes'			7.1 a
'Jelena'			5.2 b
'Goldie'			5.8 b
'Fire Fly'			5.3 b
Treatment			
AirEx			6.5 a
Closed ventilation			5.2 b
Effect	Product	Treatment	Product x Treatment
P value	*0.011*	*0.003*	*0.526ns*
2016 Season			
Treatment			**Mean mass loss (%)**
AirEx			10.2 a
Closed ventilation			2.6 b
Product			
'Soleil'			5.1 ns
'Succession'			6.3
'Veld Fire'			6.2
'Rigoletto'			7.7
Effect	Product	Treatment	Product x Treatment
P value	*0.091ns*	*<0.0001*	*0.393ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 3

Leaf colour determination (h°) of *Leucospermum* products ('Fire Fly', 'Bright Eyes', 'Goldie' and 'Jelena') recorded before- and following storage in AirEx and closed ventilation for 21 d at 1 °C in the 2015 season.

2015 Season							
Product						**Mean hue angle (°)**	
'Fire Fly'						125.3	a
'Bright Eyes'						124.5	a
'Goldie'						124.3	a
'Jelena'						120.4	b
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*<0.0001*	*0.729ns*	*0.140ns*	*0.076ns*	*0.997ns*	*0.473ns*	*0.976ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 4.

Soluble carbohydrates (mg.g^{-1}) in leaves of *Leucospermum* stems ('Bright Eyes', 'Fire Fly', 'Jelena' and 'Goldie') after 21 d of cold storage at 1 °C in AirEx and closed ventilation in the 2015 season.

2015 Season (Monosaccharides + Oligosaccharides)			
Product	**Treatment**	**Mean glucose mg.g^{-1}**	
'Bright Eyes'	AirEx	36.5	cd
	Closed ventilation	43.1	c
'Fire Fly'	AirEx	36.1	cd
	Closed ventilation	28.3	d
'Jelena'	AirEx	56.4	b
	Closed ventilation	40.5	c
'Goldie'	AirEx	37.8	cd
	Closed ventilation	67.0	a

Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.226ns*	*<0.0001*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 5

Mass loss (%) in *Leucospermum* stems after storage for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Crimson' and 'Super Gold') in the 2015 season and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Rigoletto', 'Veld Fire', 'Soleil' and 'Succession') in the 2016 season.

2015 Season			
Product			**Mean mass loss (%)**
'Crimson'			9.3 a
'Super Gold'			6.6 b
Treatment			
AirEx			7.3 ns
5-10-15 % CO_2			8.7
Effect	Product	Treatment	Product x Treatment
P value	*0.004*	*0.127ns*	*0.969ns*
2016 Season			
Treatment			**Mean mass loss (%)**
AirEx			7.6 a
5-10-15 % CO_2			5.3 b
15 % CO_2			5.0 b
Product			
'Rigoletto'			7.9 a
'Veld Fire'			6.7 b
'Soleil'			5.9 b
'Succession'			3.6 c
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*<0.0001*	*0.240ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 6

Leaf colour determination (h°**)** of *Leucospermum* stems stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Crimson' and 'Super Gold') in the 2015 and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Rigoletto', 'Soleil' and 'Veld Fire') in the 2016 season.

2015 Season							
Product		**Treatment**		**Storage**		**Mean hue angle (°)**	
'Crimson'		AirEx		Before		124.8	a
				After		125.7	a
		5-10-15 % CO_2		Before		125.1	a
				After		124.8	a
'Super Gold'		AirEx		Before		120.5	b
				After		119.7	b
		5-10-15 % CO_2		Before		120.0	b
				After		120.1	b
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*<0.0001*	*0.841ns*	*0.899ns*	*0.821ns*	*0.165ns*	*0.709ns*	*0.035*

2016 Season							
Product				**Storage**		**Mean hue angle (°)**	
'Rigoletto'				Before		129.6	a
				After		122.4	d
'Soleil'				Before		124.4	c
				After		123.1	d
'Veld Fire'				Before		127.5	b
				After		126.9	b
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Treatment	Storage x Product	Storage x Product x Treatment
P value	*<0.0001*	*0.283ns*	*<0.0001*	*0.505ns*	*0.537ns*	*<0.0001*	*0.055ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 7

Soluble carbohydrates (mg.g^{-1}**)** in leaves of *Leucospermum* stems after 21 d of storage at 1 °C in AirEx and 5-10-15 % CO_2 in the 2015 season ('Super Gold' and 'Crimson').

2015 Season (Monosaccharides + Oligosaccharides)			
Product		**Mean glucose mg.g^{-1}**	
'Crimson'		101.5	a
'Super Gold'		28.8	b
Treatment			
AirEx		63.9	ns
5-10-15 % CO_2		66.5	
Effect	Treatment	Product	Product x Treatment
P value	*0.686ns*	*<0.0001*	*0.151ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 8

Lipid peroxidation (nmol.mL^{-1}.g^{-1}) in *Leucospermum* stems during 21 d of cold storage at 1 °C in AirEx, and 5-10-15 % CO_2 ('Super Gold' and 'Crimson') in the 2015 season and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 in the 2016 season ('Soleil', 'Veld Fire' and 'Succession').

2015 Season			
Treatment		**Mean MDA nmol.mL^{-1}.g^{-1}**	
AirEx		202.3	a
5-10-15 % CO_2		116.6	b
Product			
'Super Gold'		176.6	a
'Crimson'		142.3	b
Effect	Product	Treatment	Product x Treatment
P value	*0.001*	*<0.0001*	*0.280ns*
2016 Season			
Product		**Mean MDA nmol.mL^{-1}.g^{-1}**	
'Soleil'		171.2	a
'Veld Fire'		78.9	b
'Succession'		59.9	b
Treatment			
AirEx		104.6	ns
5-10-15 % CO_2		94.6	
15 % CO_2		110.8	
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.291ns*	*0.214ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance. Lipid peroxidation expressed as malondialdehyde (MDA) content.

Table 9

Mass loss (%) in *Leucospermum* stems during storage for 21 d at 1 °C in AirEx and 2 % O_2 ('Succession', 'Soleil', 'Veld Fire', 'Bright Eyes', 'Goldie', 'Fire Fly' and 'Jelena') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Rigoletto', 'Veld Fire', 'Soleil' and 'Succession') in the 2016 season.

2015 Season			
Product	**Treatment**	**Mean mass loss (%)**	
'Succession'	AirEx	4.6	df
	2 % O_2	6.4	cd
'Soleil'	AirEx	6.6	ce
	2 % O_2	6.4	cd
'Veld Fire'	AirEx	4.1	f
	2 % O_2	4.8	df
'Bright Eyes'	AirEx	7.0	c
	2 % O_2	4.8	def
'Goldie'	AirEx	5.2	cf
	2 % O_2	6.9	c
'Fire Fly'	AirEx	5.3	cf
	2 % O_2	12.1	a
'Jelena'	AirEx	6.2	cd
	2 % O_2	10.0	b
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*
2016 Season			
Treatment		**Mean mass loss (%)**	
AirEx		6.3	b
DCA		7.4	a
2 % O_2		6.4	b
Product			
'Veld Fire'		6.3	ns
'Soleil'		6.8	
'Succession'		6.9	
'Rigoletto'		6.7	
Effect	Product	Treatment	Product x Treatment
P value	*0.631ns*	*0.021*	*0.311ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 10

Colour determination (°) of *Leucospermum* stems stored for 21 d at 1 °C in AirEx and 2 % O_2 ('Soleil', 'Veld Fire', 'Bright Eyes', 'Goldie', 'Jelena' and 'Fire Fly') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 in the 2016 season ('Veld Fire', 'Rigoletto' and 'Soleil').

2015 Season							
Product			**Storage**			**Mean hue angle (°)**	
'Soleil'			Before			122.7	c
			After			120.8	d
'Veld Fire'			Before			127.5	a
			After			127.0	a
'Bright Eyes'			Before			123.1	c
			After			123.0	c
'Goldie'			Before			124.2	bc
			After			123.8	bc
'Jelena'			Before			120.4	d
			After			120.0	d
'Fire Fly'			Before			124.9	b
			After			123.6	c
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Treatment	Storage x Product	Storage x Product x Treatment
P value	*<0.0001*	*0.933ns*	*<0.0001*	*0.766ns*	*0.454ns*	*0.006*	*0.988ns*
2016 Season							
Product		**Treatment**	**Storage**			**Mean hue angle (°)**	
'Veld Fire'		AirEx	Before			127.4	eb
			After			127.0	bde
		DCA	Before			127.0	be
			After			126.6	cef
		2 % O_2	Before			128.7	ab
			After			128.3	abc
'Soleil'		AirEx	Before			124.4	ghi
			After			123.7	hi
		DCA	Before			124.9	fh
			After			123.9	hi
		2 % O_2	Before			124.7	gh
			After			124.1	ghi
'Rigoletto'		AirEx	Before			129.2	a
			After			127.2	bde
		DCA	Before			128.8	ad
			After			122.8	i
		2 % O_2	Before			129.6	a
			After			125.9	efg
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*<0.0001*	*0.030*	*<0.0001*	*0.086ns*	*<0.0001*	*0.015*	*0.011*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 11

Soluble carbohydrates ($mg.g^{-1}$) in leaves of *Leucospermum* products after cold storage for 21 d at 1 °C in AirEx and 2 % CO_2 in the 2015 season ('Bright Eyes', 'Goldie', 'Jelena', 'Fire Fly', 'Veld Fire', 'Soleil' and 'Succession').

2015 Season (Monosaccharide + Oligosaccharide)			
Product		**Treatment**	**Mean glucose $mg.g^{-1}$**
'Bright Eyes'		AirEx	86.1 a
		2 % O_2	77.4 a
'Goldie'		AirEx	33.3 def
		2 % O_2	34.0 cdef
'Jelena'		AirEx	29.0 fg
		2 % O_2	31.2 efg
'Fire Fly'		AirEx	41.9 cd
		2 % O_2	40.1 cde
'Veld Fire'		AirEx	21.0 g
		2 % O_2	30.4 efg
'Soleil'		AirEx	44.0 bc
		2 % O_2	30.2 efg
'Succession'		AirEx	40.6 cde
		2 % O_2	52.4 b
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.992ns*	*0.009*

*****Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 12

Soluble carbohydrates (mg.g^{-1}**)** in leaves of *Leucospermum* products after cold storage for 21 d at 1 °C in AirEx, dynamic controlled atmosphere (DCA) and 2 % CO_2 in the 2016 season ('Rigoletto', 'Veld Fire', 'Soleil' and 'Succession').

		2016 Season	
Carbohydrate type		**Treatment**	**Mean glucose mg.g^{-1}**
Monosaccharides + Oligosaccharides		AirEx	89.9 b
		DCA	102.5 a
		2 % O_2	92.2 b
Polysaccharides		AirEx	15.2 c
		DCA	16.1 c
		2 % O_2	16.8 c
Starch		AirEx	13.3 d
		DCA	12.8 d
		2 % O_2	11.2 d
Carbohydrate type		**Product**	
Monosaccharides + Oligosaccharides		'Veld Fire'	80.7 c
		'Soleil'	95.1 b
		'Succession'	107.7 a
		'Rigoletto'	95.9 b
Polysaccharides		'Veld Fire'	14.2 e
		'Soleil'	18.7 d
		'Succession'	12.7 e
		'Rigoletto'	18.7 d
Starch		'Veld Fire'	10.3 ef
		'Soleil'	10.5 ef
		'Succession'	12.7 f
		'Rigoletto'	11.6 ef

Effect	Product	Carb type	Treatment	Product x Treatment	Product x Carb type	Extraction x Treatment	Product x Carb type x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*0.823ns*	*<0.0001*	*<0.0001*	*0.119ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 13

Total phenolic content (mg.g^{-1}) in leaves of *Leucospermum* stems following storage for 21 d at 1 °C in AirEx and 2 % O_2 in the 2015 ('Goldie', 'Bright Eyes', 'Veld Fire', 'Soleil', 'Jelena', 'Succession' and 'Fire Fly') and 2016 season ('Succession', 'Rigoletto', 'Veld Fire' and 'Soleil').

2015 Season			
Product			**Mean GAE mg.g^{-1}**
'Goldie'			112.6 a
'Bright Eyes'			99.5 b
'Veld Fire'			91.5 bc
'Soleil'			83.5 c
'Jelena'			57.7 d
'Succession'			50.5 d
'Fire Fly'			31.0 e
Treatment			
AirEx			76.4 ns
2 % O_2			74.0
Effect	Treatment	Product	Product x Treatment
P value	*0.295ns*	*<0.0001*	*0.968ns*
2016 Season			
Product	**Treatment**		**Mean GAE mg.g^{-1}**
'Succession'	AirEx		129.4 e
	DCA		128.3 e
	2 % O_2		143.8 de
'Rigoletto'	AirEx		162.9 cd
	DCA		224.8 a
	2 % O_2		200.2 b
'Veld Fire'	AirEx		158.7 cd
	DCA		158.7 cd
	2 % O_2		165.4 c
'Soleil'	AirEx		162.8 cd
	DCA		158.6 cd
	2 % O_2		161.4 cd
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.006*	*<0.0001*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance. Phenolic content expressed as gallic acid equivalents (GAE).

A 	**B** 	**C** 
D 	**E** 	**F** 

Fig. 1. *Leucospermum* products [‘Goldie’ (A), ‘Bright Eyes’ (B), ‘Jelena’ (C), ‘Fire Fly’ (D), ‘Succession’ (E), ‘Veld Fire’ (F), ‘Soleil’ (G), ‘Crimson’ (H), ‘Super Gold’ (I), ‘Rigoletto’ (J)] evaluated in the 2015 and 2016 seasons.

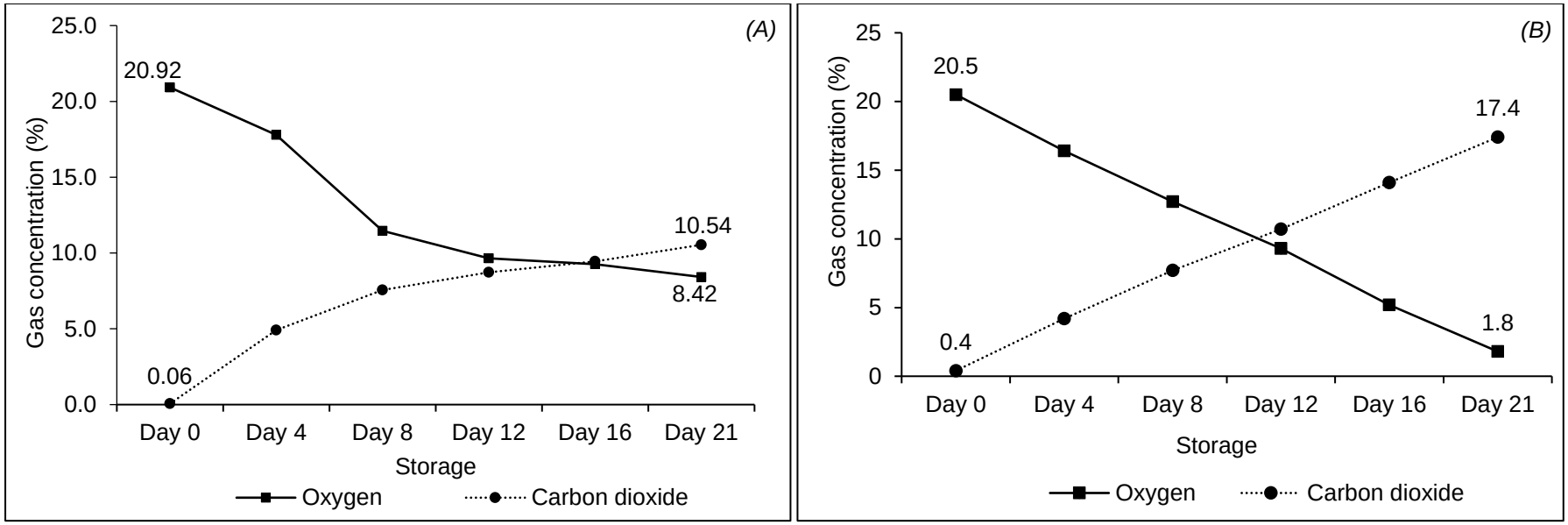
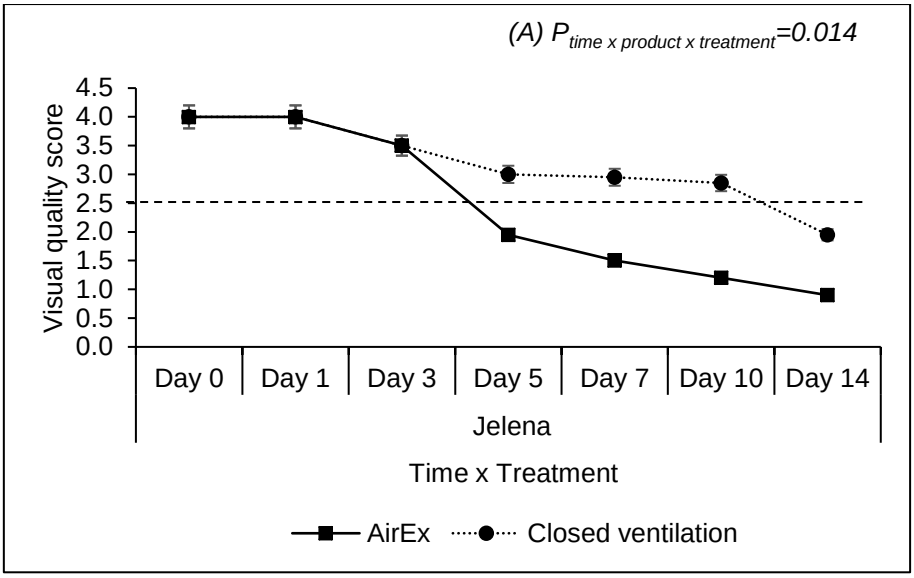
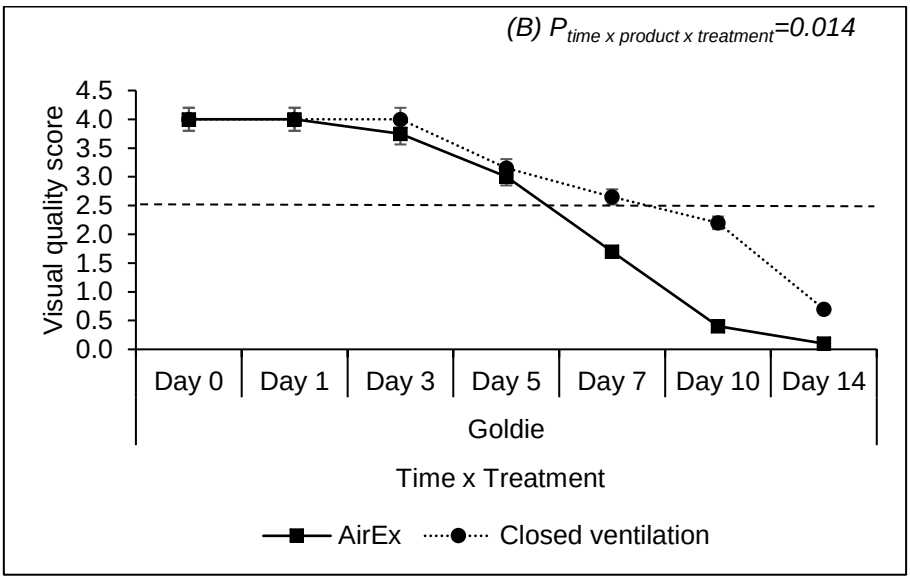
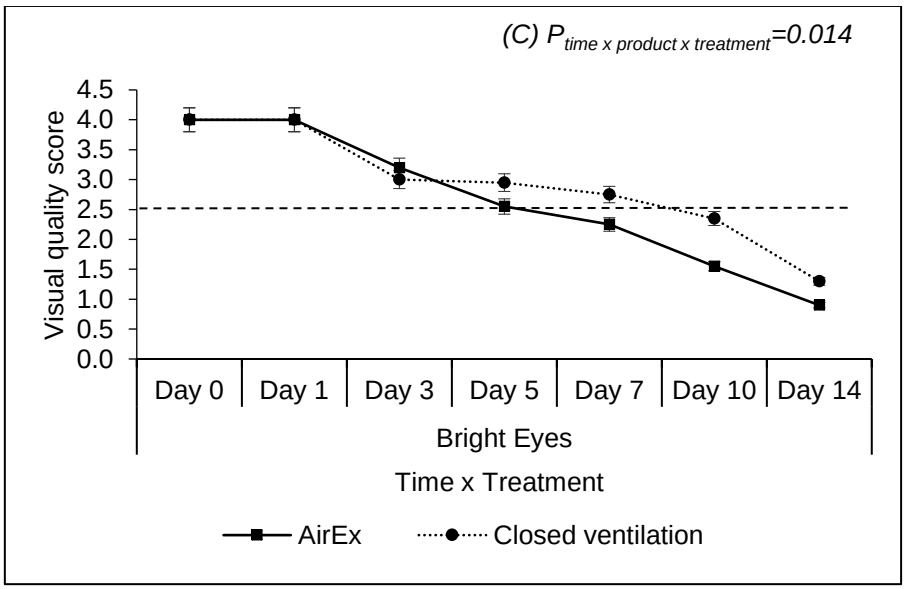
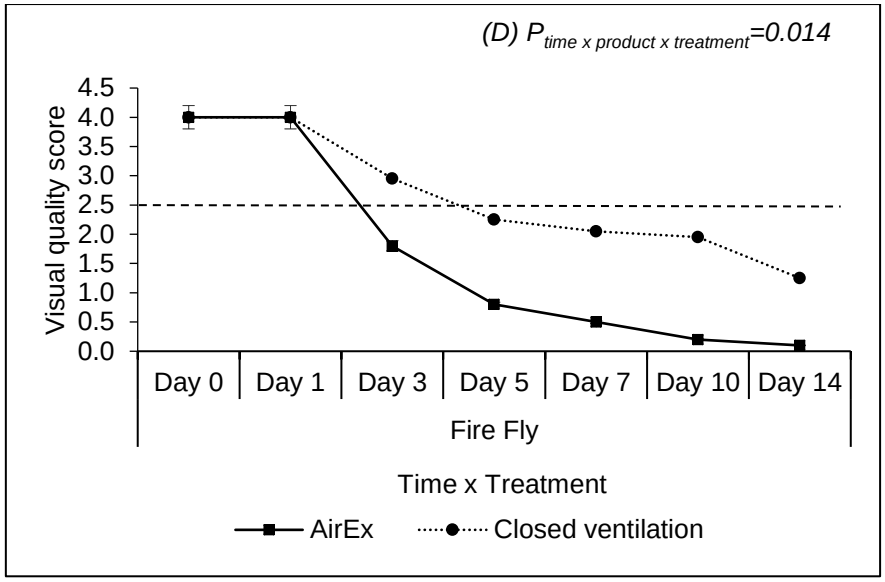


Fig. 2. Gas dynamics in a closed ventilation system monitored using HarvestWatch™ DCA system (Fig. 2A) and loaded with a mixture of *Leucadendron* ('Rosette Conebush'), *Leucospermum* ('Jelena', 'Goldie', 'Bright Eyes' and 'Fire Fly') and *Protea* ('Barbigera', 'Grandiceps' and 'Red Rex') products in 2015. Gas concentration in a closed ventilation system monitored using a hand held gas analyser (Fig. 2B) and containing a mixed load of *Leucadendron* ('Rosette Conebush') and *Leucospermum* ('Soleil', 'Succession', 'Veld Fire' and 'Rigoletto') products in the 2016 season.



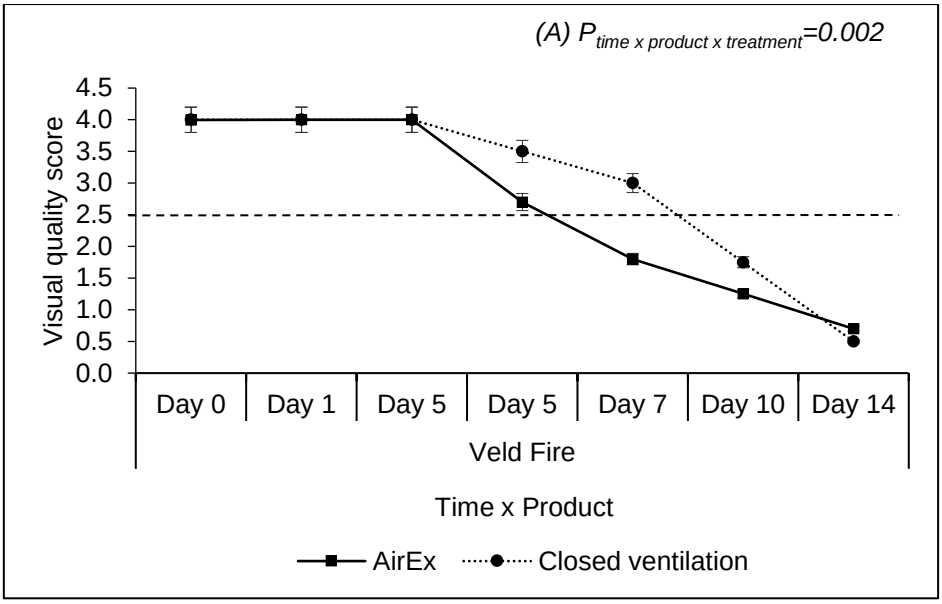


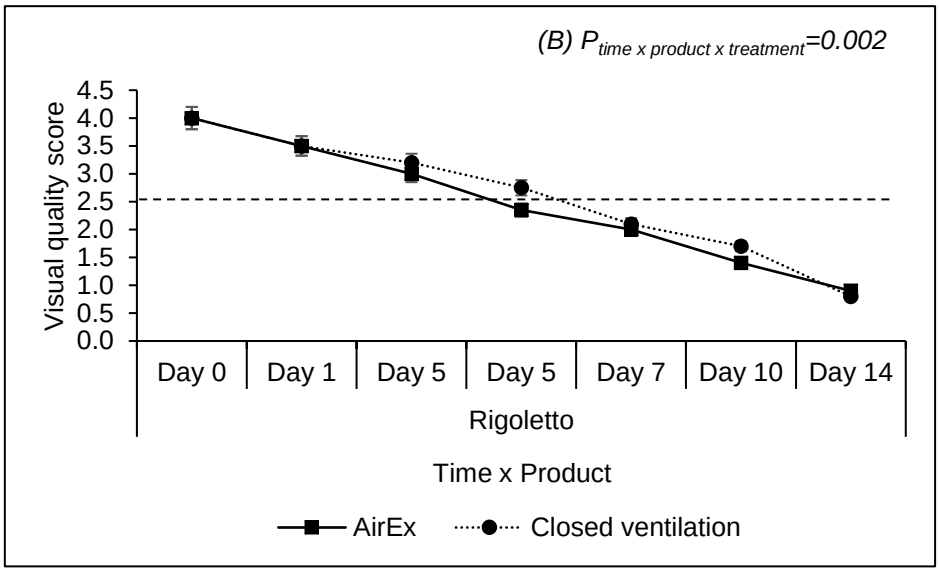


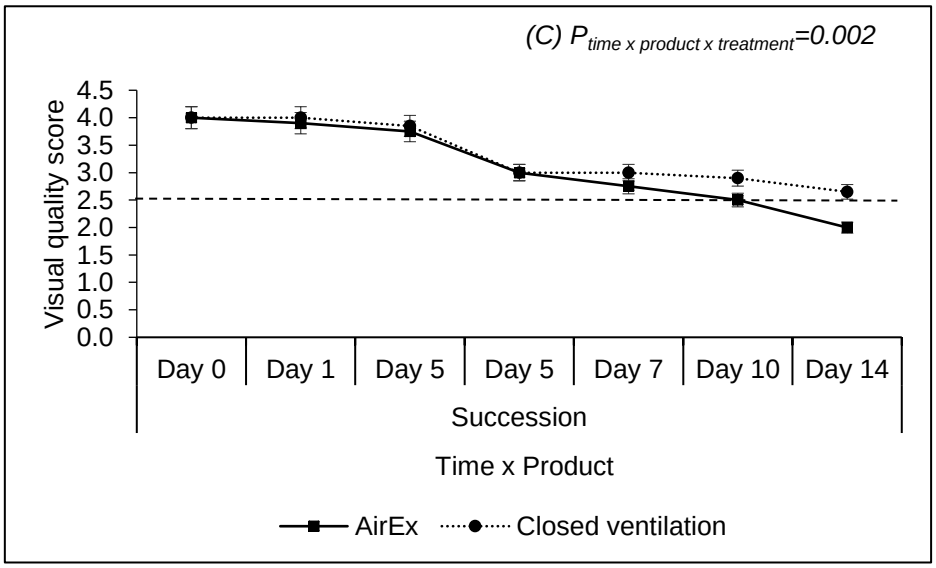


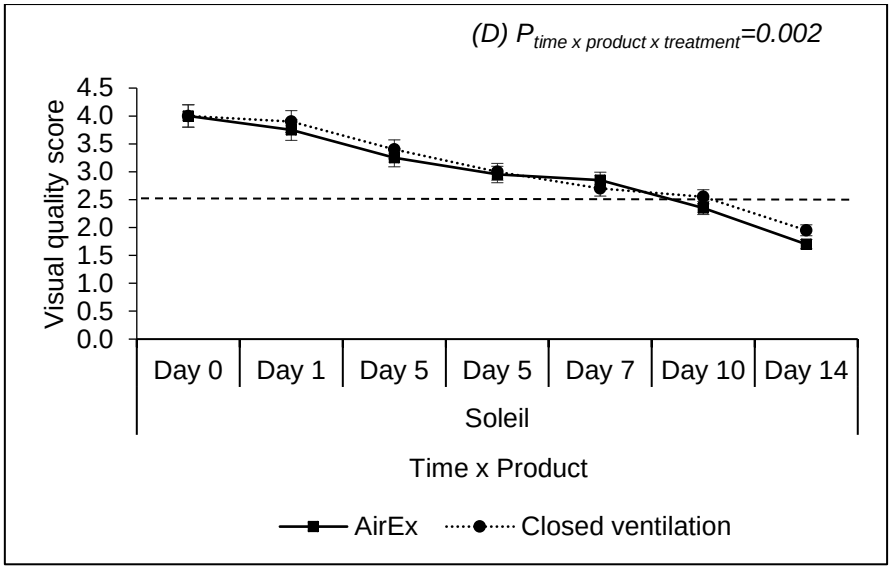
Effect	Product	Treatment	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*0.042*	*<0.0001*	*<0.0001*	*0.014*

Fig. 3. Visual quality of inflorescence of *Leucospermum* products ['Jelena'(A), 'Goldie' (B), 'Bright Eyes' (C) and 'Fire Fly' (D)] after 21 d of cold storage at 1 °C in AirEx and closed ventilation in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



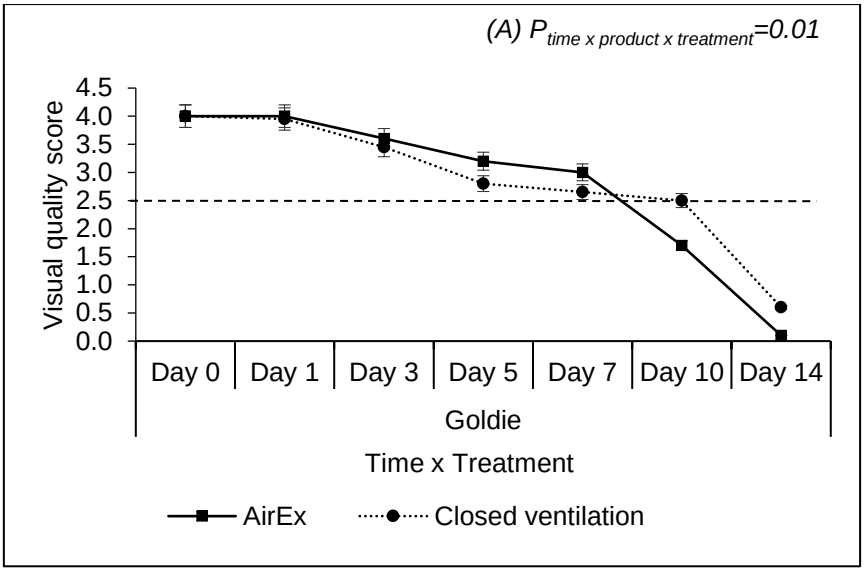


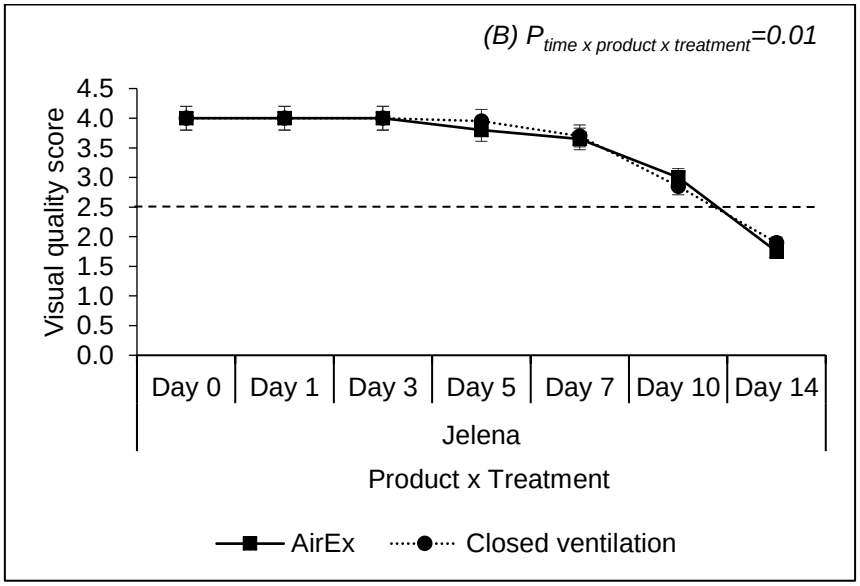


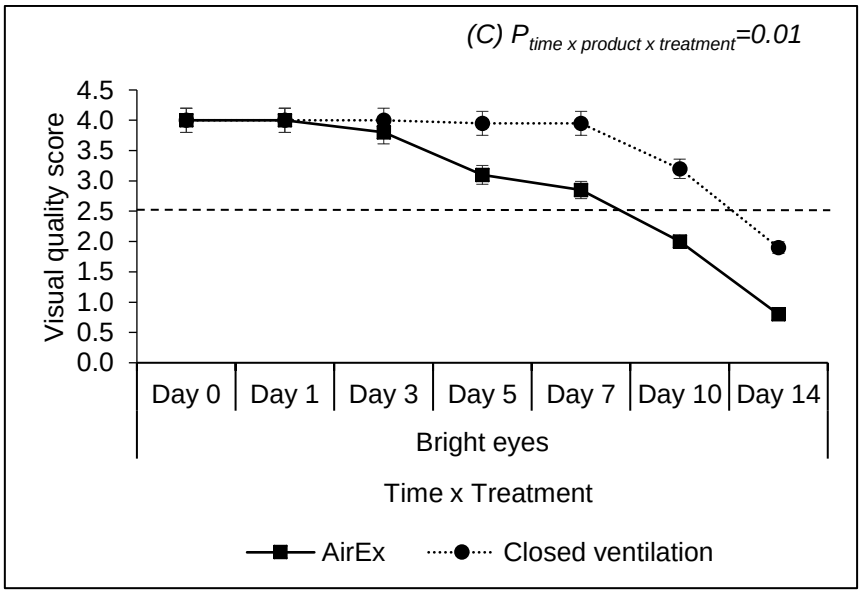


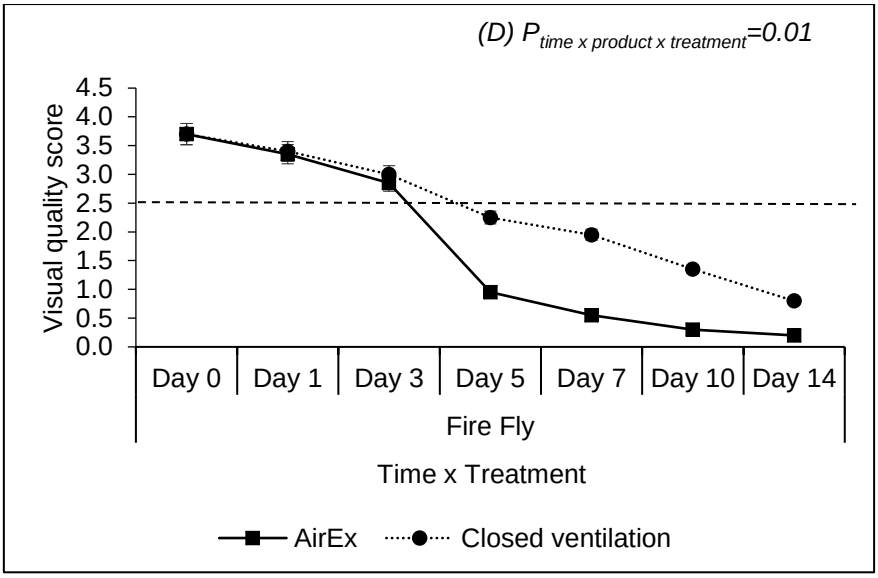
Effect	Product	Treatment	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*<0.0001*	*0.005*	*<0.0001*	*0.599ns*	*0.073ns*	*<0.0001*	*0.002*

Fig. 4. Visual quality of inflorescences of *Leucospermum* products [Veld Fire' (A), 'Rigoletto' (B), 'Succession' (C) and 'Soleil' (D)] after 21 d of cold storage at 1 °C in AirEx and closed ventilation in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



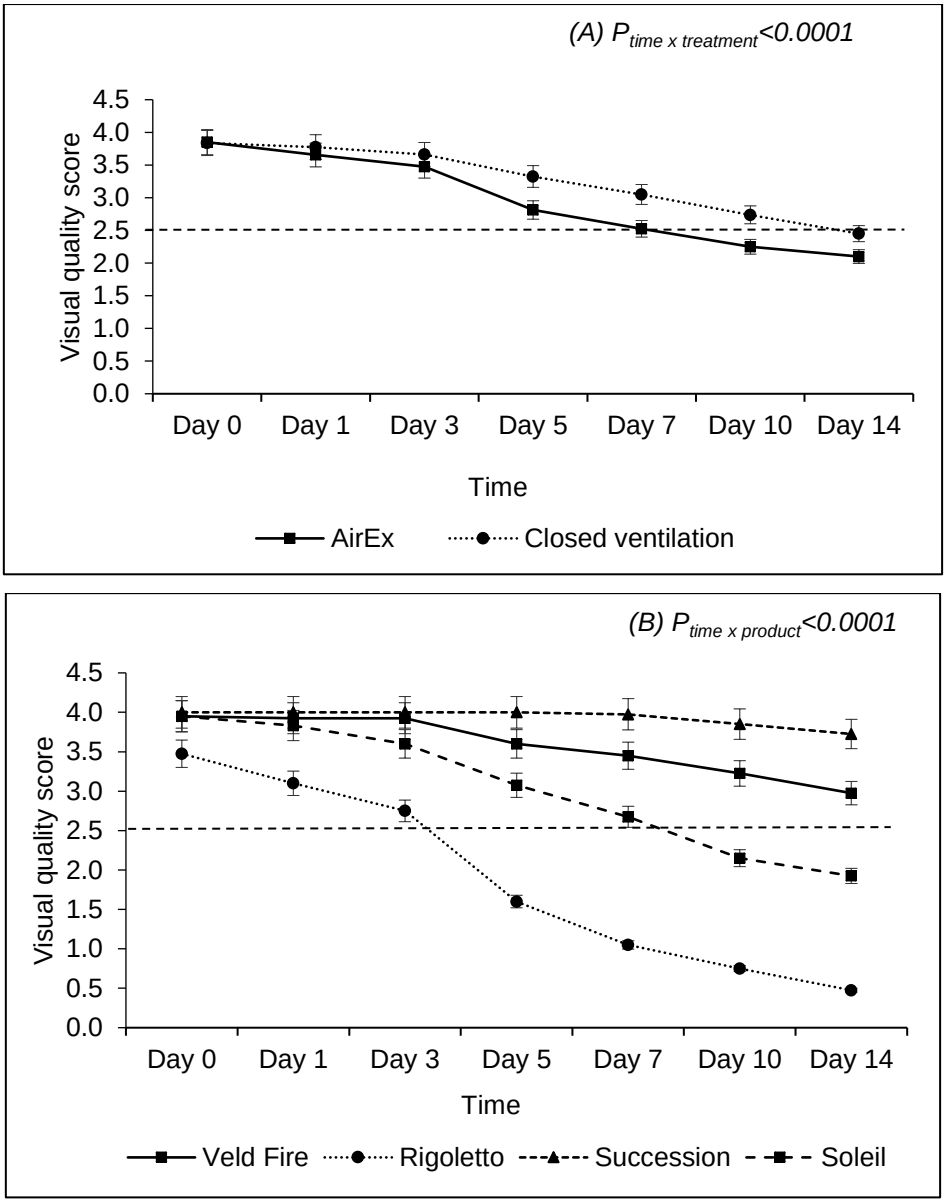






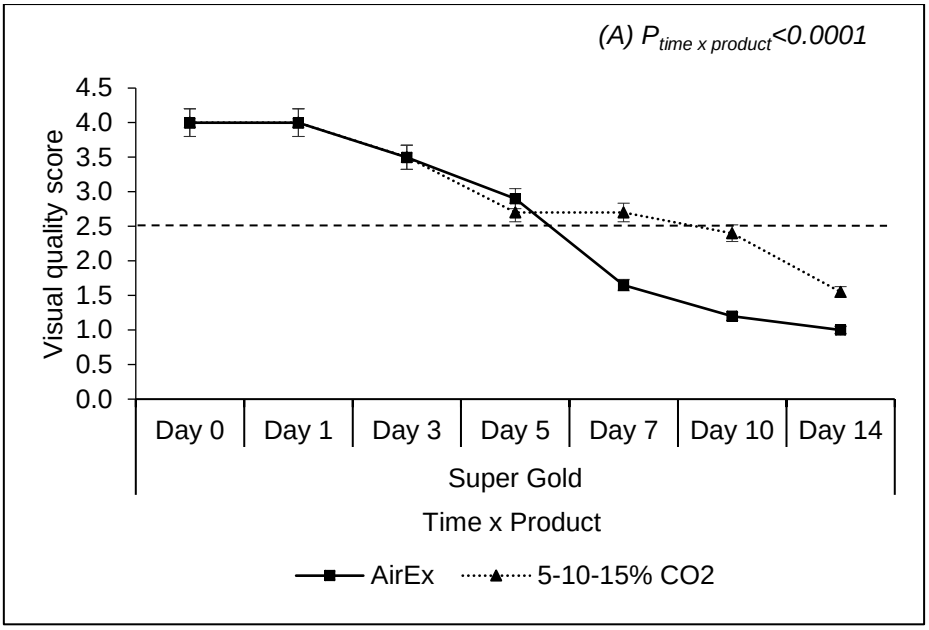
Effect	Product	Treatment	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*<0.0001*	*0.030*	*<0.0001*	*0.287ns*	*<0.0001*	*<0.0001*	*0.010*

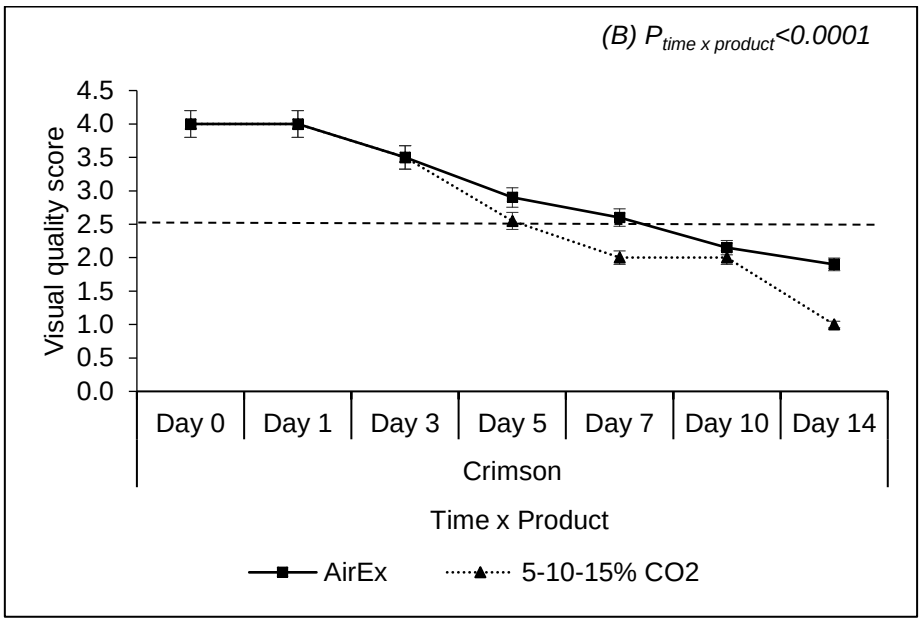
Fig. 5. Visual quality of leaves of *Leucospermum* [‘Goldie’ (A), ‘Jelena’ (B), ‘Bright Eyes’ (C) and ‘Fire Fly’ (D)] products after 21 d of cold storage at 1 °C in AirEx and closed ventilation in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.012*	*<0.0001*	*0.168ns*	*<0.0001*	*<0.0001*	*0.190ns*

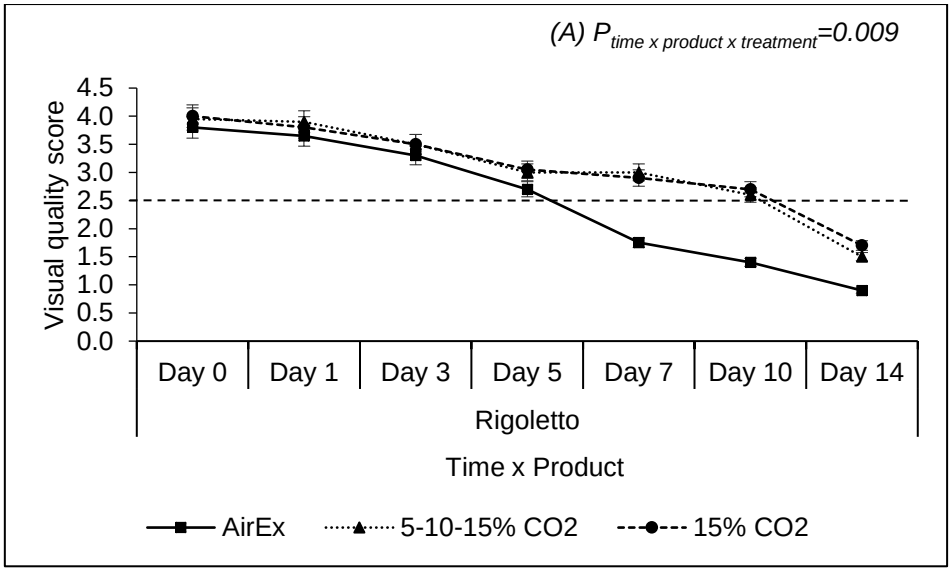
Fig. 6. Visual quality of leaves of *Leucospermum* stems ['Veld Fire', 'Rigoletto', 'Succession' and 'Soleil'] after 21 d of cold storage at 1 °C in AirEx and closed ventilation in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

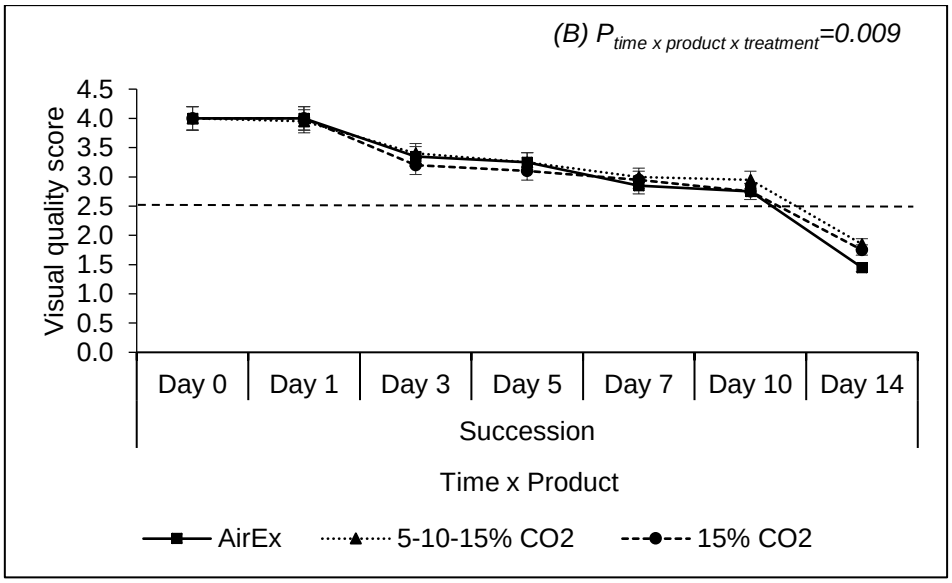


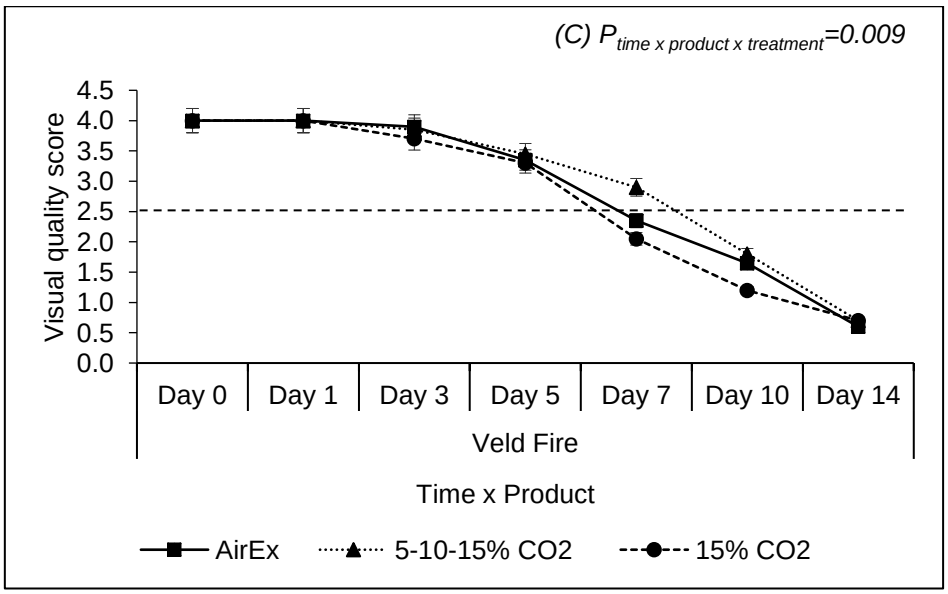


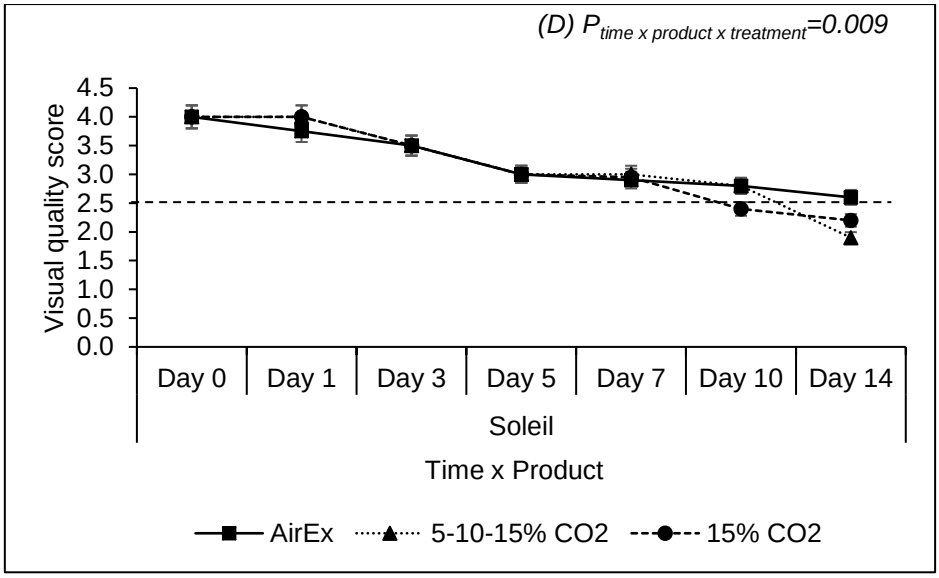
Effect	Product	Treatment	Time	Time x Product	Product x Treatment	Time x Treatment	Time x Product x Treatment
P value	*0.539ns*	*0.711ns*	*<0.0001*	*0.606ns*	*0.007*	*0.003*	*<0.0001*

Fig. 7. Visual quality of inflorescences of *Leucospermum* products ['Super Gold' (A) and 'Crimson' (B)] following 21 d of storage at 1 °C in AirEx and 5-10-15 % CO_2 in the 2015. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



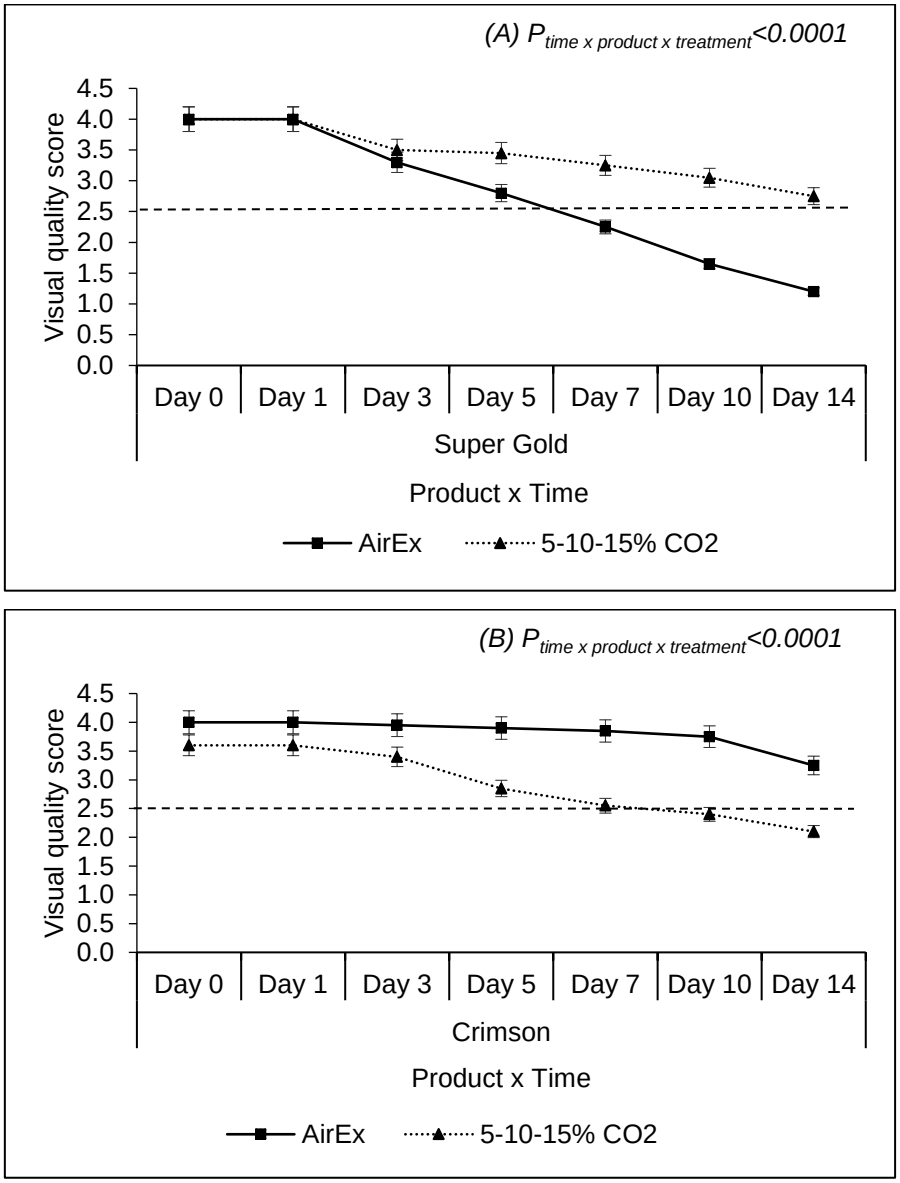






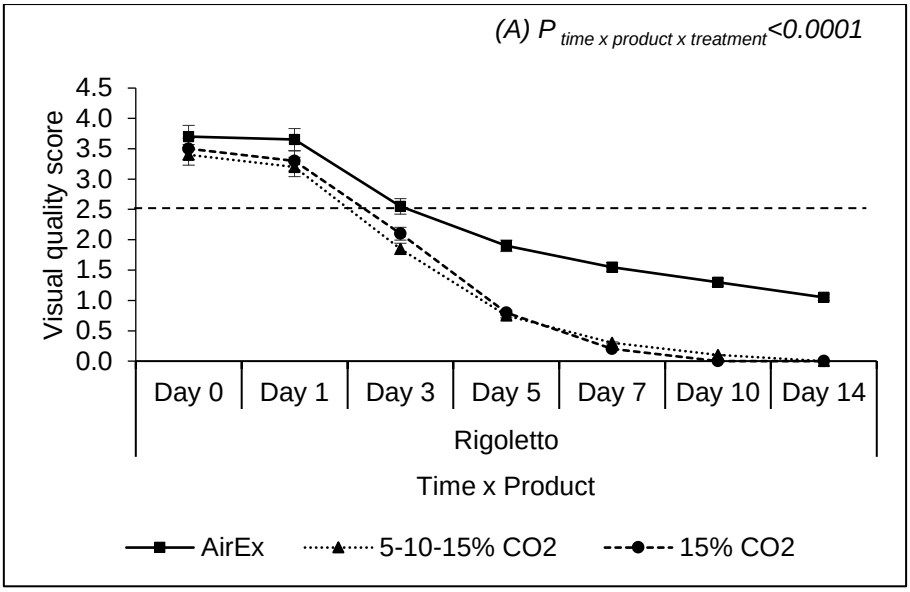
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.006*	*<0.0001*	*<0.0001*	*<0.0001*	*0.026*	*0.009*

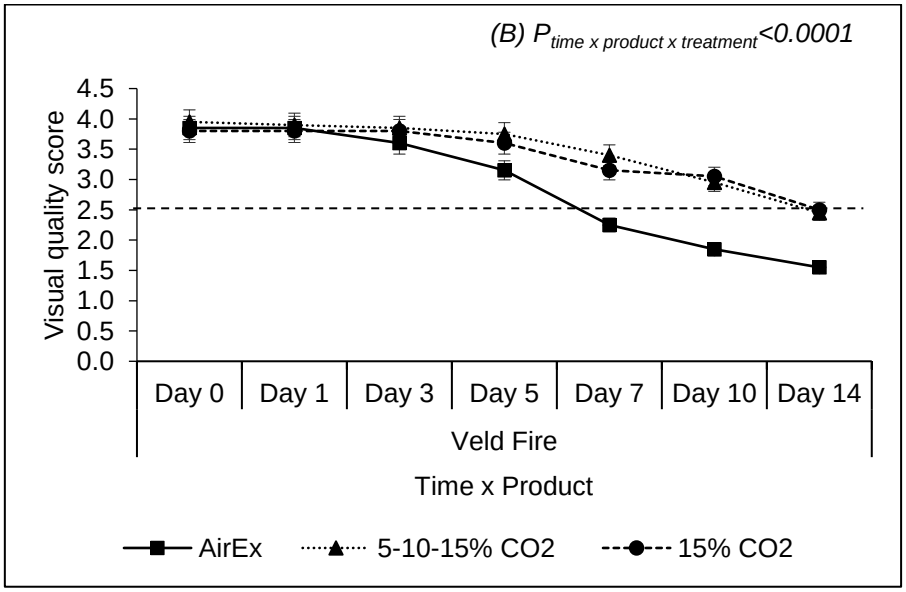
Fig. 8. Visual quality of inflorescences of *Leucospermum* products [‘Rigoletto’ (A), ‘Succession’ (B), ‘Veld Fire’ (C) and ‘Soleil’ (D)] following 21 d of storage at 1 °C in AirEx, 5-10-15 % CO_2 and 15 % CO_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

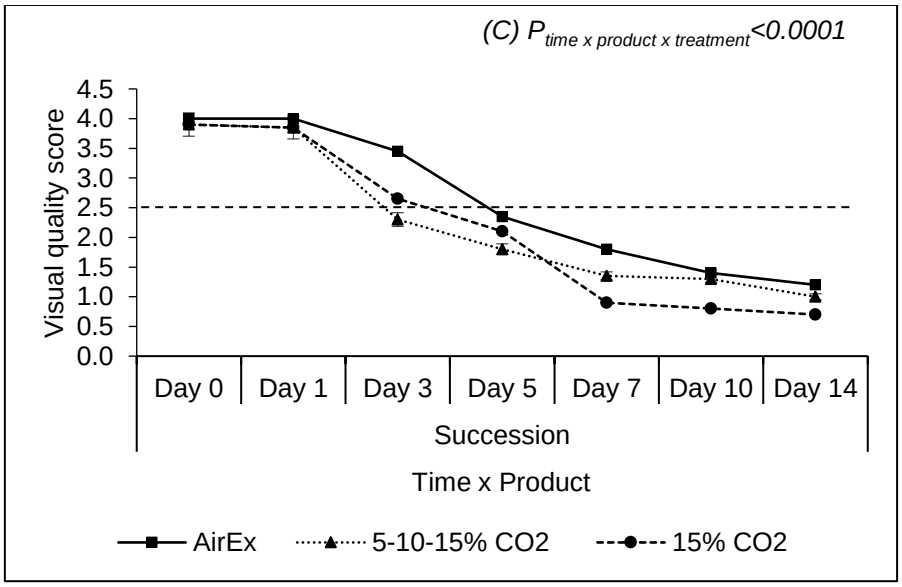


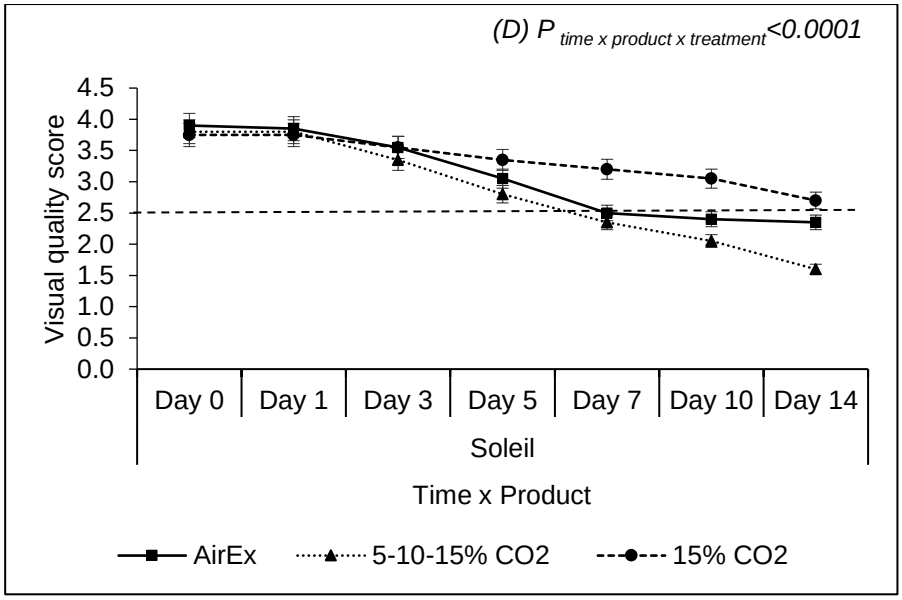
Effect	Product	Treatment	Time	Time x Treatment	Product x Treatment	Time x Product	Time x Product x Treatment
P value	*0.131ns*	*0.592ns*	*<0.0001*	*0.425ns*	*<0.0001*	*<0.0001*	*<0.0001*

Fig. 9. Visual quality of leaves of *Leucospermum* products ['Super Gold' (A) and 'Crimson' (B)] following 21 d of storage at 1 °C in AirEx and 5-10-15 % CO_2 in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.









Effect	Treatment	Product	Time	Time x Treatment	Product x Treatment	Time x Product	Time x Product x Treatment
P value	*0.435ns*	*<0.0001*	*<0.0001*	*0.855ns*	*0.052ns*	*<0.0001*	*<0.0001*

Fig. 10. Visual quality of leaves of *Leucospermum* products ['Rigoletto' (A), 'Veld Fire' (B) 'Succession' (C), and 'Soleil' (D)] following 21 d of storage at 1 °C in AirEx, 5-10-15 % CO_2 and 15 % CO_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

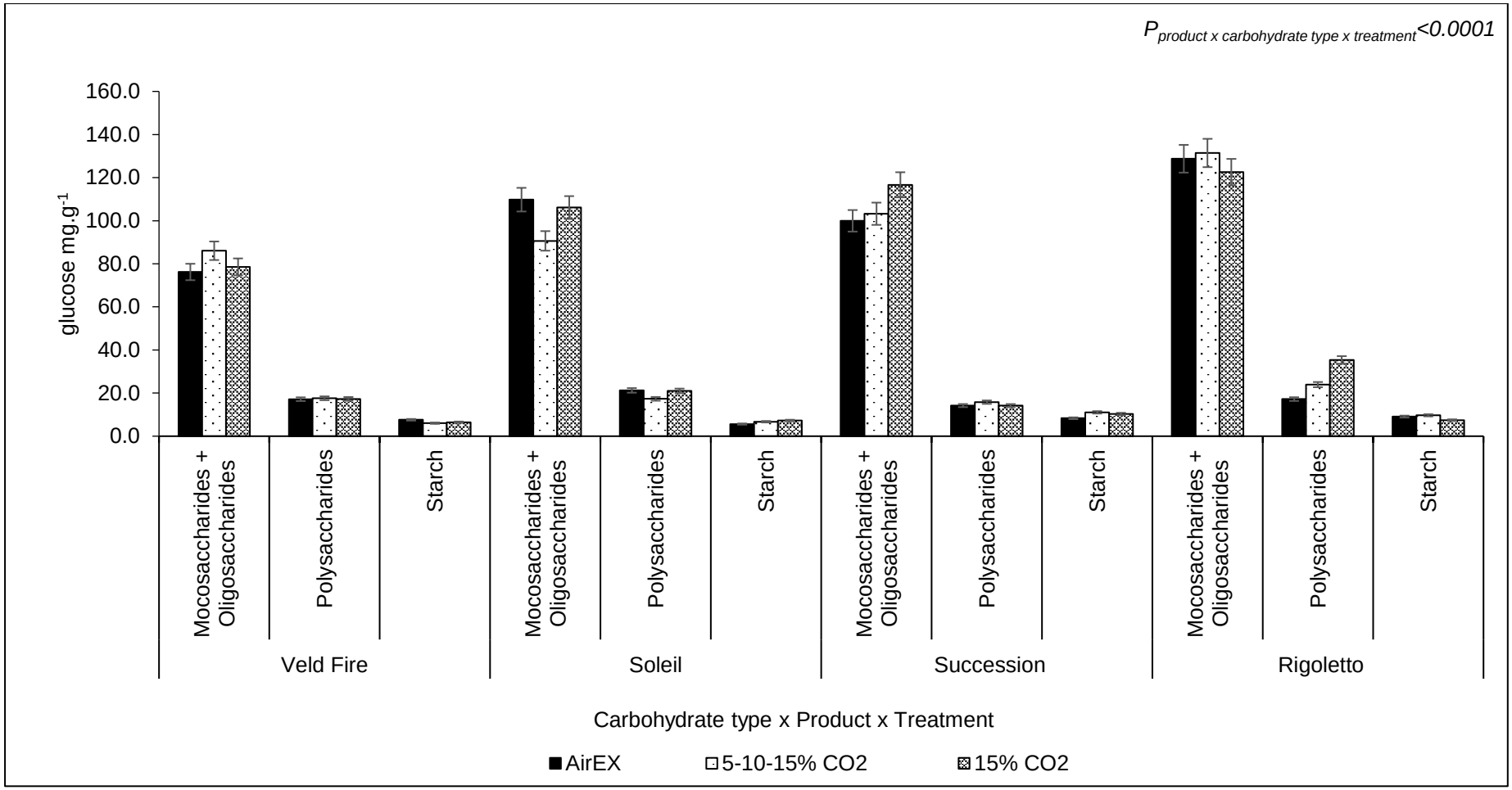
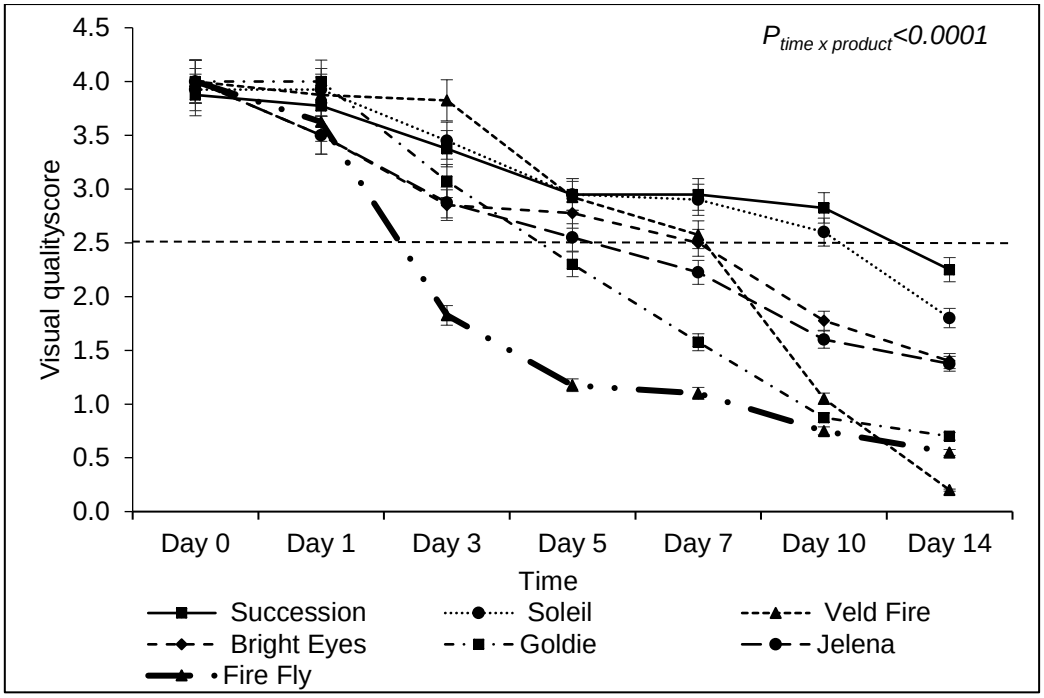
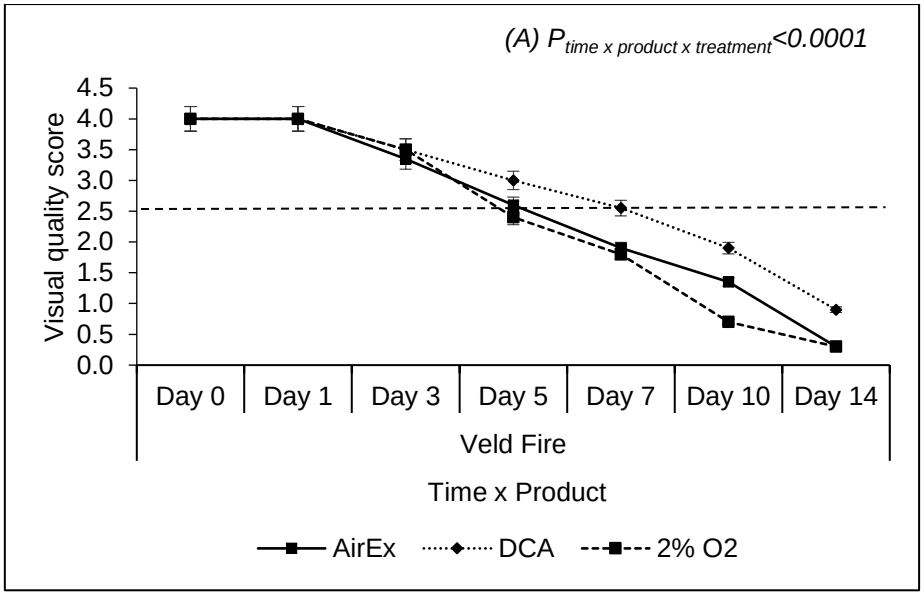


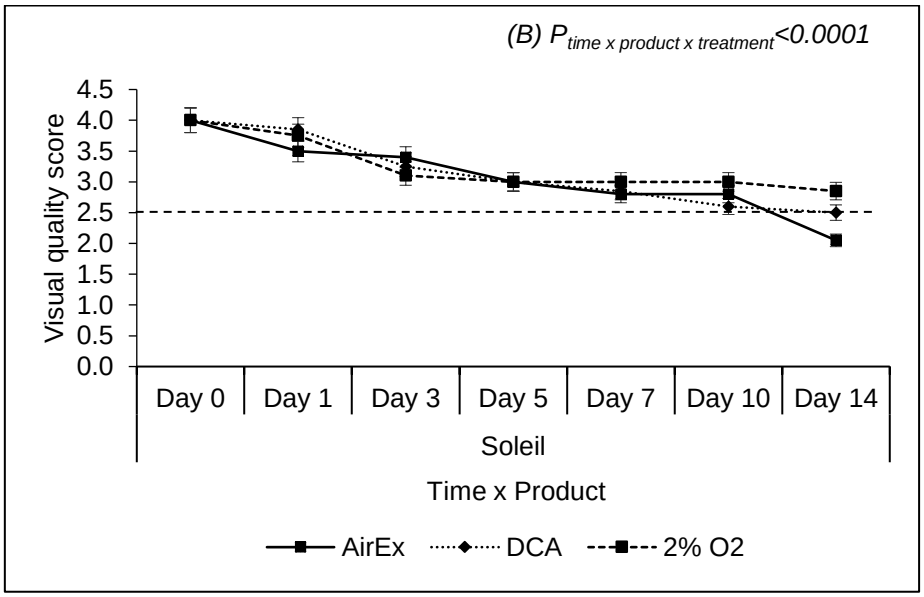
Fig. 11. Soluble carbohydrates in leaves of *Leucospermum* stems ('Veld Fire', 'Soleil', 'Succession' and 'Rigoletto') after cold storage for 21 d at 1 °C in AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments in the 2016 season.

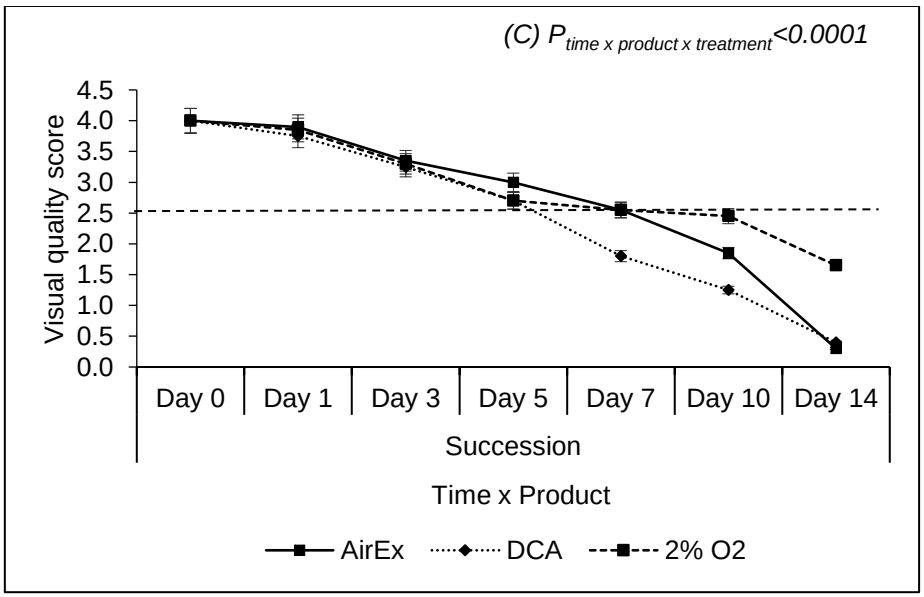


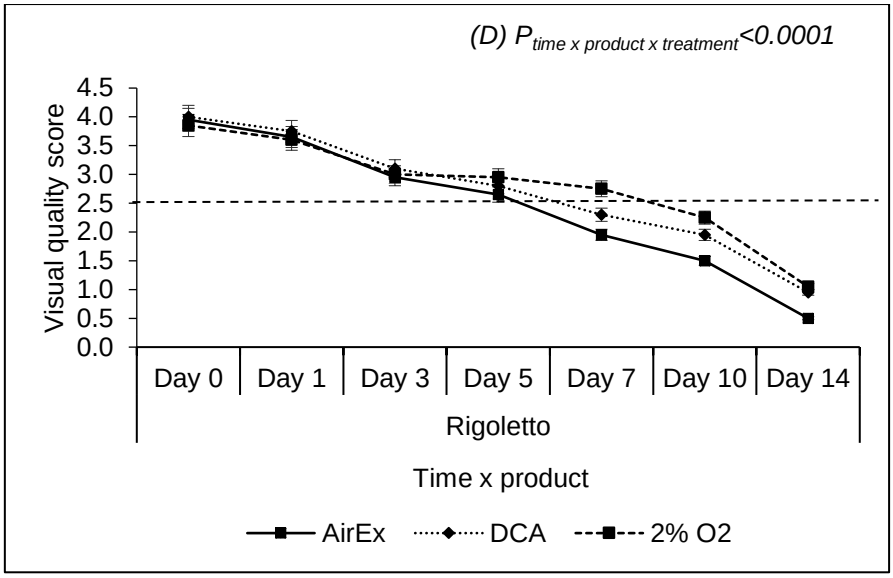
Effect	Treatment	Product	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*0.708ns*	*<0.0001*	*<0.0001*	*0.728ns*	*0.573ns*	*<0.0001*	*0.264ns*

Fig. 12. Visual quality of inflorescences of *Leucospermum* products ('Succession', 'Soleil', 'Veld Fire' 'Bright Eyes', 'Jelena' and 'Goldie') following 21 d of cold storage at 1 °C in AirEx, and 2 % O_2 treatments in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



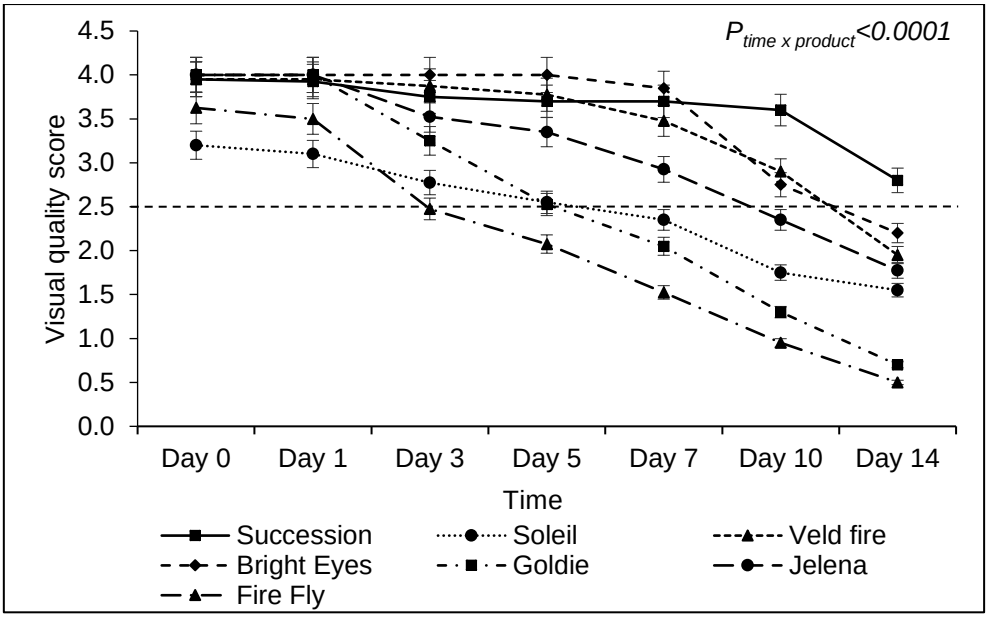






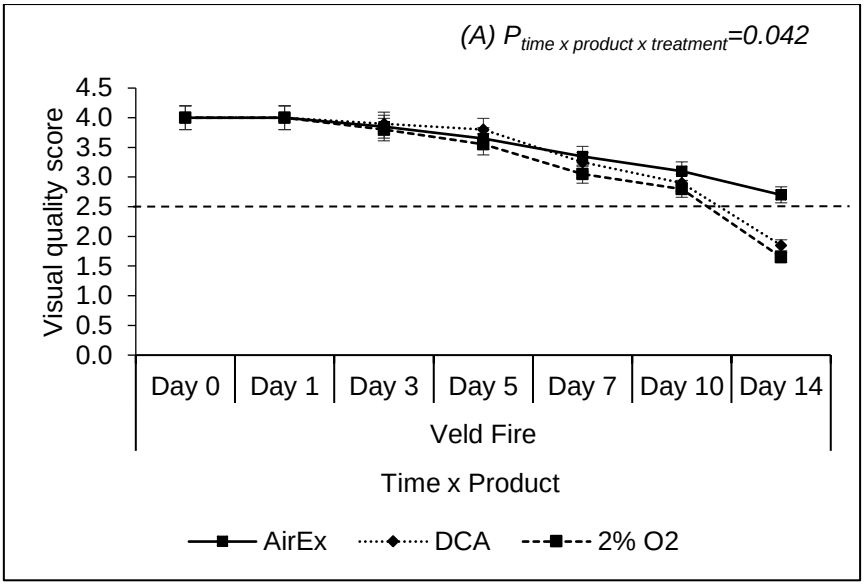
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.063ns*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

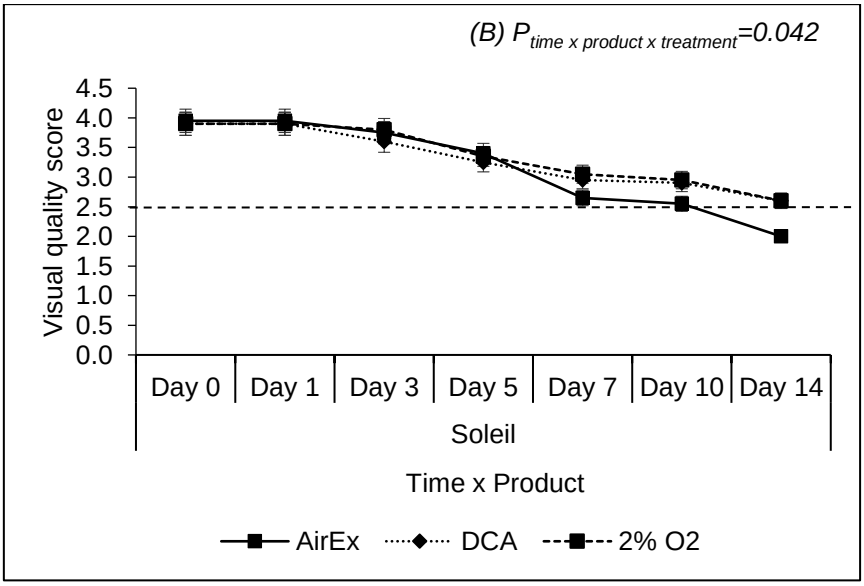
Fig. 13. Visual quality of inflorescence of *Leucospermum* products [‘Veld Fire’ (A), ‘Soleil’ (B), ‘Succession’ (C) and ‘Rigoletto’ (D)] following 21 d of storage at 1 °C in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

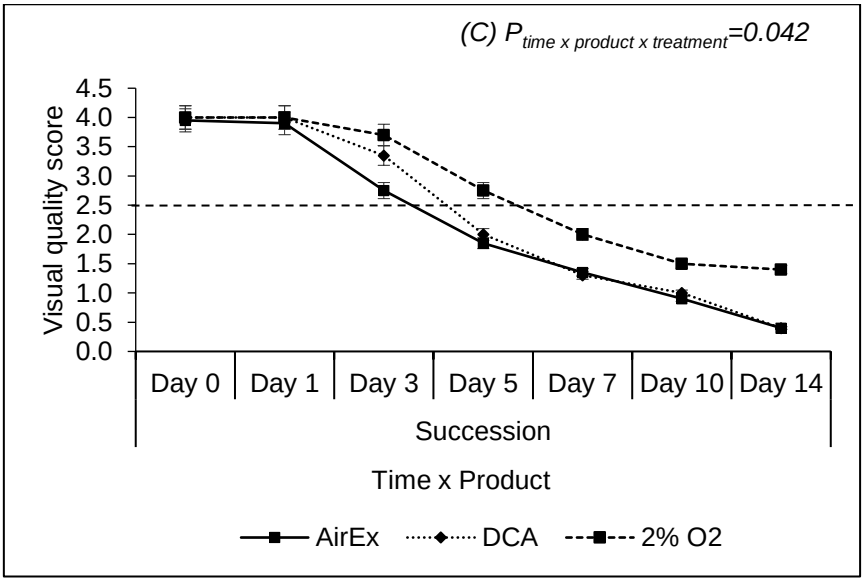


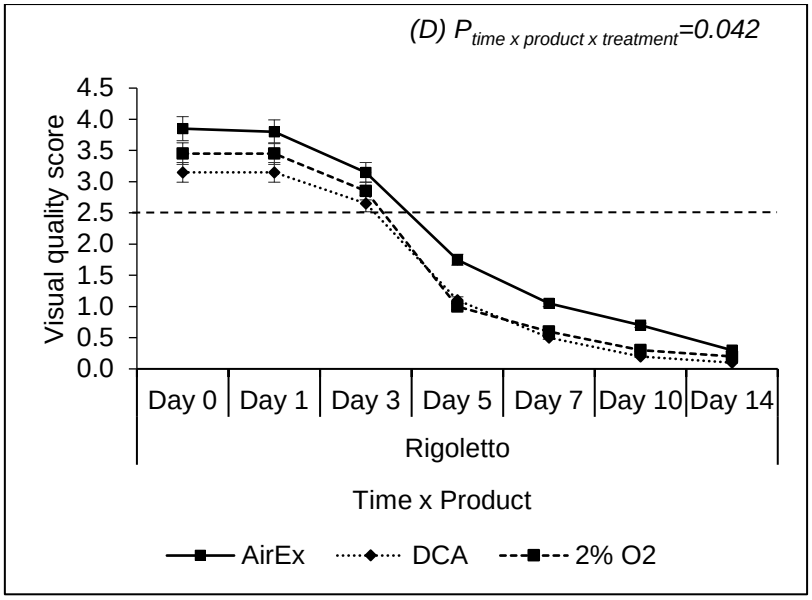
Effect	Product	Treatment	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*<0.0001*	*0.994ns*	*<0.0001*	*0.581ns*	*0.99ns*	*<0.0001*	*0.707ns*

Fig. 14. Leaf visual quality of *Leucospermum* products ('Succession', 'Soleil', Veld Fire, 'Bright Eyes', 'Jelena' and 'Goldie') following 21 d of storage at 1 °C in AirEx, and 2 % O_2 in the 2015 season. A score of 2.5 was considered the cut-off for acceptable retail quality.









Effect	Product	Treatment	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*<0.0001*	*0.581ns*	*<0.0001*	*0.157ns*	*0.979ns*	*<0.0001*	*0.042*

Fig. 15. Visual quality of leaves of *Leucospermum* products ['Veld Fire' (A), 'Soleil' (B), 'Succession' (C) and 'Rigoletto' (D)] following 21 d of cold storage at 1 °C in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 treatments in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

PAPER 4: VIABILITY OF CLOSED VENTILATION AND AUTOMATIC VENTILATION FOR SEA FREIGHT OF *PROTEA* CUT FLOWER STEMS

Abstract

Advances in reefer technology made the development of automatic ventilation technology possible. Instead of the conventional fixed ventilation during storage, the new technology can restrict gas exchange to O_2 levels near the lower O_2 limit (LOL) and the CO_2 levels to the known CO_2 toxicity limit of the particular products in transit. The gas exchange requirements of sea freighted *Protea* cut flowers from South Africa (SA) are largely unknown, which has been a significant limiting factor to the introduction of automatic ventilation shipping for these high value products. Furthermore, suitability of sea freight of *Proteaceae* cut flowers that are shipped from South Africa to Europe is debatable due to inconsistencies in product quality upon arrival at the markets. In addition, *Protea* stems suffer from a serious postharvest disorder, known as leaf blackening, that may be exacerbated by incorrect storage protocols. The low storage temperature, fixed open air exchange ventilation system (AirEx) and lengthy cold storage period have been identified as potential causes of quality loss during sea freight. The aim of this study was to collect data to evaluate the feasibility of passive modified atmosphere (closed ventilation) and automatic ventilation technology for long-term cold storage and transportation of *Protea* cut flower products. Experiments were conducted by storing a range of *Protea* cultivars in a closed ventilation system, determining the lower O_2 limit of the products, as well as evaluating the feasibility of storage of *Protea* stems at low O_2 concentrations, along with observing possible toxicity symptoms that may occur under high CO_2 storage conditions. The LOL values were determined using the HarvestWatch™ dynamic controlled atmosphere (DCA) chlorophyll fluorescence technology, whilst CO_2 toxicity at high CO_2 concentrations was determined at 15 % CO_2. Analyses conducted included determination of respiration rate, moisture loss, visual quality, carbohydrate and total phenolic content and lipid peroxidation estimations. The LOL values of the *Protea* products ranged from 0.08-0.48 % O_2. In the 2015 season, the respiration rates of the products at 5 °C ranged from 10.76-27.24 mL $CO_2.kg^{-1}.h^{-1}$ and between 12.46-17.61 mL $CO_2.kg^{-1}.h^{-1}$ in the 2016 season. Between the 5 to 15 °C temperature range, Q_{10} values ranged from 1.70 to 3.84. Monosaccharides plus oligosaccharides were the most abundant sugars, followed by

polysaccharides, and starch was the least abundant. Phenolic content was comparable among the treatments and malondialdehyde (MDA) content was highest in 'Barbigera' and 'Ice Queen'. Storage under closed ventilation resulted in better visual quality and longevity compared to AirEx stored stems, whereas products stored under high CO_2 had similar inflorescence and leaf quality than those stored in AirEx. The quality of products stored in AirEx and low O_2 was comparable, except for 'Sylvia' and 'Cynaroides', where AirEx treatment notably decreased the vase life of the stems.

Key words: Automatic ventilation; Closed ventilation shipping; CO_2 toxicity tolerance; Lower O_2 limit; *Proteaceae*

1. Introduction

Products from the *Protea* genus make up 59% of the *Proteaceae* cut flowers under production in South Africa (SA) (Gerber and Hoffman, 2014). It is the most widely known genus from the *Proteaceae* family (Janick, 2007) and is of great economic significance to the indigenous flower industry. *Protea* products are sold as either fresh or dried flowers; mainly targeted for the European Union (EU) or United Kingdom (UK) export markets. Conventionally, *Protea* cut flowers from South Africa were exported via air freight and reached their destination within 48 h. Over the past decade, the ornamentals industry has steadily moved from air- to sea freight. This is because of high air freight charges due to continuous rises in jet fuel prices and also, as sea freight produces up to 95 % less carbon per tonne of product shipped (Sechler, 2013). Despite the quality compromises encountered due to an extended cold storage period of up to 21 d, the low transport cost makes sea freight an attractive alternative (Philosoph-Hadas et al., 2007).

There is need for innovative storage technologies since sea freighting of *Proteaceae* products has been only partially successful, possibly due to an interaction between the extended storage period and the required low temperature of not more than 4°C, which favour chilling injury (CI) and leaf blackening. Additionally, oxidative stress occurs when reactive oxygen species (ROS) production exceeds the ability of the cell to maintain redox homeostasis, when exposed to stress conditions such as chilling stress (Hodges et al., 2004). Accumulation of ROS disturbs membrane lipids, proteins, and damages DNA and RNA. Apel and Hirt (2004) and Bhattacharjee (2012) reported that lipid peroxidation is caused by leakage of electrons from the electron transport system and by membranous NADPH oxidases. Chilling stress increases the ROS accumulation and this may promote membrane degradation, solute leakage and

generation of toxic aldehydes and ketones (Goliáš and Kobza, 2002; Rogers, 2012; Xie et al., 2008). Similarly, high CO_2 storage may also lead to oxidative stress, loss of antioxidant activity and membrane damage (Hodges et al., 2004; Voisine et al., 1993). Lipid peroxidation that is linked to low O_2 injury usually becomes visible after transferring the product to ambient conditions (Blokhina et al., 1999). Quantification of MDA content is an accepted method of detecting lipid peroxidation in plant tissues (Aghdam and Bodbodak, 2013).

The high possibility of oxidative stress, CI, discolouration and natural senescence during sea freighting requires a rich pool of anti-oxidants to counteract these effects and to minimise tissue damage. Plants have several compounds that are known for their antioxidant activities and these include; polyphenols, carotenoids and ascorbic acid (Lutz et al., 2015; Mlcek and Rop, 2011; Taiz et al., 2015). While Haslam, (1998) considered polyphenols to be among the most crucial natural antioxidant compounds, it is of importance to note that Philosoph-Hadas et al. (2010) reported the leaves of *Proteaceae* plants to contain large amounts of phenolic compounds. Phenolics assist in alleviating the effects of excessive ROS accumulation (Singh et al., 2009) in the presence of abiotic and biotic stresses (Taiz et al., 2015). Yet, most *Protea* products are susceptible to leaf blackening where polyphenol oxidase (PPO) or peroxidase may convert phenols to polymerized polyphenols associated with leaf blackening (Paull et al., 1980; Van Doorn, 2001; Whitehead and de Swardt, 1982).

Several researchers (Halevy and Mayak, 1981; Hodges et al., 2004; Kader, 2014; Meir et al., 1995; Nowak and Rudnicki, 1990; Philosoph-Hadas et al., 2007; Wang, 1990) have explained the role of MA technologies in lowering respiration rate, alleviating oxidative stress, decreasing ethylene sensitivity, lowering incidence of decay, alleviating symptoms of CI and retarding tissue senescence. While CA, modified atmosphere packaging (MAP) and closed ventilation have become standard storage techniques in fruit storage, MA storage has not been commercially implemented in the cut flower industry (Rani and Singh, 2014). Studies involving CA storage (Philosoph-Hadas et al. 2010) and MAP (Goliáš and Kobza, 2002) storage of cut flowers have showed that the techniques result in better storage quality if O_2 and CO_2 concentrations are maintained within the products' limits.

The AirEx ventilation system used for *Proteaceae* cut flowers is the standard technology in most reefer containers (M. Huysamer, personal communication, 7 March 2017). AirEx is characterised by a high variability in the volume of air that is exchanged through the vents, often with conflicting views by exporters on the ideal flow required in AirEx for different types of produce. The current ventilation rate in standard reefers used for *Proteaceae* shipping is usually set at 15000 L fresh air.h^{-1}. As this flow rate is derived from experience in

transporting fruit, AirEx at the current setting may be responsible for over-ventilation of ornamental products that differ vastly from fruit, since gas dynamics of *Proteaceae* products during cold storage are largely unknown. Recently advances in the sea freighting industry led to the invention of the automatic ventilation technology, which may soon become the standard ventilation technology for sea transport. The main benefit of the automatic ventilation system is that it can be adjusted to maintain ventilation to O_2 and CO_2 concentrations near toxic levels of the product throughout the entire storage period.

A closely related technique which gained commercial application in the fruit industry since 2004 is referred to as dynamic controlled atmosphere (DCA) storage. In DCA storage, the O_2 concentration varies during storage based on product response. According to Gasser et al. (2010) and Wright et al. (2012), the use of DCA technology requires knowledge of the O_2 concentration at which cellular metabolism changes from aerobic to anaerobic, also known as the lower O_2 limit (LOL). Prange et al. (2002, 2003), Weber et al. (2015) and Wright et al. (2015) explained that product response to the low O_2 levels is monitored through a respiratory quotient or chlorophyll fluorescence so as to detect levels just prior to fermentation. Sensors linked to the DCA system automatically trigger injection of fresh air when the O_2 concentration falls to near LOL levels.

As the future of automated long-term cold storage and successful sea freighting of *Protea* products from SA depend upon reliable information regarding inherent physiology parameters, the aim of this study was to obtain a better understanding of the physiology for a range of *Protea* cultivars and selections during storage, focusing on the gas dynamics during closed ventilation, as well as the limits of tolerance for low O_2 and high CO_2 of *Protea* stems. Thus, the objectives of this study were firstly to measure respiration rate at 5 and 15 °C and monitor the gas dynamics in a closed ventilation system and evaluate the feasibility of closed ventilation long-term cold storage technology for *Protea* products. The second objective was to determine the lower O_2 limits for *Protea* products, with a last objective to determine the CO_2 toxicity tolerance limit of *Protea* products. With this information available to producers and exporters optimizing of storage protocols are achievable in order to manage the entire value chain from harvest, during transport and/or storage, to maintain optimum product quality. Better yet, closed ventilation and automatic ventilation may have the ability to lower the incidence of some storage disorders and reduce chances of product rejection at the markets.

2. Materials and methods

2.1 Product source

Various *Protea* cut flower products were sourced from Floralae, Paarl (33°43'06.1"S 19°04'05.5"E), Tussenberge, Napier (34°29'10.3"S 19°44'10.7"E), Berghoff, Porterville (32°57'15.1"S 19°03'14.2"E) and Fynbloem, Riviersonderend (34°08'30.3"S 19°49'11.7"E) in the Western Cape, South Africa. The flowers were harvested mid-season and transported to the Cape Town International airport where they were held at 4 ^{o}C and collected within 24 h of harvesting. The experiments were conducted using ARC Infruitec-Nietvoorbij Stellenbosch cold rooms and Stellenbosch University Horticultural Science Department cold room and laboratory facilities. Export grade stems were used throughout this study and choice of products was dependent on availability of product (Table 1) and significance to the export market. The products that were used in the experiments included: 'Cynaroides' (*P. cynaroides*), 'Sylvia' (*P. eximia* x *P. susannae*), 'Barbigera' (*P. magnifica*), 'Ice Queen' (*P. magnifica* selections), 'Red Rex' (*P. cynaroides* selection), 'Red Magic' (*P. magnifica* selection) and 'Grandiceps' (*P. grandiceps*). 'Barbigera 1' and 'Barbigera 2' are variants of *P. magnifica* (Fig. 1).

2.2 Respiration rate

Flowers were kept in cold rooms set at 5 °C and 15 °C (± 0.5 °C) for 24 h. Five replicates were used per temperature per product and each replicate had three stems. The CO_2 produced during respiration was measured using the respiration analysis method by Jooste (2012) and Smit, (2013). The products were allowed to respire for 90 min and 10 mL of headspace gas was drawn for analysis. The gas sample was analysed on a gas chromatograph (6890N, Agilent Technologies, California, USA) fitted with a thermal conductivity detector (TCD) and a Porapak Q section (Porapaq Q/Heysep Q, 80-1000 mesh, Hayes Separations) and a Mole Sieve 5 A section (80-1000 mesh, Alltech Associates) column. The GC uses helium as a carrier gas and the oven temperature was maintained at 80 °C. Respiration rate and temperature quotient (Q_{10}) of the cut flower products were calculated using the formulas below:

$$\text{Respiration rate (mL.kg}^{-1}\text{.h}^{-1}\text{)} = \left(\frac{\%\ CO_2\ \text{produced}}{100}\right) X \left(\frac{\text{Volume free air (mL)}}{\text{Product weight (kg)}}\right) X \left(\frac{1}{\text{Time (h)}}\right)$$

$$Q_{10} = \left(\frac{\text{Respiration rate T2}}{\text{Respiration rate T1}}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

Where T_1 is 5 °C and T_2 is 15 °C.

2.3 Closed ventilation system gas dynamics

Various *Proteaceae* products were stored in a Janny MT module™ (Janny MT, France) that consists of a plastic box with a 610 L capacity and Tiempo cap lid cover, at 1 °C (±0.5 °C) for 21 d to simulate sea freight conditions. The free air inside the Janny MT boxes was approximately 45-55 % of the total volume in both the 2015 and 2016 seasons, due to the flower box arrangement at maximum capacity. The CO_2 and O_2 concentrations within the sealed bins were monitored using a HarvestWatch™ DCA system (AAFC and SAtlantic Inc., Nova Scotia, Canada) in the 2015 season. However, a hand held gas analyser (ICA, 250, Dual Analyser, Kent, U.K) was used instead in the 2016 season. This was done to achieve a more accurate simulation of commercial shipping conditions by removing less air from the small-scale, experimental closed ventilation system. The closed ventilation system contained *Leucadendron* (5.6 kg 'Rosette Conebush'), *Leucospermum* (2.15 kg 'Jelena'; 3.21 kg 'Goldie'; 2.29 kg 'Bright Eyes' and 2.56 kg 'Fire Fly') and *Protea* (9.33 kg 'Barbigera'; 7.36 kg 'Grandiceps' and 11.26 kg 'Red Rex'). Readings of gas concentrations were taken on day 0, 4, 8, 12, 16 and 21 of simulated shipping. Products in AirEx were used as the control, where a consistent ventilation rate of 15000 L fresh air.h^{-1} was maintained inside the cold room throughout the storage period. The boxes containing control stems were loosely covered with plastics to reduce air flow and simulate the conditions in a fully loaded reefer.

2.4 Determination of lower O_2 limit

The LOL values were determined according to Prange et al. (2003), with some minor adjustments. Briefly, the HarvestWatch™ DCA monitoring system, equipped with a fluorescence interactive response monitor (FIRM) sensor, was used to determine the LOL of each product. The O_2 concentration was lowered gradually over a 48 h period until the LOL was identified by a clear stress peak observed on a chlorophyll fluorescence spectrum. Product in the low O_2 atmosphere treatment was stored in 2% O_2 for the 2015 season and storage near the LOL (LOL+0.4 % O_2) was added as additional DCA treatment in the 2016 season. The CO_2 was maintained at 0.04 % in both seasons, in combination with the low O_2 regimes and all products were kept in a cold room set at 1 °C (±0.5 °C) for 21 d.

2.5 Determination of high CO_2 tolerance

A HarvestWatch™ DCA system was used to control the CO_2 concentration in a Janny MT box containing *Protea* products. In the 2015 season, products in high CO_2 tolerance treatments were stored at 5 % CO_2 during the first week of storage, then ramped to 10 % CO_2

for the second week, until a final concentration of 15 % CO_2 was maintained for the third week of storage. Storage in 15 % CO_2 for 21 d was included as a third treatment in the 2016 season. The O_2 level was maintained at 12 % in both seasons, for the high CO_2 toxicity study and temperature was maintained at 1 °C (±0.5 °C) during simulated shipping.

2.6 Mass loss

Moisture loss was determined as the difference in mass between day 0 and day 21 of storage. A scale (EEW-5000, Everyweigher, UWE, Cape Town, South Africa) accurate to 0.1 g was used. Individual *Protea* stems were used as replicates and 10 replicates were evaluated per treatment per product.

2.7 Colour determination of inflorescence and leaves

A chroma meter (CR400, Konica Minolta, Tokyo, Japan) was used to determine hue angle of leaves and inflorescences. Colour readings were taken on day 0 and day 21 of storage and 10 replicates were used per treatment. The measurements were taken on three positions on the upper half of the inflorescence, with one reading on the abaxial surface of five leaves on each stem. The chroma meter was calibrated using a standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203) prior to taking measurements and readings were taken in the CIE lightness (L*), chroma (C*), hue angle (h°) colour space. Red, yellow, green and blue colour have hue angles of 0 °, 90 °, 180 ° and 270 °, respectively (X-rite, 2007).

2.8 Visual quality evaluation

Visual quality after storage was evaluated for 14 d using 10 replicates per treatment. The samples were kept at room temperature (20 °C ±2 °C) in tap water. Visual quality indices were recorded on day 0, 1, 3, 5, 7, 10 and 14. A visual quality scale ranging from 0 to 4 was interpreted as follows: 4 - Export grade quality, 3 - Good quality, 2 - Low quality with considerable damage, 1 - Poor quality with severe damage and 0 - Product to be discarded and considered totally unacceptable.

2.9 Soluble carbohydrates

Carbohydrates were extracted and analysed in triplicate according to the method of Rohwer (2013), with slight modifications. Briefly, leaves were removed from 10 replicate stems after 21 d of cold storage, where after they were frozen in liquid N_2 and stored at -80 °C until freeze-dried and milled. Soluble monomers and oligosaccharides were extracted from 0.1 g

samples using three successive washes in 80 % ethanol. The pellet was then washed another three times (24 h each wash) in de-ionised water to extract the water-soluble polysaccharides. In a next step the pellet was heated in a sodium acetate and amyloglucosidase (AMG) buffer solution (pH=4.8) for 24 h to hydrolyse and extract the starch. The temperature was maintained at 80 °C using digital heating blocks. The amount of sugars in 10 µL of extract was determined using the Phenol-Sulphuric acid assay and absorbency was measured at 480 nm on a UV-Visible Spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia). A standard curve derived from a serial dilution of a glucose standard (0.1 mg .mL^{-1}) was used to estimate the sugars, expressed as glucose equivalents.

2.10 Lipid peroxidation

Lipid peroxidation was determined according to Hodges et al. (1999), with slight adjustments. Briefly, fresh leaves were removed from 10 replicate stems, milled in liquid N_2 and stored at -80 °C until extraction and analysis was carried out. In brief, 1.0 g fresh weight (FW) samples were homogenized in 20 mL of 80 % ethanol for each storage treatment. The probe was rinsed in 5 mL of 80 % ethanol and this was mixed with the initial extraction solution. The pooled solution was centrifuged at 4 °C for 10 min at 3000 g. A 1 mL sample from the supernatant was mixed with 1 mL thiobarbituric acid (TBA) solution and 10 µL of 1 % butylated hydroxytoluene (BHT) solution and vortexed. The solution was heated for 25 min at 95 °C. The heated solution was cooled on ice and centrifuged at 3000 g for 10 min. Absorbance was measured in glass cuvettes at 532 nm and 600 nm, using a UV–Visible Spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia). The blank solution contained 1 mL 80 % ethanol, 10 µL BHT and 1 mL TBA. The MDA content was calculated using the formula below:

MDA equivalents (nmol.g^{-1} FW) =

$$\left(\frac{\text{Volume of extraction solution (mL)}}{\text{Volume of supernatant (mL)}}\right) \text{x} \left(\frac{1}{\text{Sample mass (g)}}\right) \text{x} \left(\frac{\text{Abs 532 nm} - \text{Abs 600 nm}}{157\,000}\right) \text{x } 10^6$$

2.11 Total phenolic content

Total phenolic content was determined according to Rohwer (2016) with slight modifications. Briefly, leaves were obtained from 10 replicate stems immediately after cold storage and placed in liquid N_2 where after they were stored at -80 °C until freeze dried and milled. A 0.1 g dry weight (DW) sample of milled leaves was washed three successive times

for 20 min in 80 % ethanol, with each wash using 5 mL of ethanol and the temperature maintained at 80 °C. The three extracts were pooled to produce 15 mL of extract. A 50 µL sample from the extract was reacted with 450 µL of folin-ciocalteu (FC) reagent for 5 min. This solution was then added to 500 µL of 5.6 % Na_2CO_3, vortexed and left to react for another 90 min. The absorbance was read at 750 nm on a spectrophotometer (Varian, 50 Bio-UV-Visible Spectrophotometer, Mulgrave, Victoria, Australia). A serial dilution of gallic acid (GA) standard was used to plot a standard curve, and the amount of phenolics was expressed as gallic acid equivalents (GAE). All the extractions and analyses were done in triplicate.

3. Statistical analyses

A complete randomized design was used and analysis of variance (ANOVA) and repeated analysis of variance (RANOVA) was performed on the data using Statistica (Statistica 13.2, Statsoft Inc., USA). Means were separated by LSD test ($P \leq 0.05$).

4. Results

4.1 Closed ventilation trial

4.1.1 Gas dynamics and respiration rate

The closed ventilation system contained a total of 43.76 kg of stems that included *Leucadendron*, *Leucospermum* and *Protea* (Fig. 2). The O_2 concentration fell from 20.92 % to 8.42 %, whilst the CO_2 level rose from 0.06 % to 10.54 % as monitored by the HarvestWatch™ DCA system. In the 2015 and 2016 seasons, the respiration rates of the *Protea* products at 5 °C ranged from 10.76-27.24 mL $CO_2.kg^{-1}.h^{-1}$ and 12.46-17.61 mL $CO_2.kg^{-1}.h^{-1}$ respectively. The Q_{10} values ranged from 1.70 for 'Sylvia' to 3.8 for 'Grandiceps' between 5 and 15 °C in the 2015 season. In the 2016 season the Q_{10} values ranged from 1.94 for 'Barbigera' to 3.84 for 'Sylvia'. In the 2015 season, the respiration rates of the products ranged from 10.76-27.24 mL $CO_2.kg^{-1}.h^{-1}$ at 5 °C. In the 2016 season, the respiration rates were between 12.46-16.49 mL $CO_2.kg^{-1}.h^{-1}$ (Table 1).

4.1.2 Mass loss

The main factors, product and treatment, did not yield a significant interaction at the 5% confidence level ($p=0.090$) for mass loss (Table 2). However, both treatment ($p<0.0001$)

and product ($p<0.0001$) had a significant effect on mass loss in the 2015 season. Mass loss was significantly higher in AirEx treated stems compared to stems held under closed ventilation. The mass loss of 'Barbigera' and 'Red Rex' did not differ significantly from each other, but both were significantly higher than that recorded for 'Grandiceps'.

4.1.3 Colour determination of inflorescence and leaves

No significant ($p=0.701$) interaction between product, storage and treatment was obtained, however both storage ($p=0.037$) and product ($p<0.0001$) showed significant differences in the 2015 season (Table 3). The storage regime, thus either AirEx or closed ventilation, did not affect the inflorescence colour per se, for any of the *Protea* products tested. However, the hue angle of the inflorescences was significantly lower after storage (27.1 °) compared to before storage (29.2 °), indicating a colour change from yellow towards red. Despite this change, the quality was unaffected as the change was visually undetectable. Product comparison indicated similar hue angles for 'Grandiceps' and 'Barbigera', but with a lower value for the inflorescence of 'Red Rex'.

When considering the foliage colour, only product as a main factor had a significant effect ($p<0.0001$) as the hue angle of 'Barbigera' leaves was recorded to be higher than that of 'Red Rex' (Table 3). 'Barbigera' also had signs of leaf blackening, which despite being minor, were more visible compared to the other products.

4.1.4 Visual quality: Inflorescences

The visual quality of inflorescences was significantly ($p=0.031$) affected by an interaction between time, product and treatment in the 2015 season. Data for 'Red Rex' (Fig. 3A), 'Grandiceps' (Fig. 3B) and 'Barbigera' (Fig. 3C) revealed that differences between the treatments were insignificant on day 1, and also on day 7 for 'Barbigera', whereas storage of stems in closed ventilation generally resulted in better visual quality throughout the evaluation period than storage in AirEx.

4.1.5 Visual quality: Leaves

Leaf visual quality was significantly ($p<0.0001$) affected by an interaction between time, cultivar and treatment in the 2015 season. 'Red Rex' (Fig. 4A), 'Barbigera' (Fig. 4B) and 'Grandiceps' (Fig. 4C) stored in AirEx and closed ventilation had similar quality on day 1, and also on day 7 for 'Grandiceps'. However, quality of 'Red Rex' and 'Barbigera' stored in closed ventilation was generally superior to that of product stored in AirEx, although 'Barbigera'

products from both storage regimes had quality that was below the minimum acceptable level by day 7 of vase life evaluation.

4.1.6 Soluble carbohydrates

Carbohydrate content was significantly (p=0.043) influenced by a product and treatment interaction in the 2015 season (Table 4). Monosaccharide plus oligosaccharide content differences between the treatments were not significant in 'Barbigera' and 'Grandiceps' stems, whereas 'Red Rex' stems stored in closed ventilation had higher carbohydrate content compared to stems stored in AirEx.

4.2 High CO_2 toxicity tolerance trial

4.2.1 Mass loss

No significant product and treatment interaction (p=0.127) affected mass loss of stems in the 2015 season (Table 5). A significant treatment effect was, however, observed (p=0.004) as mass loss was more severe in product stored in AirEx compared to those stored in 5-10-15 % CO_2.

In the 2016 season however, a significant interaction (p<0.0001) between product and treatment was observed (Table 5). Mass loss differences between 'Ice Queen' products stored in AirEx, 5-10-15 % CO_2 and 15 % CO_2 were non-significant. Results for 'Barbigera' showed that moisture loss was the lowest for stems stored under the 5-10-15 % CO_2 regime, whilst the generally higher mass loss did not differ significantly for stems stored under either AirEx or 15 % CO_2. 'Sylvia' and 'Cynaroides' products both lost more moisture in AirEx storage, compared to the other treatments.

4.2.2 Colour determination of inflorescence and leaves

No significant interaction (p=0.965) between storage, product and treatment was obtained for inflorescence hue angle, although a significant storage and product interaction (p=0.004) was observed in the 2015 season (Table 6). Inflorescence hue angle readings were not significantly affected by storage for 'Barbigera', 'Grandiceps' and 'Ice Queen'. However, 'Didi' changed from yellow towards a deeper red as the hue angle for inflorescences was significantly lower after storage (25.7 °), compared to before storage (39.5 °). Despite the statistical significance, there were no visually discernible changes in quality of inflorescences. However, there was slight leaf blackening on some leaves on 'Barbigera' and 'Ice Queen'.

Results for the 2016 season produced no significant interaction between storage, product and treatment ($p=0.154$) for inflorescence hue angle (Table 7). However, both a significant product and treatment interaction ($p=0.008$) as well as a storage and product interaction ($p=0.007$) were obtained. Flower colour was not affected significantly by storage in either 'Sylvia' or 'Barbigera' products. However, for 'Ice Queen' a lowered hue angle was recorded, whereas the values for 'Cynaroides' increased with storage. Flower colour in 'Sylvia' and 'Ice Queen' were not significantly affected by storage regime. However, the mean hue angle for 'Barbigera' stored in 15 % CO_2 was higher than that of the other treatments, whereas the hue for 'Cynaroides' inflorescences stored in AirEx was lowest compared to the other treatments.

For leaf hue angle in the 2015 season no significant interaction ($p=0.633$) between storage, product and treatment was obtained (Table 8). However, both storage ($p<0.0001$) and product ($p=0.003$) significantly affected leaf hue angle. Hue angle was considerably lower after storage (119.2 °) compared to before storage (123.4 °), indicating yellowing of the leaves during storage. 'Ice Queen', 'Grandiceps' and 'Didi' had comparable leaf hue angle as well as 'Didi' and 'Barbigera 2'. 'Barbigera 1' and 'Barbigera 2' also had non-significant differences in leaf hue angle.

In the 2016 season no significant interaction between storage, product and treatment was recorded ($p=0.872$) for leaf hue angle (Table 8). However, significant interactions between storage and product ($p=0.001$) and between storage and treatment ($p<0.0001$) were obtained. AirEx did not affect leaf colour during storage, whereas products stored in 5-10-15 % CO_2 and 15 % CO_2 had significantly lower post-storage hue readings compared to pre-storage for some cultivars. This was especially the case with 'Sylvia' and 'Cynaroides' which had notably lower hue angles after storage, whereas 'Ice Queen' and 'Barbigera' were not significantly affected.

4.2.3 Visual quality: Inflorescence

Quality of inflorescences was significantly ($p<0.0001$) affected by the interaction between time, product and treatment in the 2015 season. 'Didi' and 'Grandiceps', 'Ice Queen' and 'Barbigera 1' (Figs. 5A, 5B, 5C, 5D) stored in AirEx and 5-10-15 % CO_2 had similar quality on day 1 through to day 7 of vase life. Quality differences were only notable from day 10 onwards when, in general, stems exposed to the high CO_2 regime, displayed better quality, even though stems from both treatments did not meet the minimum quality requirement anymore. 'Barbigera 2' (Fig. 5E) results revealed no significant quality differences between

the treatments on day 1, however by day 7 of vase life stems from both treatments reached an unacceptable quality for retail.

In the 2016 season a significant interaction ($p=0.005$) between time, product and treatment affected inflorescence quality. Observation on 'Ice Queen', 'Barbigera' and 'Sylvia' (Figs. 6A, 6B, 6C) indicated no significant differences among the treatments from the first day of vase life evaluation through to day 7 of vase life. However, storage in AirEx was more effective at delaying quality loss in 'Cynaroides' (Fig. 6D), whereas this treatment accelerated quality loss of 'Sylvia' inflorescences.

4.3.4 Visual quality: Leaves

Quality of leaves was significantly ($p=0.017$) affected by an interaction between time, product and treatment in the 2015 season. 'Grandiceps' (Fig. 7B) were not significantly affected by treatments between day 1 and day 7 of vase life. However, 5-10-15 % CO_2 storage resulted in better quality for 'Didi' (Fig. 7A) and 'Barbigera 2' (Fig. 7D) throughout the evaluation period. Although 'Barbigera 1' stems stored in AirEx had better leaf quality at the start (Fig. 7C), all the products reached unacceptable quality by day 5. 'Ice Queen' (Fig. 7E) stored in AirEx displayed a better quality than that of product stored in 5-10-15 % CO_2 storage.

A significant interaction ($p<0.0001$) between vase life, product and treatment affected the leaf quality in 2016 season. Differences among the treatments applied to 'Ice Queen' and 'Barbigera' (Figs. 8A, 8B) were marginal throughout the evaluation period. However, products stored in 15 % CO_2 had consistently slightly lower quality than those stored in AirEx and 5-10-15 % CO_2. Although 'Sylvia' that was stored in AirEx had superior quality compared to the other treatments on day 1 of vase life (Fig. 8C), the quality deteriorated fast so that by day 5 of vase life the quality of all the products was below the minimum acceptable for retail, regardless of treatment. Storage of 'Cynaroides' (Fig. 8D) in AirEx and 5-10-15 % CO_2 considerably delayed quality loss in the leaves during the first week of vase life, whereas exposure to 15 % CO_2 promoted rapid decline in leaf quality throughout the vase life period.

4.4.5 Soluble carbohydrates

A significant interaction ($p<0.0001$) between product and treatment for soluble carbohydrates was obtained in the 2015 season (Table 9). Storage regime did not impact on the monosaccharide plus oligosaccharide content of 'Barbigera 1', 'Didi' and 'Ice Queen'. However, 'Barbigera 2' and 'Grandiceps' stored in 5-10-15 % CO_2 had higher monosaccharide plus oligosaccharide content compared to that of the product stored in AirEx.

In the 2016 season, no significant interaction ($p=0.124$) between product, carbohydrate type and treatment was observed (Table 9). However, a significant interaction between product and carbohydrate type was obtained ($p<0.0001$) as 'Ice Queen' and 'Cynaroides' had significantly higher monosaccharide plus oligosaccharide content than that recorded for 'Barbigera' and 'Sylvia', in that order. Both the polysaccharide and starch content were found not to vary among the cultivars studied.

4.2.6 Lipid peroxidation

In the 2015 season, no significant interaction between product and treatment was found ($p=0.449$), whilst the various products differed significantly ($p<0.0001$) amongst themselves in terms of MDA values, which is considered a reliable estimation of the degree of lipid peroxidation (Table 10). The MDA content was found highest in 'Barbigera 1', 'Barbigera 2' and 'Ice Queen', whilst values from 'Grandiceps' and 'Didi' were significantly lower, but did not differ from one another. However, storage regime did not impact significantly on the estimated lipid peroxidation values.

Interaction between product and treatment was non-significant ($p=0.827$) in the 2016 season (Table 10). However, 'Ice Queen' had a significantly higher MDA content than that recorded for 'Barbigera', 'Sylvia' and 'Cynaroides', which produced similar values on analysis. As in the 2015 season, storage regime did not impact differently on the various products.

4.3 Lower O_2 limit trial

4.3.1 Mass loss

Mass loss was significantly ($p<0.0001$) affected by an interaction between product and treatment in the 2015 season (Table 11). Results for 'Barbigera', 'Grandiceps', 'Red Magic', and 'Red Rex' revealed that storage treatment did not have a significant effect on mass loss. However, a significant higher mass loss was recorded for stems of 'Cynaroides' stored in 2 % O_2, compared to AirEx.

Similar to the 2015 season, mass loss was significantly ($p<0.0001$) affected by an interaction between product and treatment in the 2016 season (Table 11). Storage in DCA and 2 % O_2 favoured higher mass loss in 'Ice Queen'. Differences between treatments were insignificant in 'Barbigera', whereas storage in 2% O_2 resulted in a significantly higher mass loss in 'Sylvia' compared to the other treatments, which did not differ significantly from each

other. Mass loss of 'Cynaroides' was highest in product stored in DCA and lowest in product stored in 2 % O_2.

4.3.2 Colour determination of inflorescence and leaves

No significant interaction between storage, product and treatment was obtained (p=0.207) for inflorescence head hue angle in the 2015 season (Table 12). A significant storage and product interaction however, emerged (p=0.0391). Results indicated that the period of storage, irrespective the storage treatment, did not affect the hue angle readings for 'Red Magic', 'Grandiceps', 'Barbigera' and 'Red Rex'. Only 'Cynaroides' inflorescences had significantly lower hue angle following storage.

Interaction between storage, product and treatment did not significantly (p=0.199) affect the inflorescence hue angle in the 2016 season (Table 13). However, interactions between storage and product (p<0.0001) and that of product and treatment (p=0.007) had a significant effect on inflorescence hue angle. Inflorescence hue angle readings for 'Sylvia', 'Ice Queen' and 'Cynaroides' were not affected by the storage period. Nonetheless, hue values for 'Barbigera' increased with storage from 24.9 ° before storage to 32.4 ° after storage, which implies a change from red towards yellow colour. Despite the statistical significance of the hue change, there were no was visually detectable signs of colour change. The storage regimes of AirEx, DCA and 2 % O_2 did not significantly affect inflorescence colour of 'Sylvia', 'Ice Queen' or 'Cynaroides'. However, inflorescences of 'Barbigera' stored in DCA had elevated hue angle values compared to stems stored in the other treatments.

No significant interaction between storage, product and treatment (p=0.111) affected the leaf hue angle in the 2015 season (Table 14). Hue angle was, however, significantly (p<0.0001) affected by an interaction between storage and product. Leaf hue angles of 'Red Magic', 'Grandiceps' and 'Red Rex' were not significantly affected by the storage period. However, there was a significant decrease in leaf hue angle of 'Barbigera' and 'Cynaroides' (115 ° and 122.9 ° before storage and 107.8 ° and 120.4 ° after storage, respectively). This indicates a change towards yellow from green colour, however colour change was visually undetectable except for slight leaf blackening which was present on some leaves.

No significant interaction (p=0.171) between storage, product and treatment was reported in the 2016 season (Table 14). Only storage (p=0.003) and product (p<0.0001) had a significant influence on leaf hue angle. Hue angle of the products was generally lower after storage compared to before storage. 'Cynaroides' and 'Ice Queen' recorded similar hue leaf

angles which were significantly higher than those recorded for ‘Barbigera’ and ‘Sylvia’, where ‘Sylvia’ displayed the lowest leaf hue angle in all products.

4.3.3 Visual quality: Inflorescence

No significant interaction ($p=0.175$) between time, product and treatment occurred in the 2015 season for inflorescence quality. Inflorescence quality was significantly affected by the interaction between time and treatment ($p=0.004$) and between time and product ($p<0.0001$). There were insignificant differences between quality of product stored in AirEx and 2 % O_2 before day 3 (Fig. 9A). However, the stems stored in 2 % O_2 had better quality than those stored in AirEx from day 3 until the end of evaluation. Inflorescences of ‘Cynaroides’, ‘Red Rex’ and ‘Red Magic’ had superior quality on the first day of vase life (Fig. 9B). By day 7 of vase life ‘Red Rex’ inflorescence scored the lowest quality, below that of saleability, whilst all the other products were able to retain similar and acceptable quality for retail.

In the 2016 season, inflorescence quality was significantly ($p=0.007$) affected by an interaction between time, product and treatment. The data for ‘Ice Queen’, ‘Barbigera’ and ‘Cynaroides’ (Figs. 10A, 10B, 10C) revealed that there were slight differences among the treatments, throughout the evaluation period. However, ‘Sylvia’ stems stored in AirEx had significantly better post-storage quality than stems stored in DCA and 2 % O_2 (Fig. 10D).

4.3.4 Visual quality: Leaves

No significant interaction ($p=0.196$) between time, product and treatment occurred in the 2015 season. However, leaf quality was significantly affected by an interaction between time and treatment ($p=0.049$) and between time and product ($p<0.0001$). Product stored in 2 % O_2 had consistently higher quality than the product stored in AirEx (Fig. 11A). All the products had significantly higher quality indices than ‘Barbigera’, on the first day of vase life. ‘Grandiceps’ maintained export grade quality throughout the entire evaluation period, whilst ‘Cynaroides’, ‘Barbigera’ and ‘Red Rex’ scored reduced indices of below 2.5 on day 7 of vase life (Fig. 11B).

In the 2016 season, leaf quality was significantly ($p<0.0001$) affected by an interaction between time, product and treatment. Quality differences among treatments were minor, throughout the vase life evaluation for ‘Ice Queen’ and ‘Barbigera’ (Figs. 12A, 12B). However, despite the initial likeness in leaf quality between treatments, ‘Cynaroides’ and ‘Sylvia’ stems

that were stored in AirEx had a significant and rapid decline in quality, compared to stems stored in DCA and 2 % O_2 (Figs. 12C, 12D).

4.3.5 Soluble carbohydrates

No significant interaction ($p=0.369$) between product and treatment was obtained for carbohydrate content in the 2015 season (Table 15). The various cultivars, however, differed significantly ($p<0.0001$) with respect to soluble mono- and oligosaccharides. 'Grandiceps' stems were shown to contain carbohydrate levels that were significantly higher than in any of the other cultivars, including 'Red Magic', which scored the second highest carbohydrate content. 'Barbigera' and 'Cynaroides' did not differ significantly from each other, but were significantly lower than that reported in 'Red Magic' and higher than levels recorded in 'Red Rex'.

Carbohydrate content was significantly ($p<0.0001$) affected by an interaction between product, treatment and carbohydrate type in the 2016 season (Fig. 13). Monosaccharide plus oligosaccharide content data for 'Sylvia' and for 'Cynaroides' showed that concentrations of the sugars did not differ significantly among the treatments. In 'Barbigera', monosaccharides plus oligosaccharides were more abundant in stems stored in AirEx and lowest in product stored in DCA. However, the opposite was observed for 'Ice Queen', where storage in DCA resulted in the highest monosaccharide plus oligosaccharide content, with storage in AirEx resulting in the lowest concentration of the sugars. Polysaccharide and starch content of the various cultivars remained similar among the storage treatments in all the products.

4.3.6 Total phenolic content

A significant ($p=0.049$) interaction between product and treatment was obtained in the 2015 season (Table 16). There were insignificant variations in total phenolics between the AirEx and 2 % O_2 treatments in 'Cynaroides', 'Red Rex', 'Grandiceps' and 'Red Magic'. However, storage of 'Barbigera' in 2 % O_2 resulted in higher phenolic content than storage in AirEx.

In the second season, a significant ($p=0.006$) product and treatment interaction impacted on total phenolic content. Differences among storage treatments were insignificant in 'Sylvia' and 'Ice Queen'. Comparison of 'Barbigera' treated in AirEx and DCA revealed minor differences, similar to the phenolic content recorded in 'Barbigera' stems stored in DCA and 2 % O_2 treatments. However, 'Barbigera' stems stored in AirEx had significantly higher total phenolic content than stems stored in 2% O_2. Storage of 'Cynaroides' stems in 2 % O_2

resulted in a significantly higher concentration of total phenolics compared to stems exposed to AirEx and DCA treatments (Table 16).

5. Discussion

5.1 Gas dynamics and respiration rates

Findings on *Leucadendron* and *Leucospermum* (Paper 3 and 4) indicated that gas sampling using the HarvestWatch™ system resulted in disturbance of the closed ventilation system due to pressure decrease inside the Janny MT boxes during sampling. It is thus possible that the O_2 concentration could have decreased below the observed level and for the CO_2 level to have risen above the recorded concentration, when not exposed to changes introduced through gas sampling. Additionally, the gas dynamics in a closed ventilation setup are dependent on the respiration rate of the products, which is affected by temperature, gas concentration, harvesting maturity, growing conditions and product type (Kader, 2014). The closed ventilation system results in a passive MA, which lowers metabolic activity and raises the RH, resulting in lower moisture loss due to a smaller vapour pressure deficit between the plant tissue and the storage atmosphere (Wills et al., 2007).

Although a 45-55 % free air content was achieved inside the closed ventilation Janny MT boxes, the amount of free air in a reefer used for sea freight shipping is lower than in a Janny MT box (< 35 % free air). Consequently, different results will likely be obtained if a commercial trial is to be conducted, as the O_2 depletion and CO_2 accumulation may occur faster than in a Janny MT box, which has more free air and is under better temperature management. Saltveit (2016) outlined that at higher temperatures, the Q_{10} is often smaller than at lower temperatures and it differs between different temperature ranges. Similarly, Ekman et al. (2008) observed that the Q_{10} is greater between 0-10 °C (3.4) compared to between 10-20 °C (1.4). The results from the study on Geraldton wax cv. My Sweet Sixteen proved that the 0-2 °C temperature range had the highest Q_{10} within the 0-40 °C temperature range. Additionally, the estimated respiration rate at 1 °C will differ (may be higher or lower) from the actual respiration rate in a commercial reefer since the effects of temperature, gas concentration, storage duration and chilling stress were not accounted for in the estimate. Therefore, the O_2 and CO_2 sensors on automated reefers are more reliable than estimation using the Q_{10}.

5.2 Mass loss

Mass loss was generally greater in stems stored in AirEx compared to closed ventilation. AirEx in standard reefers promotes greater changes in RH and increased air movement due to continuous ventilation and fixed fan speed, compared to closed ventilation with relatively static air and advanced reefers that have variable fan speed. Gas dynamics are likely to vary under commercial shipping due to O_2 depletion and CO_2 accumulation occurring faster than in a Janny MT box, which has more free air and better temperature management. A thicker boundary layer, as would occur in the absence or with a reduction of air movement, such as under closed ventilation, result in lower moisture loss from fresh products. Moisture loss from fresh produce depends on the steepness of the water vapour gradient between the plant tissue and the surrounding air along with the diffusional resistance of the transpiration pathway (Taiz et al., 2015; Wills et al., 2007). Taiz et al. (2015) also acknowledged that numerous anatomical and morphological structures also influence the thickness of the boundary layer. *Protea* plants have sclerophyllous leaves that contain dense trichomes and papillae which assist with water conservation (Leonhardt and Criley, 1999).

The optimum RH for storage of most cut flower products is considered around 95 % (Reid, 2016; Vigneault et al., 2009), with lower RH levels promoting moisture loss. There was no RH control in the AirEx reefers used for *Proteaceae* and as a result the RH greatly fluctuated between ventilation cycles (Huysamer, personal communication, 7 March 2017). Moisture loss is enhanced when fresh products are held below the optimum RH during storage or retail (Herppich et al., 1999). Product comparison revealed that mass loss was greater in 'Barbigera' and 'Red Rex' compared to 'Grandiceps'. However, the physiological traits of the products, amount of leaves present on stems after processing and pre-harvest conditions are highly variable among producers and they all affect moisture loss from produce. Storage in 5-10-15 % CO_2 resulted in consistently lower mass loss than AirEx. Although storage in 15 % CO_2 gave similar results to storage in 5-10-15 % CO_2, the risk of CO_2 damage makes 5-10-15 % CO_2 treatment the best alternative since the stepped increase approach was generally harmless to all the products.

Storage in AirEx and 2 % O_2 had similar effects on mass loss of products in the 2015 season, whereas varying findings were obtained in the 2016 season. However, DCA storage resulted generally in greater mass loss than AirEx or 2 % O_2. Despite these recorded differences, mass loss was generally negligible. Placement of products close to the ventilation valves in such a small volume experimental system as a Janny MT box could also promote

mass loss in DCA and 2 % O_2 systems, therefore unintentionally compromising results. Additionally, DCA storage involves repeated pumping of gases into the atmosphere around the products. This may have promoted moisture loss since the DCA system used was not equipped with a gas humidifier.

5.3 Colour determination of inflorescences and leaves

The AirEx and closed ventilation treatments did not have a significant effect on hue angles of inflorescences and differences in colour were visually indistinguishable (personal observation). Post-storage hue angle measurements for inflorescences and leaves were generally lower when stored in AirEx compared to before storage. This shows increase in red colour in inflorescences and yellowing of the leaves from green. Despite the variances in inflorescences of products stored in AirEx, pre- and post-storage hue angle differences were generally not significant when comparing products stored in 5-10-15 % CO_2 with those stored under 15 % CO_2 treatments. Differences in hue angle were mainly distinguishable between products and this was likely due to inherent genetic differences in pigment types, combinations and concentrations. The leaves of the *Protea* stems generally had a lower hue after storage, regardless of the storage atmosphere. This shows a general yellowing of the leaves during storage, presumably due to a loss of chlorophyll resulting from prolonged lack of light. Differences in inflorescence and leaf hue angles between AirEx, DCA and 2 % O_2 regimes were generally minor and unimportant, in both seasons. Although there were some significant differences in hue, they were not visually detectable. According to van Doorn and van Lieburg (1993) darkness, as would be the case in sea freighting of ornamental stems, transforms the phytochrome to the Pfr form, which may activate physiological processes, which include colour change primary in foliage, but also in petals.

5.4 Visual quality: Inflorescence

Storage in closed ventilation generally resulted in better inflorescence quality than AirEx. Similar observations were made by Aros et al. (2017) who recommended closed ventilation storage for tulips at 0 °C for extended longevity. Storage in MAP and closed ventilation increased vase life by slowing respiration rate, lowering water loss from the flowers and assisted in maintaining a stable RH around the product (Bishop et al., 2007). In our study, storage of the product in AirEx and 5-10-15 % CO_2 resulted in similar inflorescence quality during the first 7 d of vase life evaluation in the first season. However, beyond 7 d product stored in 5-10-15 % CO_2 generally displayed better inflorescence quality. The high CO_2 could

have resulted in the extended vase life since it lowers respiration rate and possibly resulted in the higher carbohydrate content, as observed in some products in the 2015 season. Data from the second season revealed that AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments resulted in comparable inflorescence quality. However, product stored in 5-10-15 % CO_2 generally had slightly superior inflorescence quality. Yet, the observed differences may have been due to product variation or harvesting maturity. Quality differences between AirEx, DCA and 2 % O_2 treatments were negligible in both seasons and longevity of inflorescences was highly dependent on product type.

5.5 Visual quality: Leaves

Storage in closed ventilation resulted in better leaf quality than storage in AirEx. Generally there was less chilling damage on the leaves of product stored in closed ventilation. Furthermore, the progression of leaf blackening during vase life was slower in product stored in closed ventilation compared to product stored in AirEx. Ferreira (1983) and Paull and Dai (1990) reported that leaf blackening is escalated by water stress, which is promoted by the reduced RH around the products in AirEx ventilation. The better vase life quality which was observed in stems stored under closed ventilation and 5-10-15 % CO_2 could have resulted from lower moisture stress since mass loss was lower in these products compared to those under AirEx.

Products that were stored in AirEx and 5-10-15 % CO_2 had greater visual quality longevity compared to products stored in 15 % CO_2. There was an increased incidence of CI in product stored in 15 % CO_2. It was also observed that 'Barbigera' products stored in high CO_2 had a more rapid development of leaf blackening during vase life. The high CO_2 concentration possibly caused membrane damage and lipid peroxidation since the MDA content, which is an indication of lipid peroxidation (Hodges et al., 2004), was also high in 'Barbigera' compared to the other products. High CO_2 storage has been reported to cause leaf discolouration in roses (Hammer et al., 1990; Tshwenyane et al., 2012).

Quality differences between product stored in AirEx, DCA and 2 % O_2 were generally negligible. Where differences were noteworthy, product stored in DCA and 2 % O_2 had superior quality compared to product stored in AirEx. The reduced ventilation rate in DCA and 2 % O_2 decreases moisture loss and possibly reduces incidence of CI and leaf blackening, which are aggravated by dehydration (Paull et al., 1980; Whitehead and de Swardt, 1982). Product comparison showed that the leaf quality of 'Grandiceps' hardly changed during the evaluation period. Differences between products were likely due to genetics, harvesting conditions and

maturity, growing conditions and post-harvest handling. Longevity of 'Sylvia' and 'Cynaroides' was significantly reduced by storage in AirEx, which may be an indication that the products are not suitable for long-term cold storage at 1 °C at the currently used ventilation rate. Furthermore, the quick decline in quality of 'Sylvia' and 'Cynaroides' stored under AirEx could be due to the lower carbohydrate content, compared to the other products.

5.6 Soluble carbohydrates

The AirEx and closed ventilation treatments had minor influence on monosaccharide plus oligosaccharide content of *Protea* products in both seasons. However, closed ventilation led to a higher monosaccharide plus oligosaccharide content in 'Red Rex'. 'Grandiceps' generally had the highest monosaccharide plus oligosaccharide content compared to the other products. Furthermore, its quality was consistently high throughout the evaluation period, which could be linked to its high carbohydrate content and lack of leaf blackening signs. Leaf blackening has been attributed to carbohydrate depletion in the leaves (McConchie et al., 1991), thus a high carbohydrate content may be the reason for high longevity in 'Grandiceps'. Generally, the AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments had negligible differences in content of monosaccharides plus oligosaccharides, polysaccharides and starch. However, in the 2015 season some products had higher monosaccharide plus oligosaccharide levels when they were stored in 5-10-15 % CO_2 compared to AirEx. This may have been due to lower respiration rate under high CO_2 atmosphere (Kader, 2004). Comparison of the products showed that monosaccharides plus oligosaccharides were more abundant in 'Ice Queen' and least abundant in 'Sylvia'. Despite this, the results varied considerably between seasons and no specific trend was observed.

Although there were inconsistencies in some products, the monosaccharide plus oligosaccharide content was not significantly affected by AirEx, DCA and 2 % O_2 treatments. Polysaccharide content and starch content were also not affected by the AirEx and low O_2 treatments. Monosaccharides plus oligosaccharides were the most abundant sugars in the products, regardless of the storage atmosphere. Extended cold storage of cut flowers has been reported to initiate starch and sucrose degradation, resulting in a higher concentration of reducing sugars (Van Doorn and Han, 2011; Walton et al., 2010). Furthermore, the authors noted that an increase in reducing sugar levels is a defence mechanism against chilling or frost injury in plants.

5.7 Lipid peroxidation

The AirEx, 5-10-15 % and 15 % CO_2 storage regimes generally had a similar impact on the MDA levels, for both seasons. However, lipid peroxidation did vary considerably among the products. 'Barbigera' and 'Ice Queen', which are both selections of *Protea magnifica*, recorded the highest MDA concentrations. The elevated MDA levels determined in 'Barbigera' may be connected to its high susceptibility to leaf blackening (Windell, 2012). It has been reported that leaf blackening in *Protea* is linked to the peroxidase and PPO activity (Hernández et al., 2012; Van Doorn, 2001).

5.8 Total phenolic content

The AirEx, DCA and 2 % O_2 treatments resulted in non-significant differences in total phenolics, in both seasons. Van Rheede van Oudtshoorn (1963) reported that phenolic compounds are abundant in *Proteaceae*. There is need for further investigation of phenolic activity in *Protea* since they play a role in leaf blackening (Dai and Paull, 1995; Malan, 2012). Phenolic compounds turn brown or black when oxidized or polymerized under stress conditions (Ferreira, 1983; Paull et al., 1980).

6. Conclusions and recommendations

In a closed ventilation system with 45-55 % free air that is monitored using a HarvestWatch™ DCA system and containing mixed *Proteaceae* products, the O_2 level fell to about 8.5 % and the CO_2 level rose approximately 10 %. However, results from chapter 3 and 4 showed that the gas dynamics in a closed ventilation system vary depending on respiration rate of the products, volume of free air and gas sampling method. The respiration rates of the *Protea* products ranged from 10.76-27.24 mL $CO_2.kg^{-1}.h^{-1}$ at 5 °C, in the 2015 and 2016 seasons. The Q_{10} values for the 5-15 °C were between 1.7 and 3.84.

Mass loss was higher in product stored in AirEx compared to those under closed ventilation and high CO_2 atmosphere, and results for AirEx and low O_2 treatments were generally comparable. The AirEx and closed ventilation treatments had non-significant visually detectable effects on the colour of inflorescences and leaves. Similarly, the change in hue angle of inflorescences and leaves of products stored in AirEx, high CO_2 and low O_2 were minor. Although the 5-10-15 % CO_2 and 15 % treatments resulted in greater leaf colour loss than AirEx in the second season, the changes in hue angle were visually indistinguishable and did not reduce the visual quality of the products.

Products stored in closed ventilation had better inflorescence and leaf visual quality than products stored in AirEx. Storage in AirEx and high CO_2 resulted in similar inflorescence and leaf quality among the products. There were inconsistencies in leaf quality responses to the high CO_2 treatments among the products. However, the 15 % CO_2 treatment led to faster inflorescence and leaf deterioration of 'Cynaroides' and 'Ice Queen'. The quality and longevity of products stored in AirEx and the low O_2 were comparable, although AirEx treatment notably decreased the vase life of 'Sylvia' and 'Cynaroides'.

The AirEx, high CO_2 and low O_2 treatments had minor influence on the quantity of soluble carbohydrates and differences in carbohydrate content were likely due to pre-harvest and product dependent factors. Monosaccharides plus oligosaccharides were the most abundant sugars, followed by polysaccharides. Starch was the least abundant carbohydrate group in the leaves. The differences in quantity of polysaccharides and starch were minor between products stored in AirEx and those in low O_2. Similarly, differences in quantity of polysaccharides and starch were insignificant between the AirEx and high CO_2 treatments.

The AirEx, 5-10-15 % and 15 % CO_2 treatments had insignificant effects on the MDA levels in the products. However, lipid peroxidation was variable among the products and appeared to occur more in 'Barbigera' and 'Ice Queen', which are both susceptible to leaf blackening. The AirEx, DCA and 2 % O_2 treatments did not have a significant effect on total phenolic content of the products. The concentration of total phenolics was variable among the products. In most cases, closed ventilation, low O_2 and high CO_2 resulted in visual quality and longevity which was similar or better than that of products stored in AirEx. This showed that low O_2, closed ventilation and high CO_2 storage are suitable for use during long-term cold storage of some *Protea* products. However, the O_2 level should be maintained above the lower O_2 limits of the products, which ranged from 0.08-0.48 % O_2. Thus, automatic ventilation may be set to maintain a minimum of 2 % O_2 and maximum of 15 % CO_2 concentration in the reefers during long-term sea freight shipping of *Protea* cut flower products. Addition of CO_2 scrubbers may help to further prevent injection of external air into the storage atmosphere if the maximum CO_2 limit is reached before the minimum O_2 limit.

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8. Tables and Figures

Table 1

Lower O_2 limit (LOL) values, respiration rates, temperature quotients (Q_{10}) and trial dates for *Protea* products in the 2015 and 2016 seasons.

2015 Season									
Product	**Respiration at 5 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Respiration at 15 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Q_{10}**	**Estimated respiration at 1 °C**	**LOL (O_2 %)**	**Respiration rate date**	**CO_2 toxicity tolerance trial date**	**Closed ventilation trial date**	**LOL trial Date**
'Sylvia'	27.24	46.37	1.70	22.03	0.17	20-May	-	-	-
'Pink Ice'	25.88	54.41	2.10	19.23	0.20	27-May	-	-	-
'Sussara'	21.65	54.89	2.54	14.91	-	27-May	-	-	-
'Barbigera'	13.56	31.11	2.29	9.73	0.16	02-Jun	24-Aug & 14-Oct	11-Oct	13-Sep
'Cynaroides'	16.04	47.30	2.95	10.41	0.25	29-Oct	-	-	28-Oct
'Ice Queen'	10.76	33.52	3.12	6.83	-	20-Nov	24-Aug	-	-
'Grandiceps'	14.42	54.73	3.80	8.45	0.08	14-Nov	24-Aug	11-Oct	13-Sep
'Red Rex'	16.96	49.71	2.93	11.03	0.25	14-Nov	-	11-Oct	20-Nov
'Red Magic'	-	-	-	-	0.16	-	-	-	13-Sep
'Didi'	-	-	-	-	-	-	24-Aug	-	-
2016 Season									
Product	**Respiration at 5 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Respiration at 15 °C (mL $CO_2.kg^{-1}.h^{-1}$)**	**Q_{10}**	**Estimated respiration at 1 °C**	**LOL (O_2 %)**	**Respiration rate date**	**CO_2 toxicity tolerance trial date**	**Closed ventilation trial date**	**LOL trial date**
'Barbigera'	12.46	24.12	1.94	9.56	0.48	01-Aug	28-Jul	-	28-Jul
'Cynaroides'	17.61	49.75	2.83	11.62	0.16	03-Sep	01-Sep	-	-
'Sylvia'	13.27	51.00	3.84	7.75	0.23	09-Jun	09-Jun	-	09-Jun
'Ice Queen'	16.49	32.81	1.99	12.52	0.32	01-Aug	28-Jul	-	28-Jul
'Red Rex'	-	-	-	-	-	-	-	-	02-Sep

Table 2

Mass loss (%) of *Protea* products ('Barbigera', 'Red Rex', and 'Grandiceps') stored in AirEx and closed ventilation at 1 °C for 21 d in the 2015 season.

2015 Season			
Treatment			**Mean mass loss (%)**
AirEx			3.900 a
Closed ventilation			2.504 b
Product			
'Barbigera'			3.565 a
'Red Rex'			3.634 a
'Grandiceps'			2.407 b
Effect	Product	Treatment	Product x Treatment
P value	*0.001*	*<0.0001*	*0.090ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 3

Colour determination (h°) of inflorescence and leaves of *Protea* products ('Grandiceps', 'Red Rex' and 'Barbigera') stored in AirEx and closed ventilation for 21 d at 1 °C in the 2015 season.

2015 Season Inflorescences							
Storage					**Mean hue angle (°)**		
After					29.2 a		
Before					27.1 b		
Product							
'Grandiceps'					31.6 a		
'Barbigera'					29.4 a		
'Red Rex'					23.5 b		
Effect	Treatment	Product	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*0.426ns*	*<0.0001*	*0.037*	*0.738ns*	*0.382ns*	*0.915ns*	*0.701ns*
Leaves							
Product					**Mean Hue angle (°)**		
'Barbigera'					129.4 a		
'Red Rex'					123.5 b		
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*<0.0001*	*0.437ns*	*0.241ns*	*0.796ns*	*0.707ns*	*0.107ns*	*0.144ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 4

Soluble carbohydrates (mg.g^{-1}) in leaves of *Protea* products ('Red Rex', 'Barbigera' and 'Grandiceps') after 21 d of storage at 1 °C in AirEx and closed ventilation in the 2015 season.

	2015 season (Monosaccharides + Oligosaccharides)		
Product	**Treatment**	**Mean glucose mg.g^{-1}**	
'Barbigera'	AirEx	67.2	b
	Closed ventilation	64.8	b
'Grandiceps'	AirEx	83.9	a
	Closed ventilation	75.1	ab
'Red Rex'	AirEx	30.5	d
	Closed ventilation	46.7	c
Effect	Treatment	Product	Product x Treatment
P value	*0.688ns*	*<0.0001*	*0.043*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 5

Mass loss (%) of *Protea* products stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Grandiceps', 'Didi', 'Ice Queen', 'Barbigera 1' and 'Barbigera 2') and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Ice Queen', 'Barbigera', 'Sylvia' and 'Cynaroides') in the 2016 season.

2015 Season			
Treatment			**Mean mass loss (%)**
AirEx			6.9 a
5-10-15 % CO_2			4.7 b
Product			
'Grandiceps'			4.0 ns
'Didi'			6.3
'Ice Queen'			6.3
'Barbigera 1'			5.4
'Barbigera 2'			7.0
Effect	Product	Treatment	Product x Treatment
P value	*0.085ns*	*0.004*	*0.127ns*

2016 Season			
Product	**Treatment**		**Mean mass loss (%)**
'Ice Queen'	AirEx		2.7 de
	5-10-15 % CO_2		2.4 de
	15 % CO_2		3.5 cd
'Barbigera'	AirEx		2.4 cd
	5-10-15 % CO_2		1.8 e
	15 % CO_2		3.1 cd
'Sylvia'	AirEx		6.7 a
	5-10-15 % CO_2		4.9 b
	15 % CO_2		4.7 b
'Cynaroides'	AirEx		8.0 a
	5-10-15 % CO_2		4.0 bc
	15 % CO_2		3.3 cd
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 6

Colour determination (h°**)** of inflorescences of *Protea* products stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Barbigera 1', 'Barbigera 2', 'Grandiceps', 'Ice Queen' and 'Didi') in the 2015 season.

2015 Season							
Product			**Storage**			**Mean hue angle (°)**	
'Barbigera 1'			Before			27.1	cd
			After			27.4	cd
'Barbigera 2'			Before			19.5	de
			After			22.4	de
'Grandiceps'			Before			30.4	c
			After			27.3	cd
'Ice Queen'			Before			108.6	a
			After			104.5	a
'Didi'			Before			39.5	b
			After			25.7	cd
Effect	Treatment	Product	Storage	Product x Treatment	Storage x Treatment	Storage x Product	Storage x Product x Treatment
P value	*0.676ns*	*<0.0001*	*0.012*	*0.803ns*	*0.971ns*	*0.004*	*0.965ns*

Table 7

Colour determination (h°) of inflorescences of *Protea* products stored for 21 d at 1 °C in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Sylvia', 'Ice Queen', 'Barbigera' and 'Cynaroides') in the 2016 season.

2016 Season			
Product	**Treatment**	**Mean hue angle (°)**	
'Sylvia'	AirEx	27.5	bcd
	5-10-15 % CO_2	28.0	bc
	15 % CO_2	27.1	bcde
'Ice Queen'	AirEx	99.8	a
	5-10-15 % CO_2	105.0	a
	15 % CO_2	103.9	a
'Barbigera'	AirEx	24.6	cde
	5-10-15 % CO_2	21.9	e
	15 % CO_2	32.2	b
'Cynaroides'	AirEx	22.2	de
	5-10-15 % CO_2	30.2	b
	15 % CO_2	30.5	b
Product	**Storage**		
'Sylvia'	Before	27.2	cd
	After	27.9	cd
'Ice Queen'	Before	105.3	a
	After	100.5	b
'Barbigera'	Before	25.9	d
	After	26.6	cd
'Cynaroides'	Before	25.3	d
	After	30.0	c

Effect	Product	Treatment	Storage	Storage x Treatment	Storage x Product	Product x Treatment	Storage x Product x Treatment
P value	*<0.0001*	*0.002*	*0.757ns*	*0.500*	*0.007*	*0.008*	*0.154ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 8

Colour determination (h°)of leaves of *Protea* products stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Barbigera', 'Grandiceps', 'Ice Queen' and 'Didi') in the 2015 season and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Sylvia', 'Ice Queen', 'Barbigera' and 'Cynaroides') in the 2016 season.

2015 Season							
Storage						**Mean hue angle (°)**	
Before						123.4	a
After						119.2	b
Product							
'Ice Queen'						124.3	a
'Grandiceps'						123.4	a
'Didi'						121.7	ab
'Barbigera 2'						119.0	bc
'Barbigera 1'						118.0	c
Effect	Treatment	Product	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*0.208ns*	*0.003*	*<0.0001*	*0.737ns*	*0.452ns*	*0.434ns*	*0.633ns*
2016 Season							
Treatment			**Storage**			**Mean hue angle (°)**	
AirEx			Before			120.7	ab
			After			121.6	a
5-10-15 % CO_2			Before			121.0	a
			After			118.6	bc
15 % CO_2			Before			122.0	a
			After			116.6	c
Product							
'Sylvia'			Before			121.9	ab
			After			116.4	d
'Ice Queen'			Before			119.1	bcd
			After			118.7	cd
'Barbigera'			Before			120.8	abc
			After			120.2	abc
'Cynaroides'			Before			123.1	a
			After			120.4	bc
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*0.15ns*	*0.287ns*	*<0.0001*	*0.024*	*0.001*	*<0.0001*	*0.872ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 9

Soluble carbohydrates (mg.g^{-1}) in leaves of *Protea* products after 21 d of storage at 1 °C in AirEx and 5-10-15 % CO_2 ('Barbigera', 'Didi', 'Grandiceps' and 'Ice Queen') in the 2015 season and in AirEx, and 5-10-15 % CO_2 and 15 % CO_2 ('Sylvia', 'Barbigera', 'Ice Queen' and 'Cynaroides') in the 2016 season.

2015 Season (Monosaccharides + Oligosaccharides)							
Product	**Treatment**				**Mean glucose mg.g^{-1}**		
'Barbigera 1'	AirEx				50.9	e	
	5-10-15 % CO_2				64.9	de	
'Barbigera 2'	AirEx				39.4	f	
	5-10-15 % CO_2				53.8	e	
'Didi'	AirEx				52.1	e	
	5-10-15 % CO_2				47.3	ef	
'Grandiceps'	AirEx				87.6	b	
	5-10-15 % CO_2				117.2	a	
'Ice Queen'	AirEx				76.1	c	
	5-10-15 % CO_2				69.6	cd	
Effect	Product		Treatment		Product x Treatment		
P value	*<0.0001*		*<0.0001*		*<0.0001*		
2016 Season							
Product	**Carbohydrate type**				**Mean glucose mg.g^{-1}**		
'Sylvia'	Monosaccharides + Oligosaccharides				80.1	c	
	Polysaccharides				24.3	d	
	Starch				7.5	e	
'Barbigera'	Monosaccharides + Oligosaccharides				101.7	b	
	Polysaccharides				18.4	d	
	Starch				6.1	e	
'Ice Queen'	Monosaccharides + Oligosaccharides				110.9	a	
	Polysaccharides				18.6	d	
	Starch				6.9	e	
'Cynaroides'	Monosaccharides + Oligosaccharides				107.0	ab	
	Polysaccharides				21.0	d	
	Starch				8.8	e	
Treatment							
AirEx					42.3	ns	
5-10-15 % CO_2					42.4		
15 % CO_2					43.1		

Effect	Treatment	Product	Carb type	Carb type x Treatment	Product x Treatment	Product x Carb type	Product x Carb type x Treatment
P value	*0.877ns*	*<0.0001*	*<0.0001*	*0.893ns*	*0.144ns*	*<0.0001*	*0.124ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 10

Lipid peroxidation (nmol.mL^{-1}.g^{-1}**)** in *Protea* products stored for 21 d at 1 °C in AirEx and 5-10-15 % CO_2 ('Barbigera 1' and 'Barbigera 2', 'Ice Queen', 'Grandiceps' and 'Didi') in the 2015 season and in AirEx, 5-10-15 % CO_2 and 15 % CO_2 ('Ice Queen', 'Barbigera', 'Sylvia' and 'Cynaroides') in the 2016 season.

2015 Season			
Product		**Mean MDA nmol.mL^{-1}.g^{-1}**	
'Barbigera 1'		86.2	a
'Barbigera 2'		77.4	ab
'Ice Queen'		74.4	b
'Grandiceps'		45.5	c
'Didi'		37.5	c
Treatment			
AirEx		64.3	ns
5-10-15 % CO_2		64.1	
Effect	Treatment	Product	Product x Treatment
P value	*0.953ns*	*<0.0001*	*0.449ns*
2016 Season			
Product		**Mean MDA nmol.mL^{-1}.g^{-1}**	
'Ice Queen'		81.2	a
'Barbigera'		69.2	b
'Sylvia'		65.3	b
'Cynaroides'		58.5	b
Treatment			
AirEx		72.4	ns
5-10-15 % CO_2		66.3	
15 % CO_2		67.0	
Effect	Treatment	Product	Product x Treatment
P value	*0.373ns*	*<0.0001*	*0.827ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance. Lipid peroxidation expressed as malondialdehyde (MDA) content.

Table 11

Mass loss (%) of *Protea* products stored for 21 d at 1 °C in AirEx and 2 % O_2 ('Barbigera', 'Grandiceps', 'Red Magic' and 'Cynaroides') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Ice Queen', 'Barbigera', 'Sylvia' and 'Cynaroides') in the 2016 season .

2015 Season		
Product	**Treatment**	**Mean mass loss (%)**
'Barbigera'	AirEx	2.7 cd
	2 % O_2	2.2 d
'Grandiceps'	AirEx	2.6 cd
	2 % O_2	2.3 cd
'Red Magic'	AirEx	3.4 cd
	2 % O_2	3.8 cd
'Red Rex'	AirEx	7.3 b
	2 % O_2	7.2 b
'Cynaroides'	AirEx	4.0 c
	2 % O_2	9.3 a
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.006*	*<0.0001*
2016 Season		
Products	**Treatment**	**Mean mass loss (%)**
'Ice Queen'	AirEx	3.0 fg
	DCA	4.8 de
	2 % O_2	3.4 efg
'Barbigera'	AirEx	2.5 g
	DCA	3.1 fg
	2 % O_2	2.8 fg
'Sylvia'	AirEx	7.0 bc
	DCA	6.2 bc
	2 % O_2	10.4 a
'Cynaroides'	AirEx	5.6 dc
	DCA	7.5 b
	2 % O_2	4.0 ef
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.037*	*<0.0001*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 12

Colour determination (h°) of inflorescences of *Protea* products stored at 1 °C for 21 d in AirEx and 2 % O_2 (‘Red Magic’, ‘Grandiceps’, ‘Barbigera’, ‘Cynaroides’ and ‘Red Rex’)in the 2015 season.

2015 Season							
Product			**Storage**			**Mean hue angle (°)**	
‘Red Magic’			Before			19.2	c
			After			20.4	c
‘Grandiceps’			Before			29.4	ab
			After			32.7	a
‘Barbigera’			Before			19.5	c
			After			19.1	c
‘Cynaroides’			Before			27.1	b
			After			22.7	c
‘Red Rex’			Before			28.5	ab
			After			28.4	ab
Effect	Product	Treatment	Storage	Product x Treatment	Storage x Treatment	Storage x Product	Storage x Product x Treatment
P value	*<0.0001*	*0.209ns*	*0.931ns*	*0.967ns*	*0.631ns*	*0.0391*	*0.207ns*

Table 13

Colour determination (h°) of inflorescences of *Protea* products stored at 1 °C for 21 d in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Sylvia', 'Ice Queen', 'Barbigera' and 'Cynaroides') in the 2016 season.

2016 Season			
Product	**Storage**	**Mean hue angle (°)**	
'Sylvia'	Before	27.9	bc
	After	26.1	c
'Ice Queen'	Before	104.6	a
	After	101.3	a
'Barbigera'	Before	24.9	cd
	After	32.4	b
'Cynaroides'	Before	20.0	e
	After	21.3	de
Product	**Treatment**		
'Sylvia'	AirEx	28.8	c
	DCA	25.5	cd
	2 % O_2	26.7	cd
'Ice Queen'	AirEx	101.9	a
	DCA	103.7	a
	2 % O_2	103.1	a
'Barbigera'	AirEx	24.0	cd
	DCA	38.3	b
	2 % O_2	23.7	cd
'Cynaroides'	AirEx	19.9	d
	DCA	20.2	d
	2 % O_2	21.8	cd

Effect	Product	Treatment	Storage	Storage x Treatment	Product x Treatment	Storage x Product	Storage x Product x Treatment
P value	*<0.0001*	*0.126ns*	*0.293ns*	*0.605ns*	*0.007*	*<0.0001*	*0.199ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 14

Colour determination (h°) of leaves of *Protea* products stored for 21 d at 1 °C in AirEx and 2 % O_2 ('Red Magic', 'Grandiceps', 'Barbigera', 'Cynaroides' and 'Red Rex') in the 2015 season and in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Sylvia', 'Ice Queen', 'Barbigera' and 'Cynaroides') in the 2016 season.

2015 Season							
Product			**Storage**		**Mean hue angle (°)**		
'Red Magic'			Before		117.4	cde	
			After		115.7	de	
'Grandiceps'			Before		125.0	ab	
			After		124.1	ab	
'Barbigera'			Before		115.0	e	
			After		107.8	f	
'Cynaroides'			Before		122.9	a	
			After		120.4	bcd	
'Red Rex'			Before		122.6	ab	
			After		122.0	abc	
Effect	Treatment	Storage	Product	Product x Treatment	Storage x Treatment	Storage x Product	Storage x Product x Treatment
P value	*0.945ns*	*<0.0001*	*<0.0001*	*0.825ns*	*0.531ns*	*<0.0001*	*0.111ns*
2016 Season							
Storage					**Mean hue angle (°)**		
Before					121.0	a	
After					118.5	b	
Product							
'Cynaroides'					123.1	a	
'Ice Queen'					120.6	ab	
'Barbigera'					119.3	b	
'Sylvia'					115.9	c	
Effect	Treatment	Product	Storage	Product x Treatment	Storage x Product	Storage x Treatment	Storage x Product x Treatment
P value	*0.286ns*	*<0.0001*	*0.003*	*0.470ns*	*0.743ns*	*0.655ns*	*0.171ns*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 15

Soluble carbohydrates in leaves of *Protea* products after 21 d of cold storage at 1 °C in AirEx, and 2 % O_2 (‘Grandiceps’, ‘Red Magic’, ‘Barbigera’, ‘Cynaroides’ and ‘Red Rex’) in the 2015 season.

2015 Season (Monosaccharides + Oligosaccharides)			
Product		**Mean glucose mg.g^{-1}**	
‘Grandiceps’		77.8	a
‘Red Magic’		65.9	b
‘Barbigera’		51.4	c
‘Cynaroides’		50.1	c
‘Red Rex’		40.1	d
Treatment			
AirEx		56.2	ns
2 % O_2		57.9	
Effect	Treatment	Product	Product x Treatment
P value	*0.503ns*	*<0.0001*	*0.369ns*

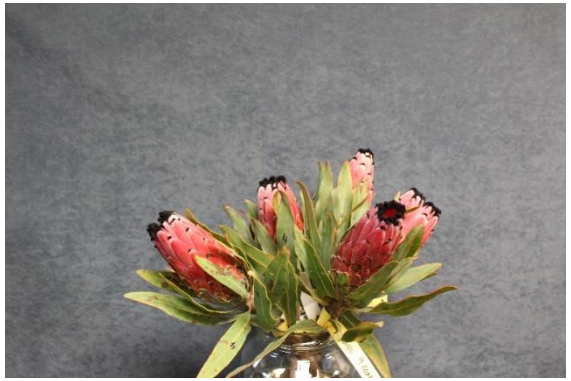
*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance.

Table 16

Total phenolic content (nmol.mL^{-1}.g^{-1}) in leaves of *Protea* products following storage of 21 d at 1 °C in AirEx and 2 % O_2 ('Barbigera', 'Cynaroides', 'Grandiceps' and 'Red Magic') in the 2015 season and AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 ('Sylvia', 'Barbigera', 'Ice Queen' and 'Cynaroides') in the 2016 season.

2015 Season			
Product	**Treatment**	**Mean**	**GAE nmol.mL^{-1}.g^{-1}**
'Barbigera'	AirEx	89.1	g
	2 % O_2	110.9	def
'Cynaroides'	AirEx	120.1	cd
	2 % O_2	121.5	cde
'Red Rex'	AirEx	144.1	b
	2 % O_2	129.4	bc
'Grandiceps'	AirEx	187.8	a
	2 % O_2	186.4	a
'Red Magic'	AirEx	103.5	eg
	2 % O_2	99.2	fg
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.879ns*	*0.049*
2016 Season			
Product	**Treatment**	**Mean**	**GAE nmol.mL^{-1}.g^{-1}**
'Sylvia'	AirEx	158.8	cd
	DCA	154.6	cd
	2 % O_2	148.4	d
'Barbigera'	AirEx	171.7	c
	DCA	162.3	cd
	2 % O_2	152.3	d
'Ice Queen'	AirEx	157.6	cd
	DCA	159.5	cd
	2 % O_2	164.5	cd
'Cynaroides'	AirEx	199.9	b
	DCA	192.5	b
	2 % O_2	227.3	a
Effect	Product	Treatment	Product x Treatment
P value	*<0.0001*	*0.422ns*	*0.006*

*Mean separation within main factors was done by LSD (5 %) and means with different letters are significantly different from each other and ns shows no significance. Phenolic content was expressed as gallic acid equivalents (GAE).

A 	**B** 	**C** 
D 	**E** 	**F** 

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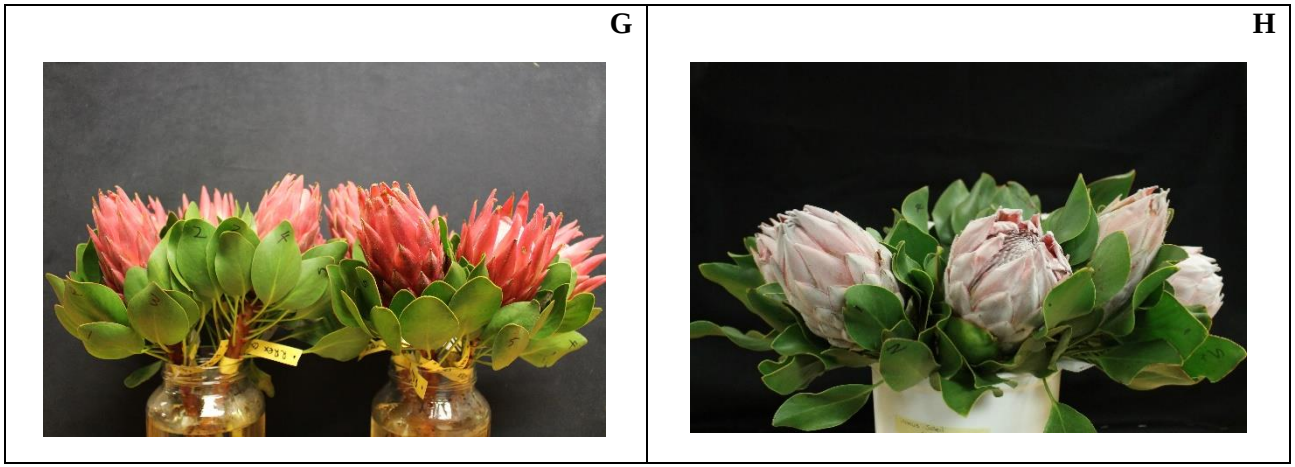


Fig. 1. *Protea* products [‘Barbigera’ (A), ‘Grandiceps’ (B), ‘Ice Queen’ (C), ‘Red Magic’ (D), ‘Sylvia’ (E), ‘Didi’ (F), ‘Red rex’ (G), ‘Cynaroides’ (H)] evaluated in the 2015 and 2016 seasons.

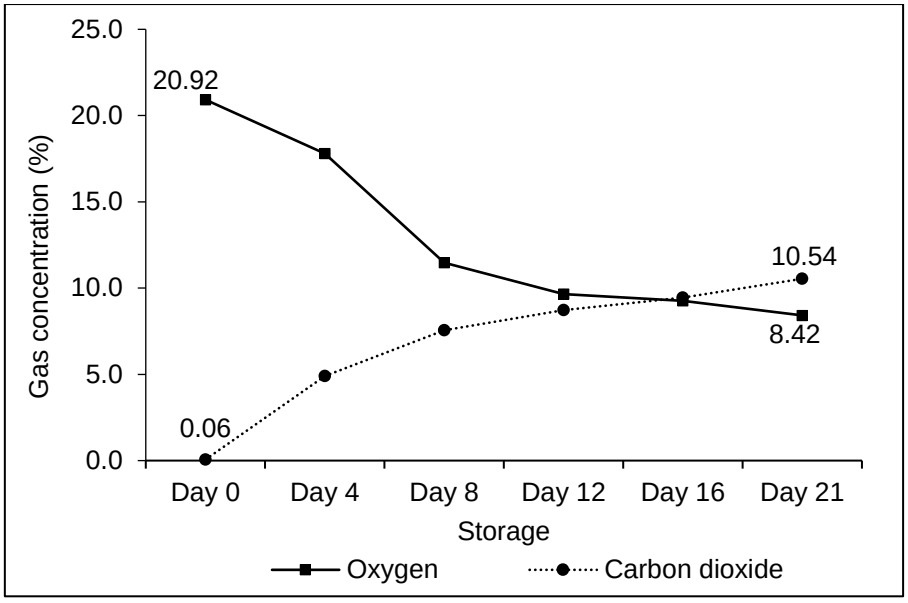
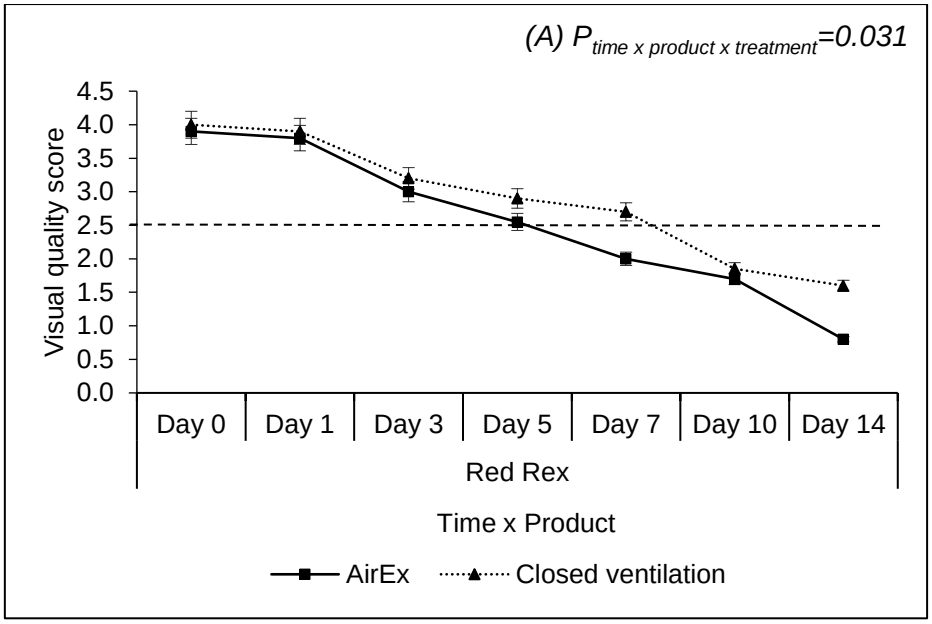
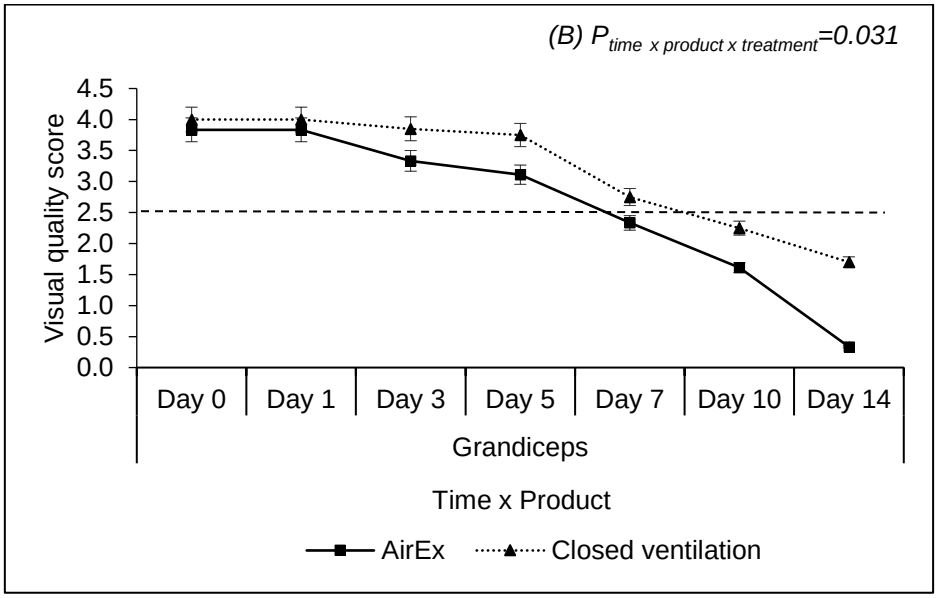
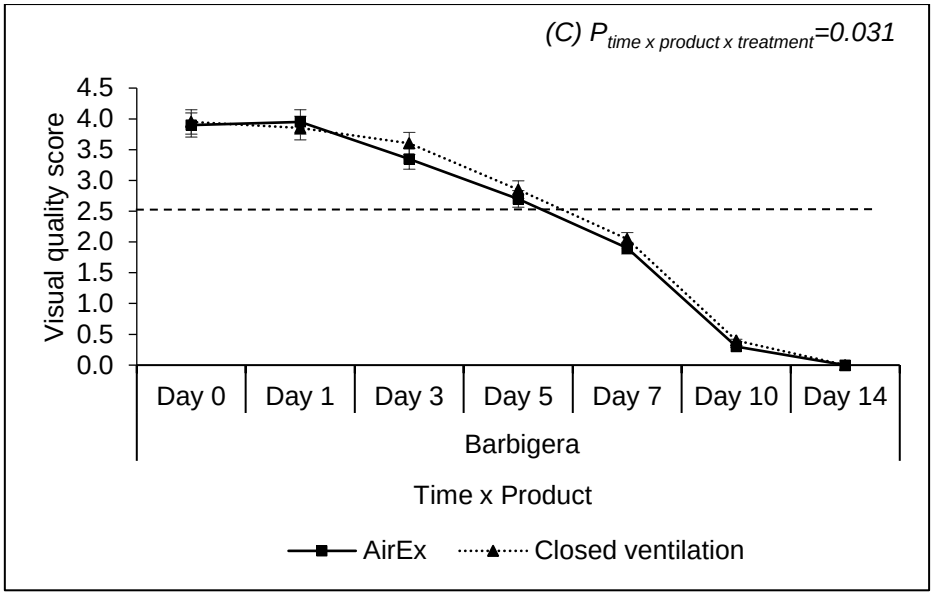


Fig. 2. Gas dynamics in a closed ventilation system monitored using HarvestWatch™ DCA system. The Janny MT box contained a mixture of *Leucadendron* ('Rosette Conebush'), *Leucospermum* ('Jelena', 'Goldie', 'Bright Eyes' and 'Fire Fly') and *Protea* ('Barbigera', 'Grandiceps' and 'Red Rex') products in the 2015 season.

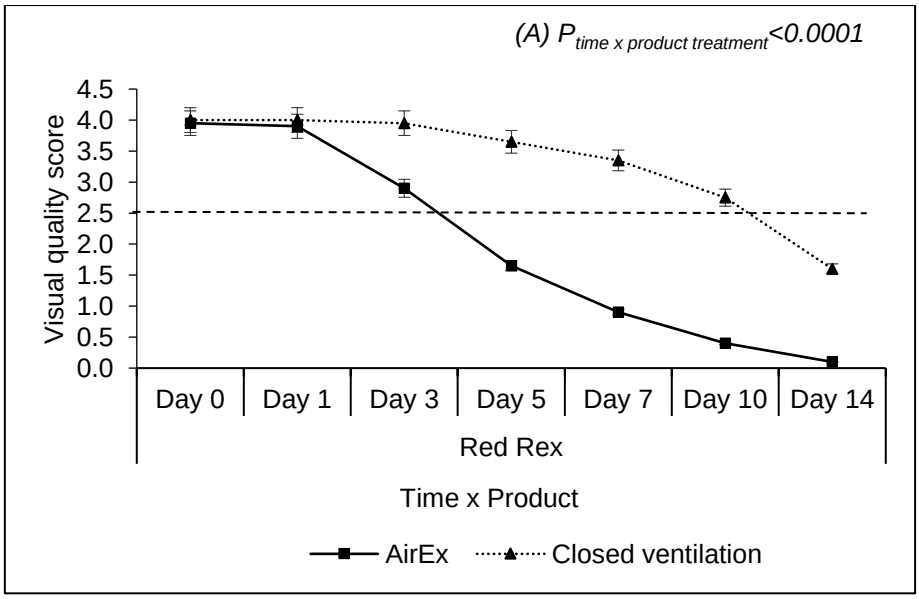


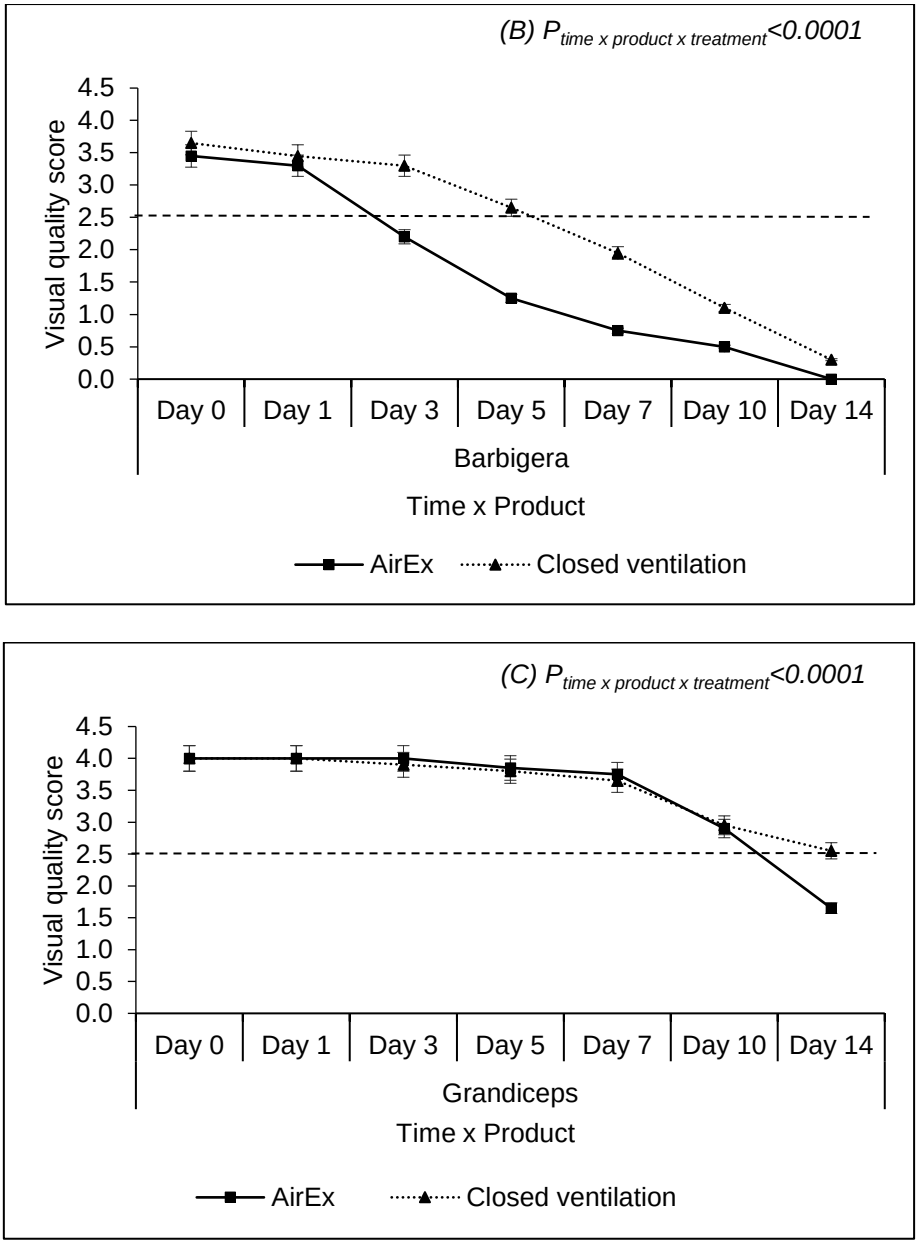




Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*0.043*	*<0.0001*	*<0.0001*	*0.031*

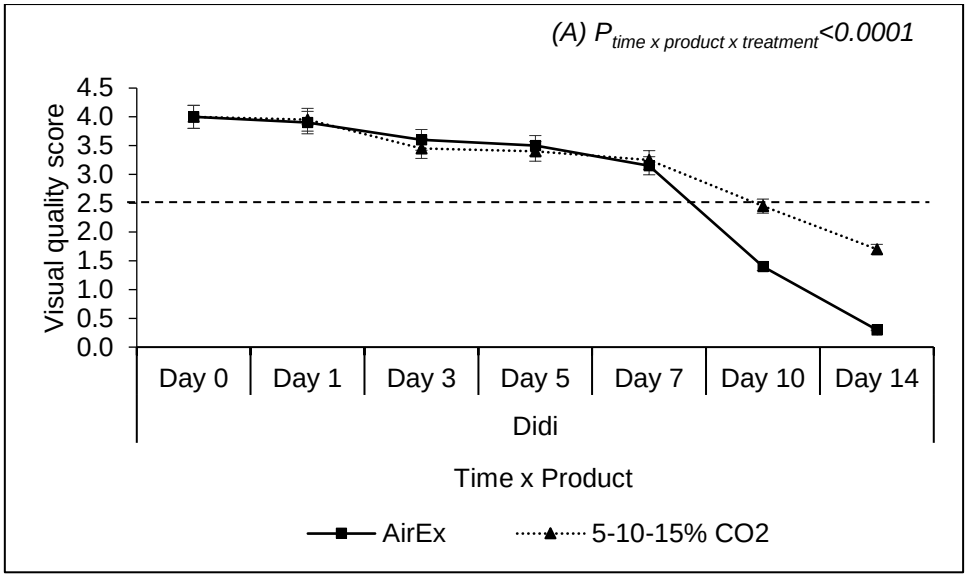
Fig. 3. Inflorescence visual quality of *Protea* products ['Red Rex' (A), 'Grandiceps' (B) and 'Barbigera' (D)] after 21 d of storage at 1 ^{o}C in AirEx and closed ventilation in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

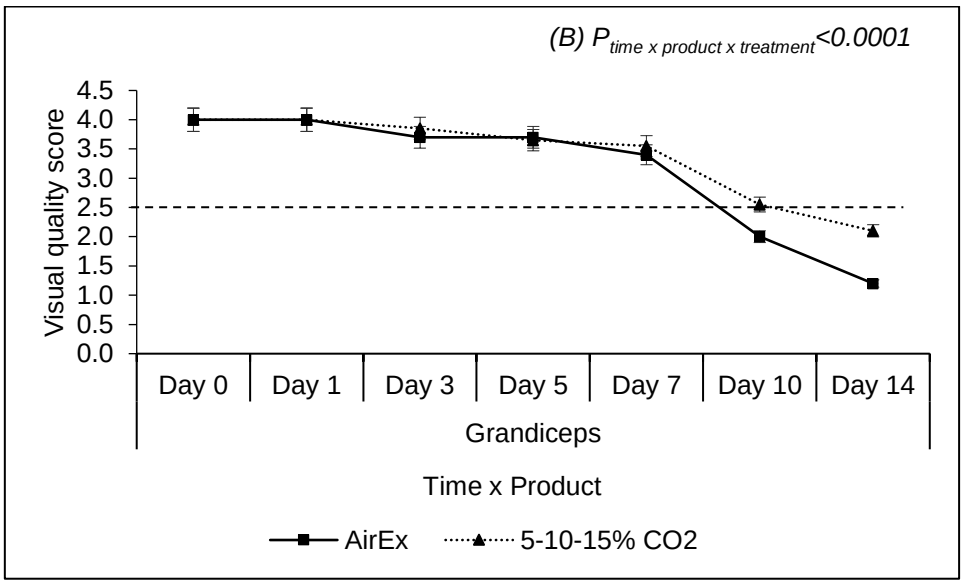


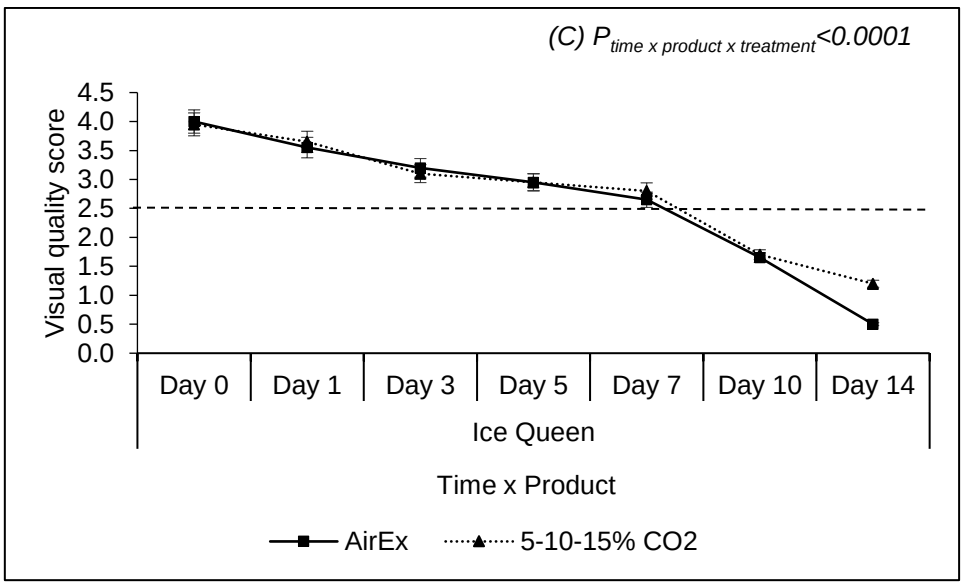


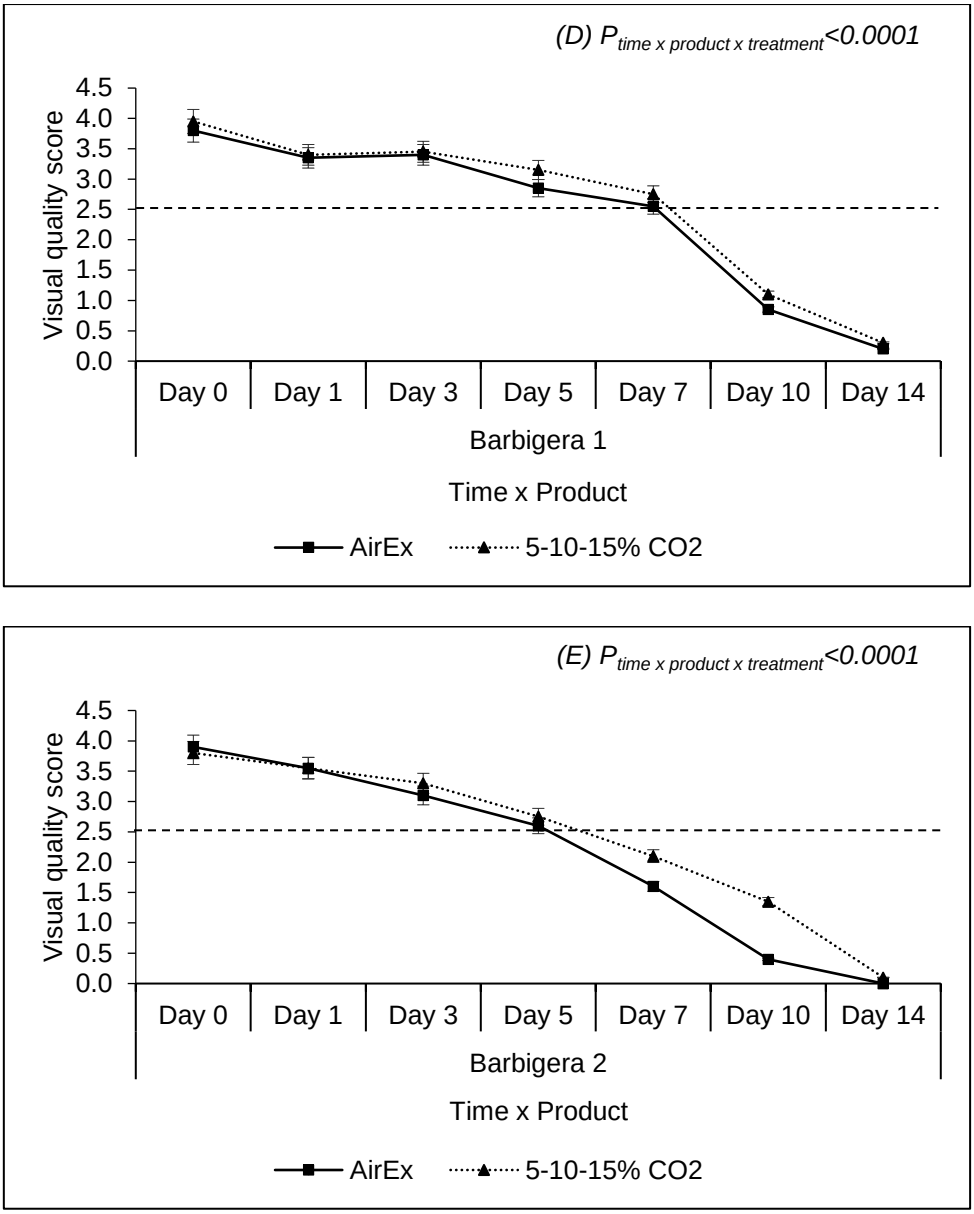
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*0.012*	*<0.0001*	*<0.0001*	*<0.0001*

Fig. 4. Leaf visual quality of *Protea* products ['Red Rex' (A), 'Barbigera' (B) and 'Grandiceps' (C)] after 21 d of storage at 1 °C in AirEx and closed ventilation in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



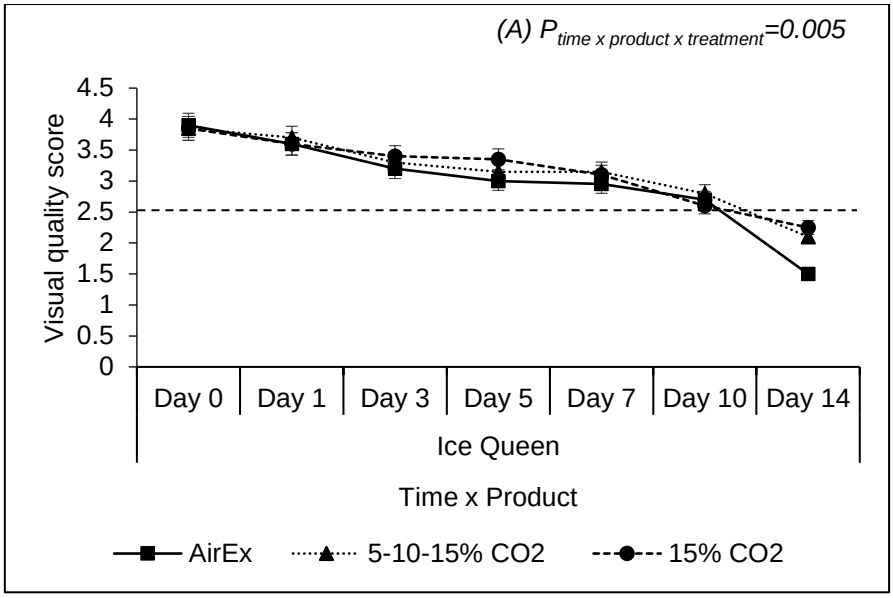


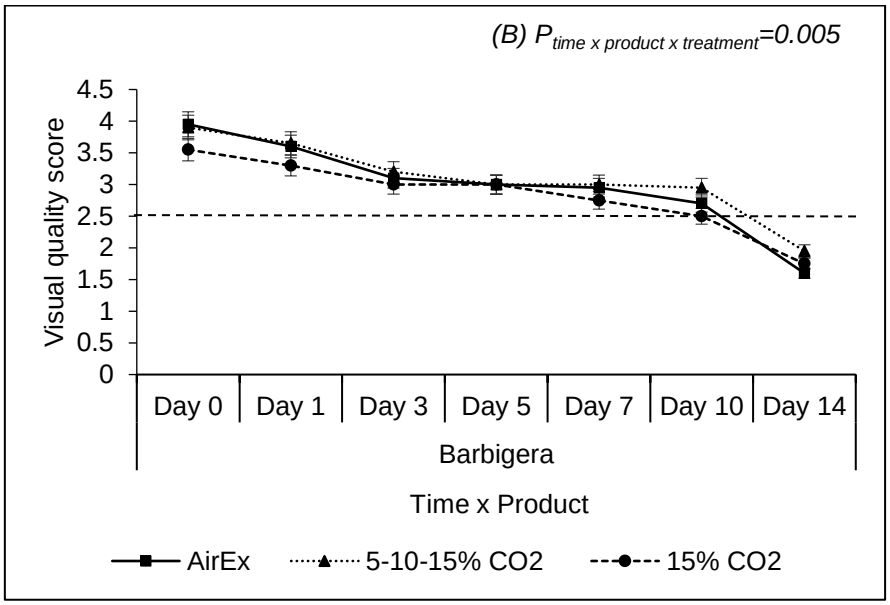


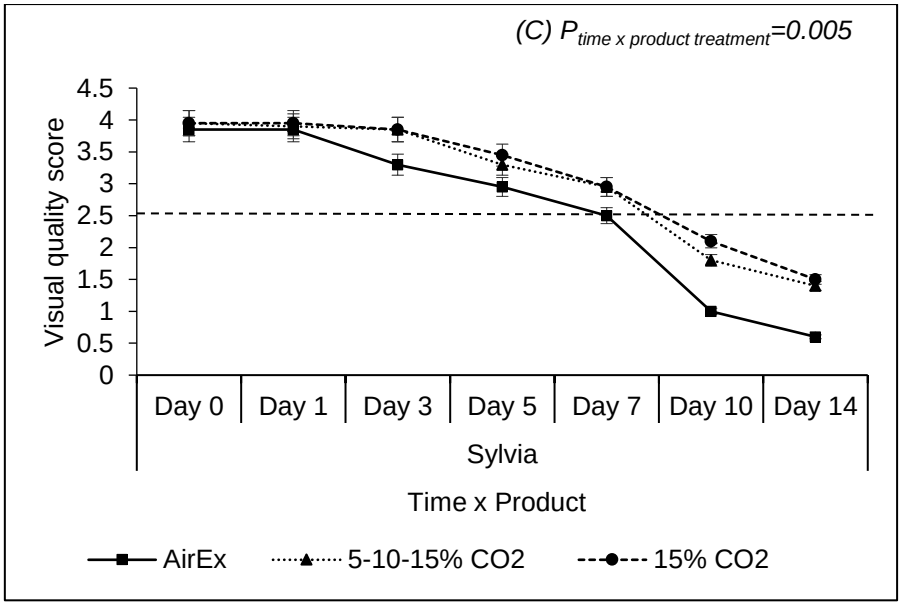


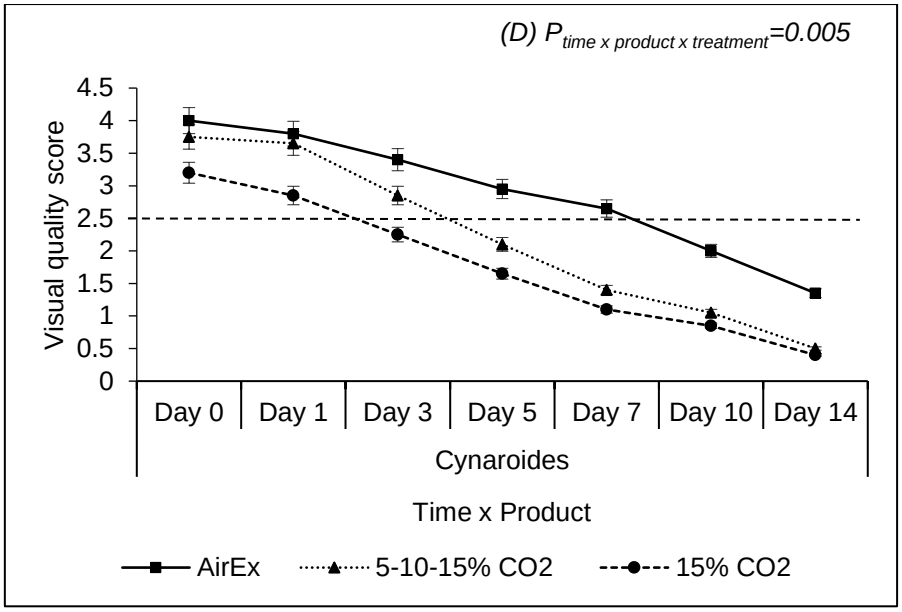
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*0.822ns*	*<0.0001*	*<0.0001*	*<0.0001*

Fig. 5. Inflorescence visual quality of *Protea* products ['Didi' (A), 'Grandiceps' (B), 'Ice Queen', (C) 'Barbigera 1' (D) and 'Barbigera 2' (E)] after 21 d of storage at 1 °C in AirEx and 5-10-15 % CO_2 in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



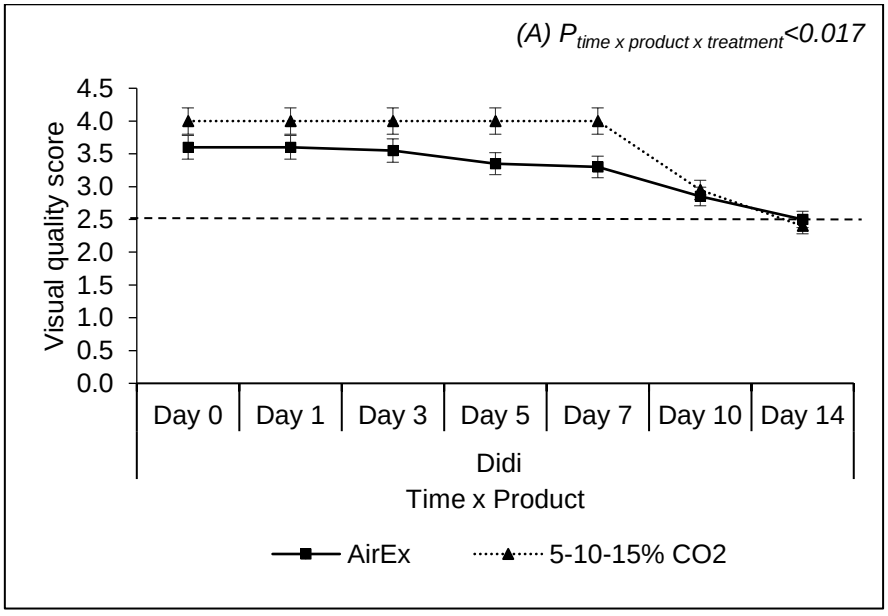


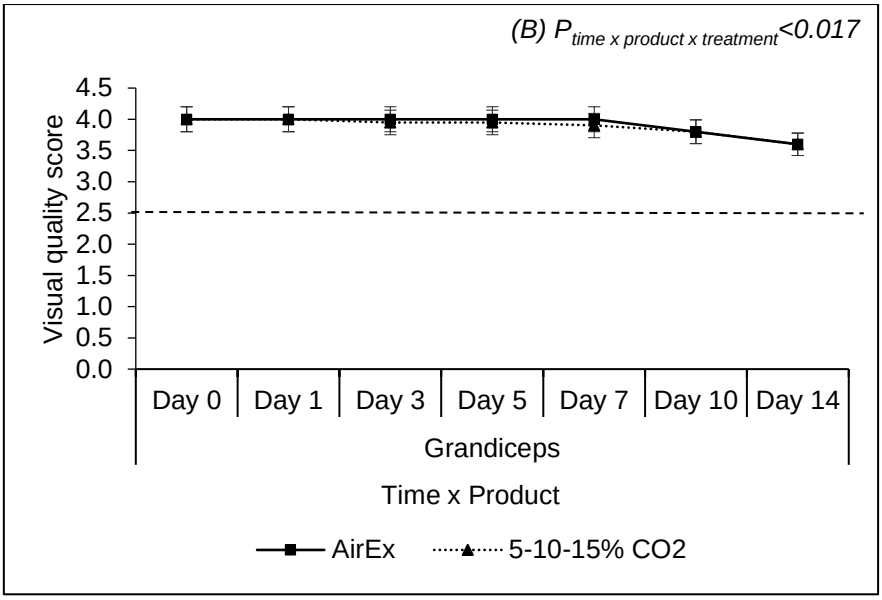


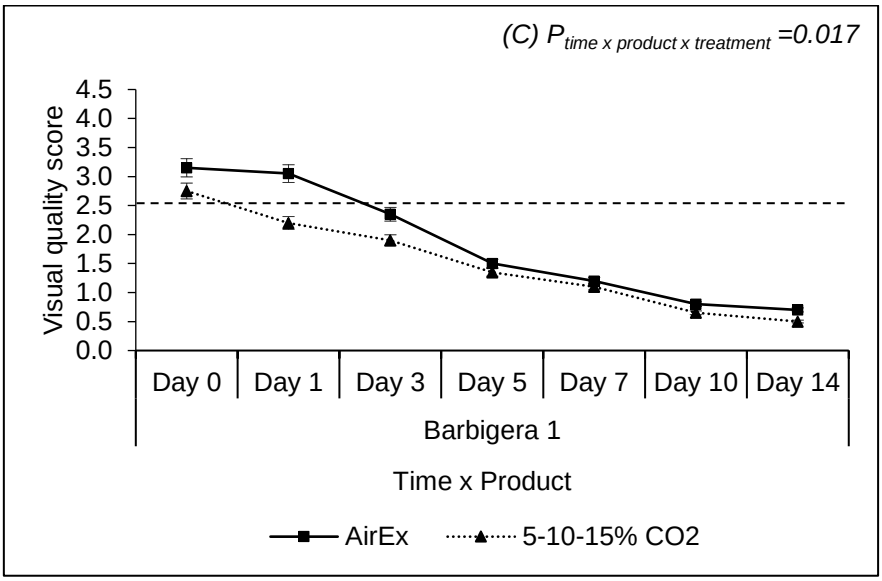


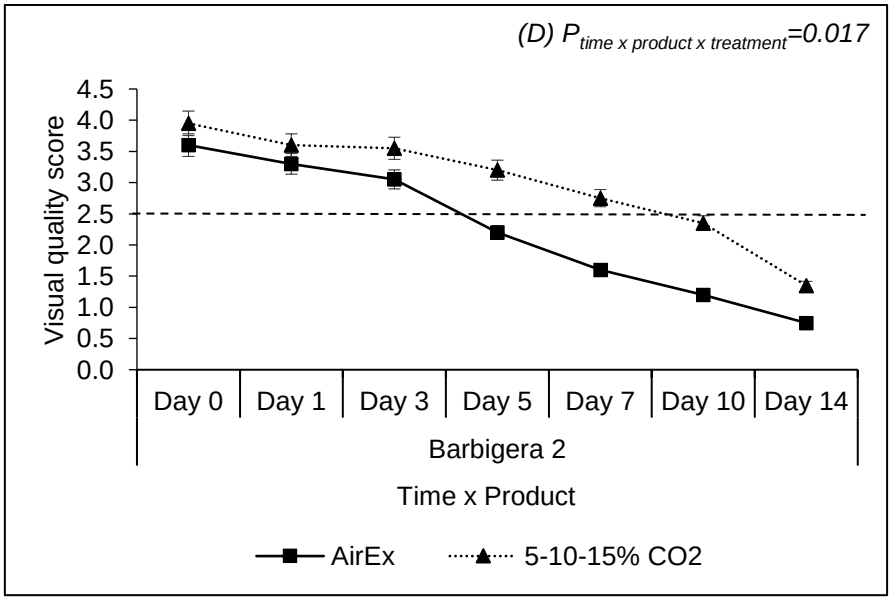
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.191ns*	*<0.0001*	*<0.0001*	*<0.0001*	*0.007*	*0.005*

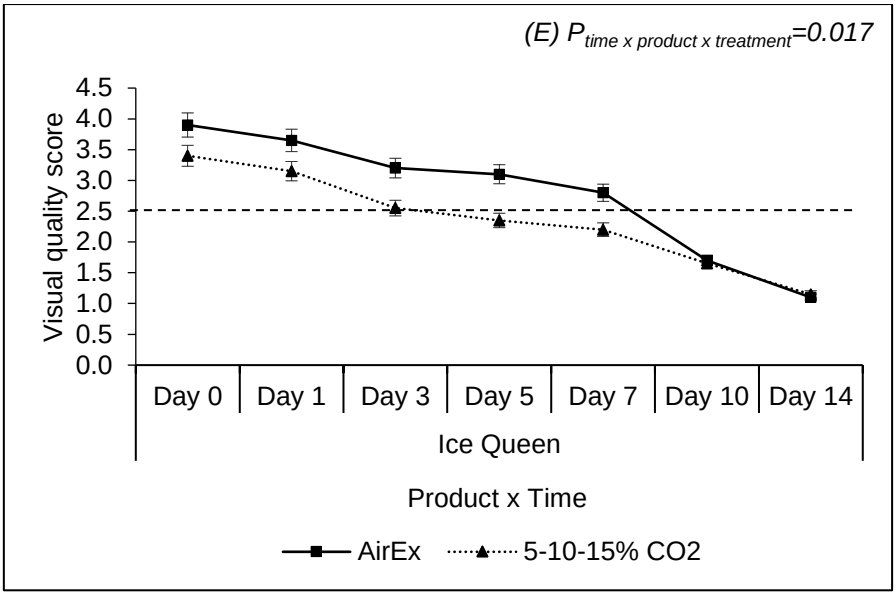
Fig. 6. Inflorescence visual quality of *Protea* products [‘Ice Queen’ (A), ‘Barbigera’ (B), ‘Sylvia’ (C) and ‘Cynaroides’ (D)] after storage in AirEx, 5-10-15 % CO_2 and 15 % CO_2 for 21 d at 1 °C in 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.





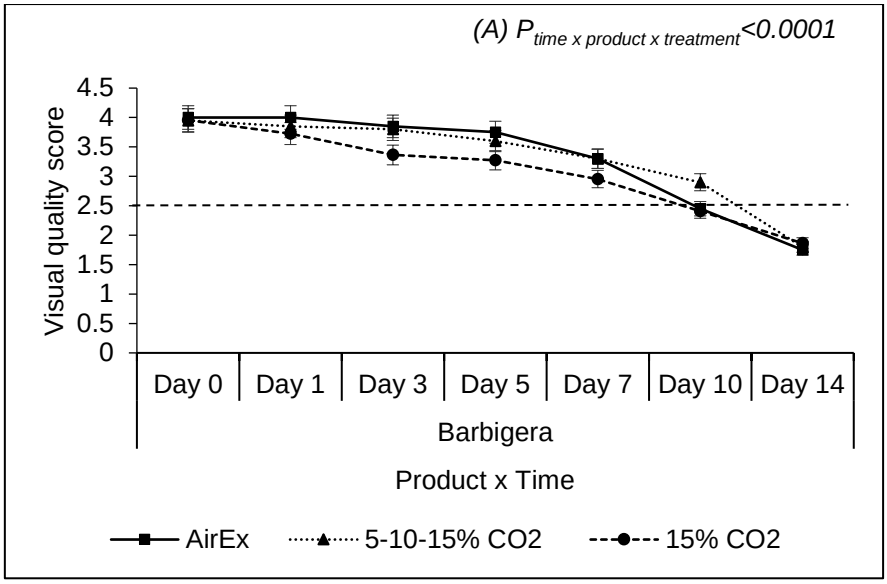


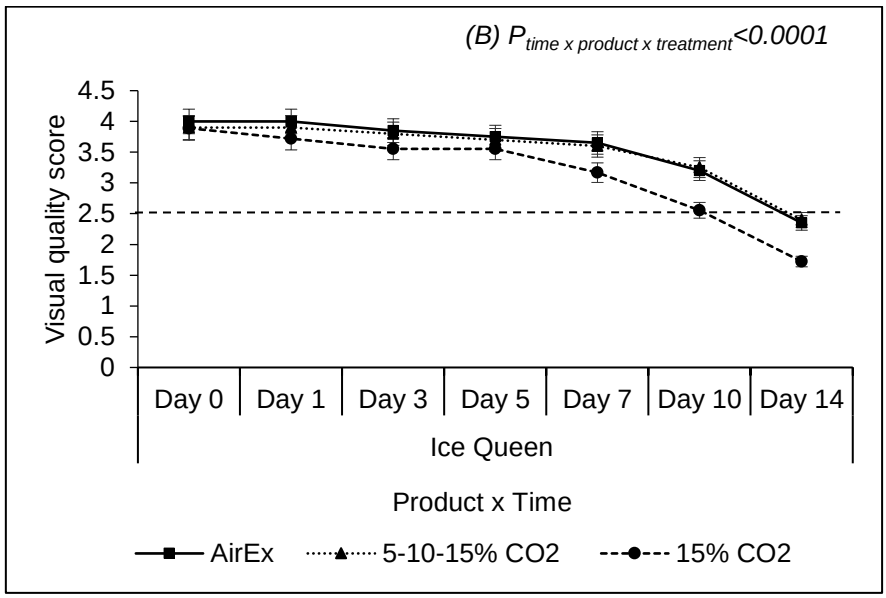


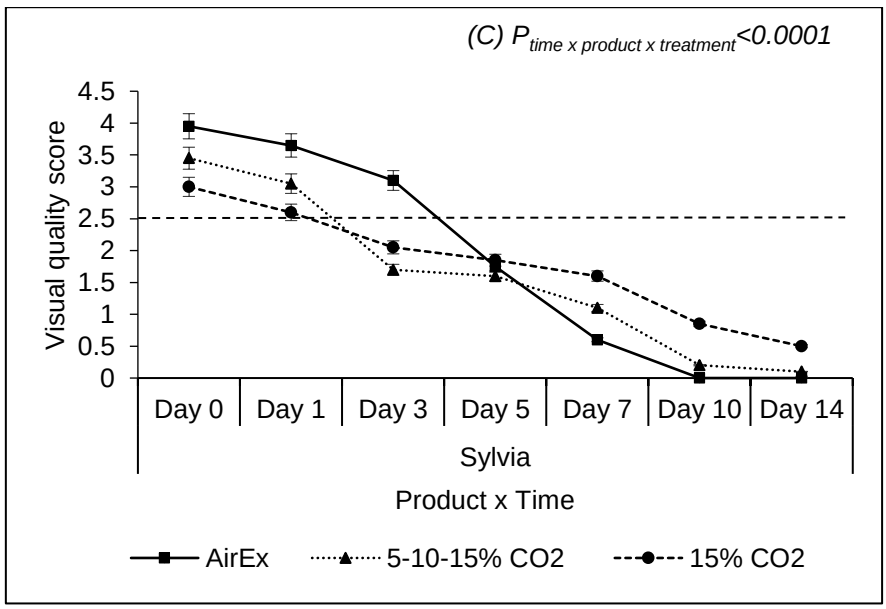


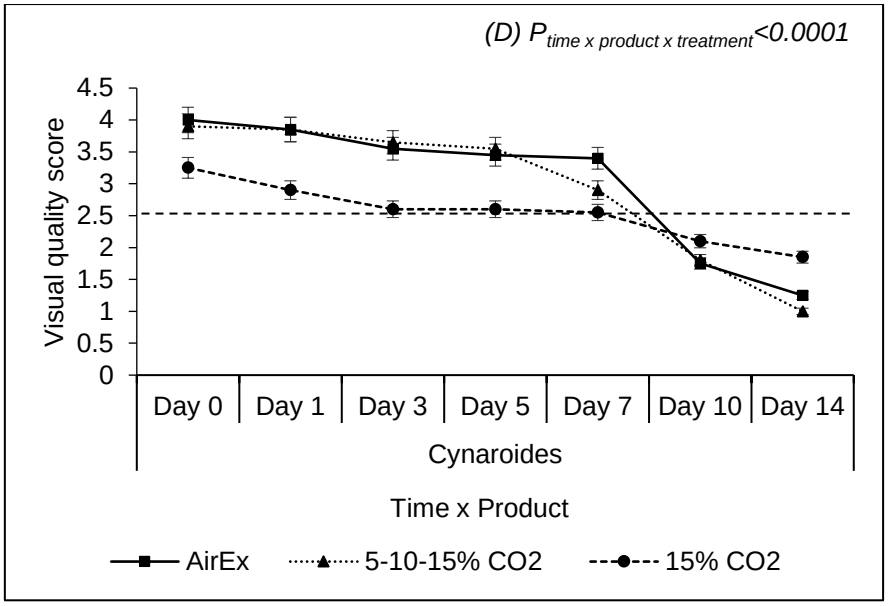
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.681ns*	*<0.0001*	*0.087ns*	*<0.0001*	*0.086ns*	*0.017*

Fig. 7. Leaf visual quality of *Protea* products ['Didi' (A), 'Grandiceps' (B), 'Barbigera 1' (C) 'Barbigera 2' (D) and 'Ice Queen' (E)] after 21 d of storage at 1 °C in AirEx and 5-10-15 % CO_2 in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



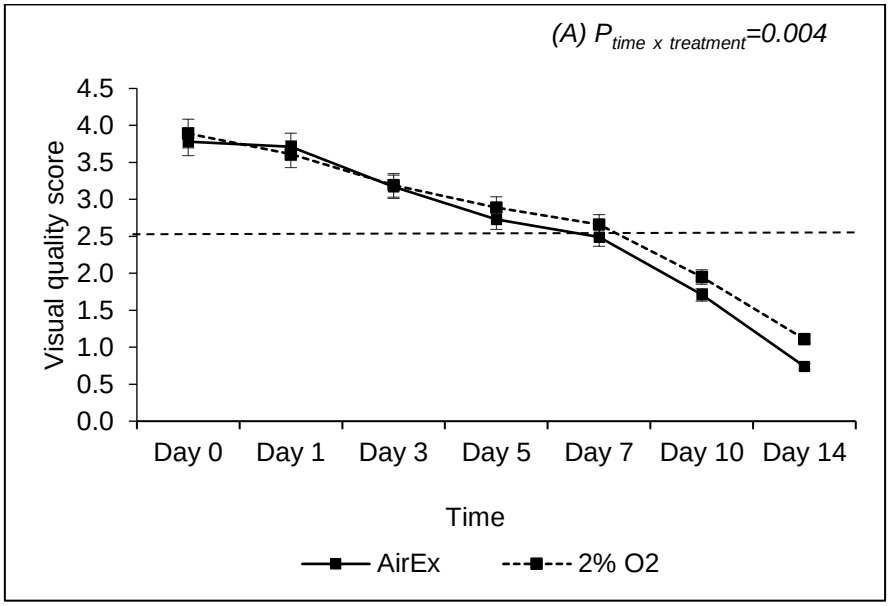


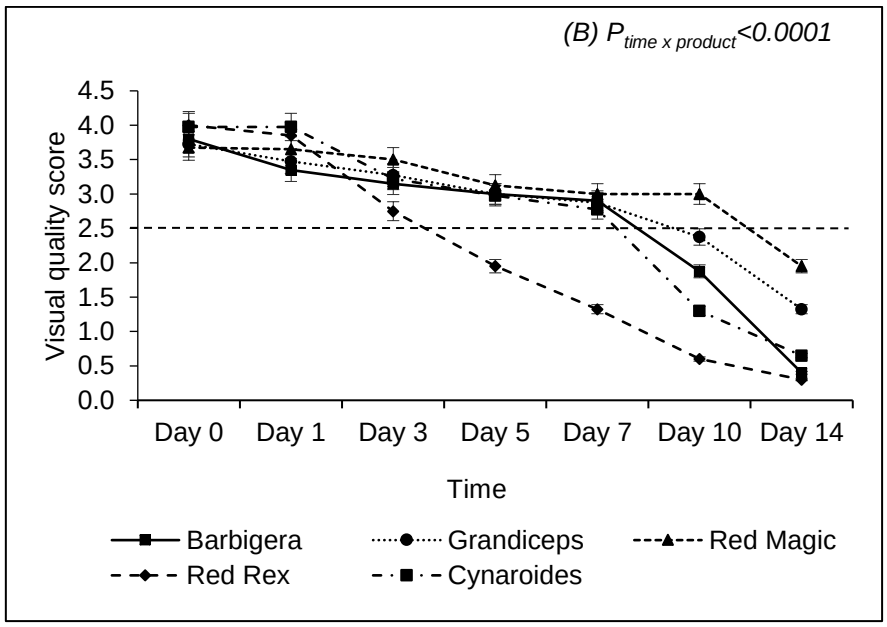




Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.249ns*	*<0.0001*	*0.937ns*	*<0.0001*	*<0.0001*	*<0.0001*

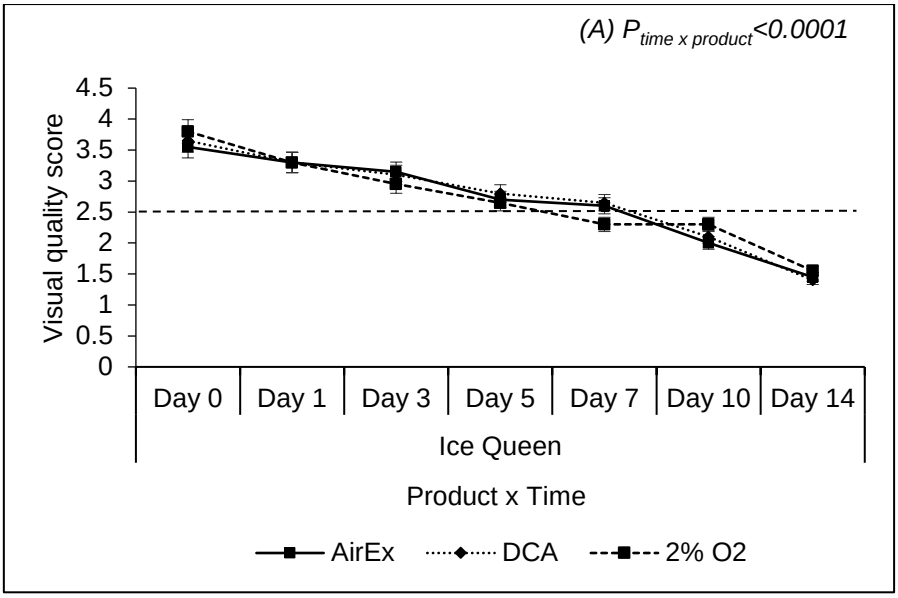
Fig. 8. Leaf visual quality of *Protea* products [‘Ice Queen’ (A), ‘Barbigera’ (B), ‘Sylvia’ (C) and ‘Cynaroides’ (D)] after 21 d of storage at 1 °C in AirEx, 5-10-15 % CO_2 and 15 % CO_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

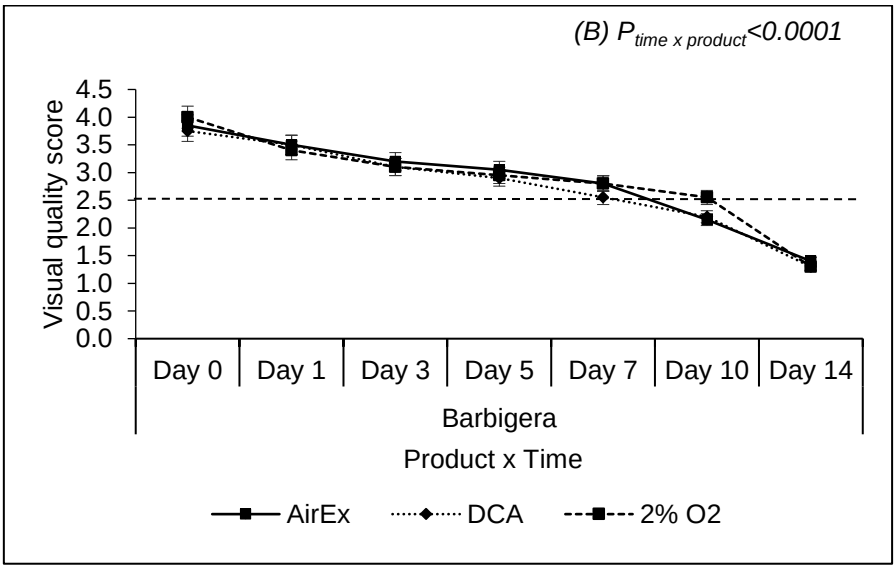


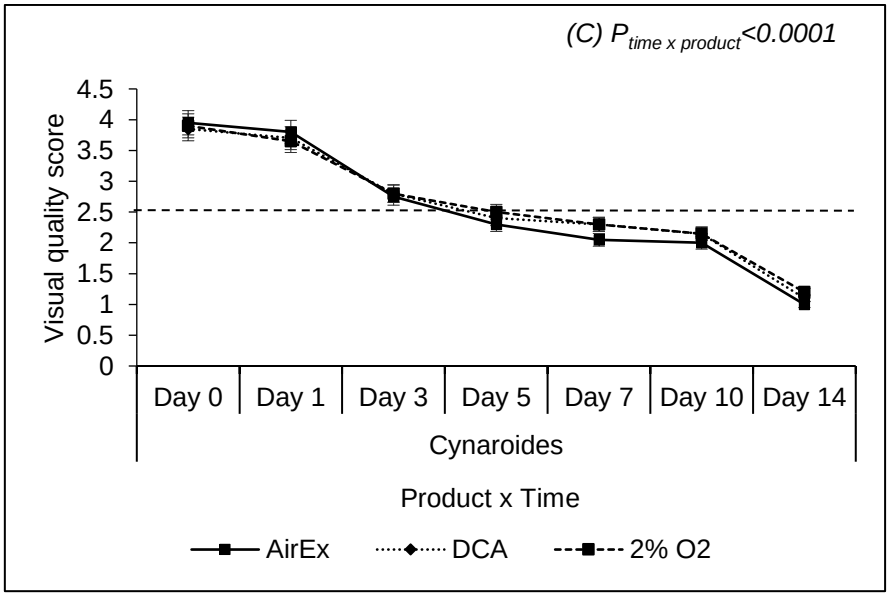


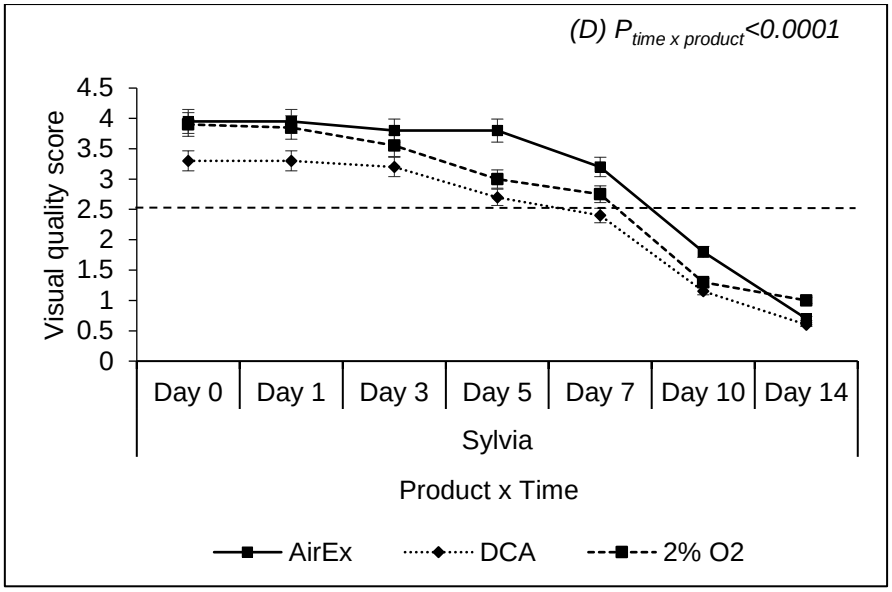
Effect	Treatment	Product	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*0.103ns*	*<0.0001*	*<0.0001*	*0.692ns*	*<0.0001*	*0.004*	*0.175ns*

Fig. 9. Effect of time x treatment (9A) and time x product (9B) on inflorescence quality of *Protea* products ('Barbigera', 'Grandiceps', 'Red Magic', 'Red Rex' and 'Cynaroides') after 21 d of storage at 1 °C in AirEx and 2 % O_2 in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.



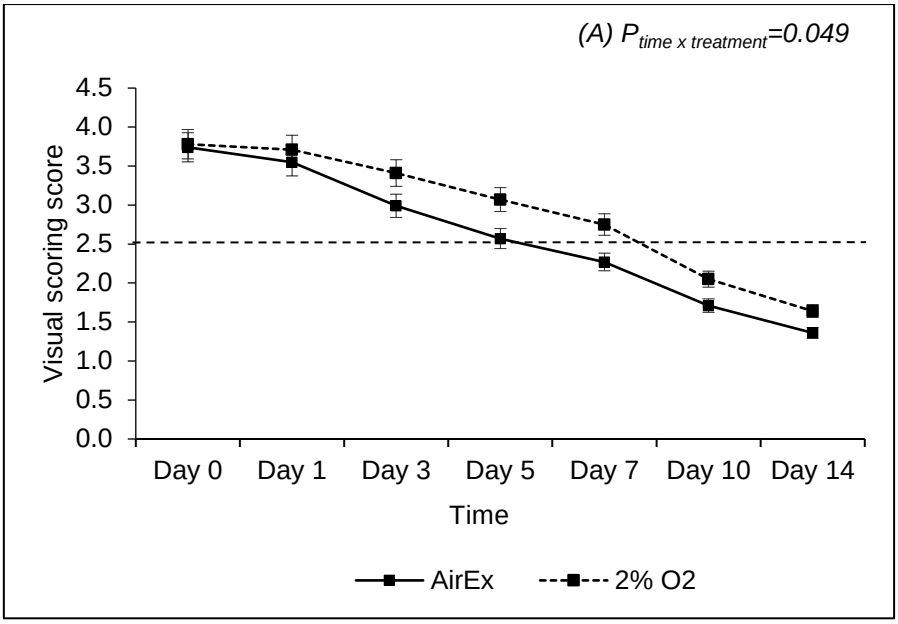


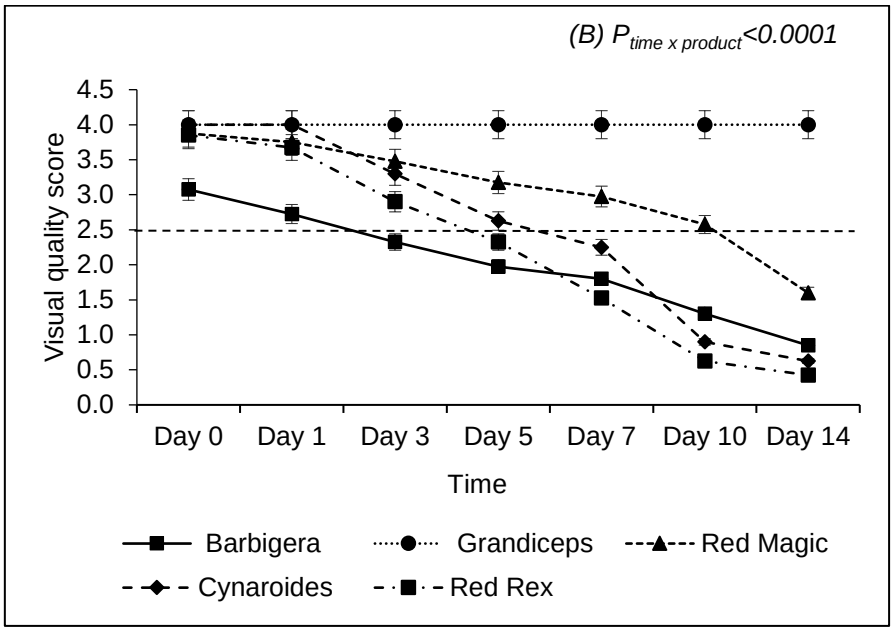




Effect	Product	Treatment	Time	Product x Treatment	Time x Treatment	Time x Product	Time x Product x Treatment
P value	*0.146ns*	*0.132ns*	*<0.0001*	*0.069ns*	*0.078ns*	*<0.0001*	*0.007*

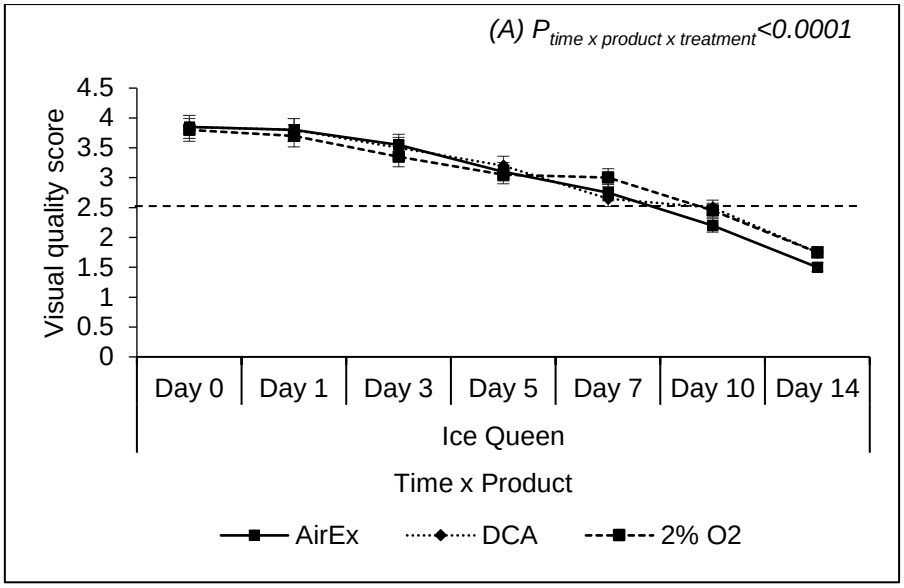
Fig. 10. Inflorescence quality of *Protea* products [‘Ice Queen’ (A), ‘Barbigera’ (B), ‘Cynaroides’ (C) and ‘Sylvia’ (D)] after 21 d of storage at 1 °C in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

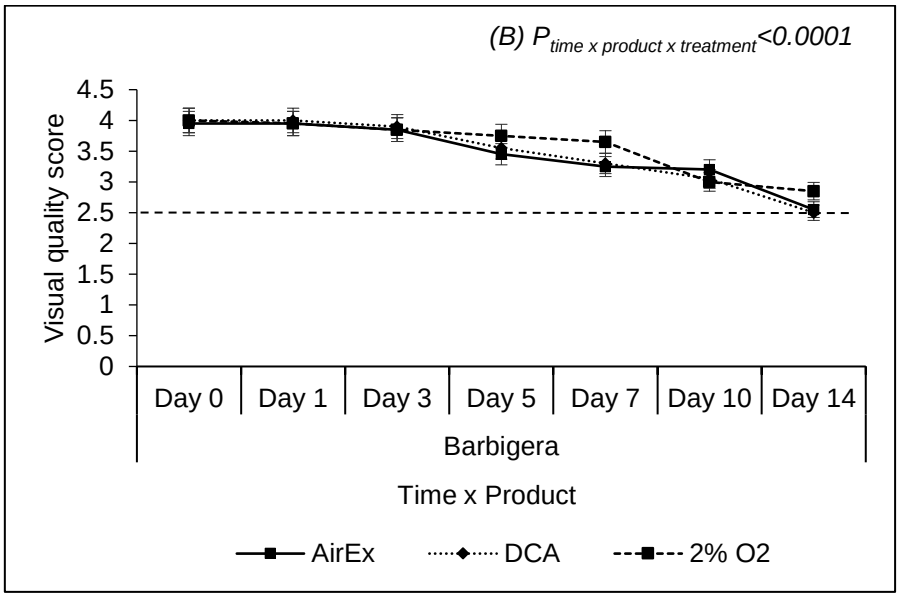


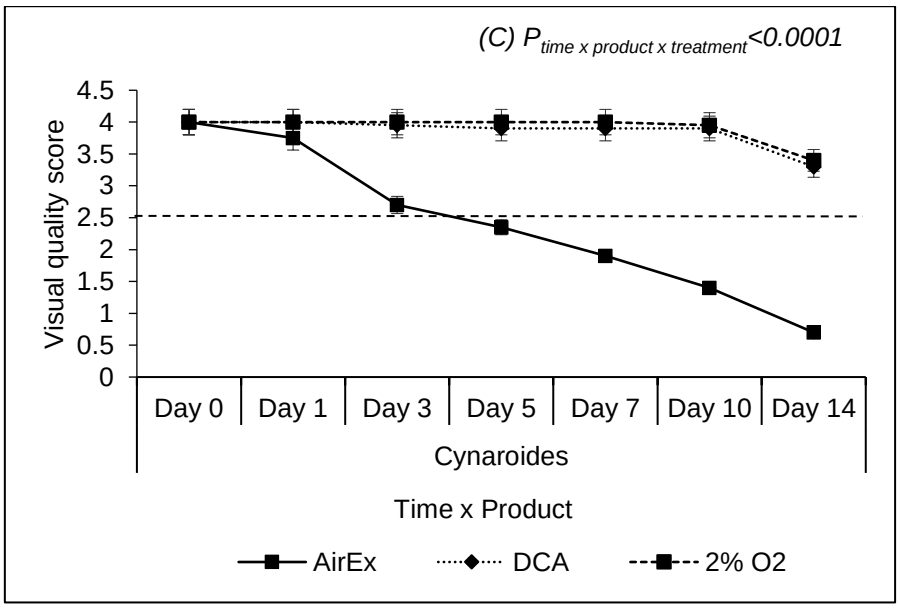


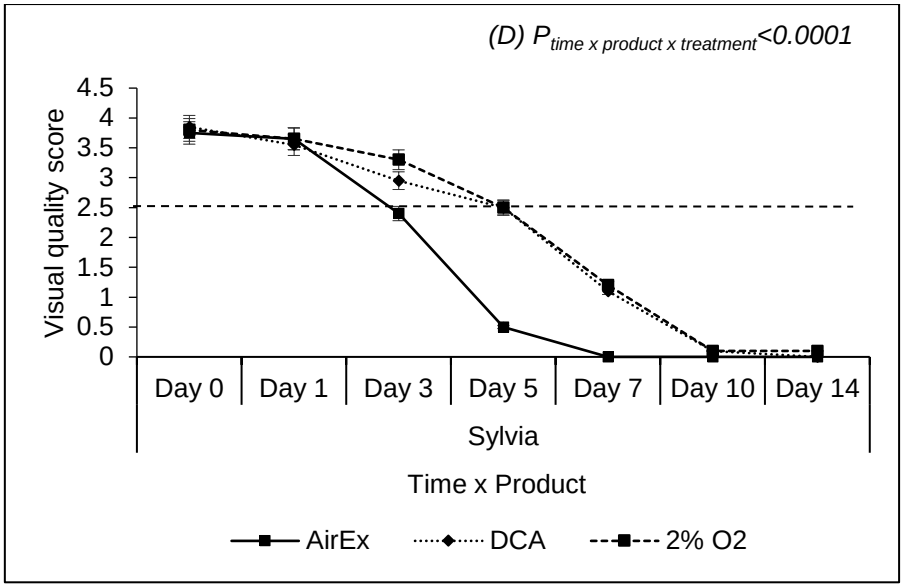
Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*0.046*	*<0.0001*	*0.165ns*	*<0.0001*	*<0.049*	*0.196ns*

Fig. 11. Effect of time x treatment (11A) and time x product (11B) on leaf visual quality of *Protea* products ('Barbigera', 'Grandiceps', 'Red Magic', 'Red Rex' and 'Cynaroides') after 21 d of cold storage in AirEx and 2 % O_2 at 1 °C in the 2015 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.









Effect	Product	Treatment	Time	Product x Treatment	Time x Product	Time x Treatment	Time x Product x Treatment
P value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

Fig. 12. Leaf visual quality of *Protea* products ('Ice Queen' (A), 'Barbigera' (B), 'Cynaroides' (C) and 'Sylvia' (D)) after 21 d of storage at 1 °C in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 in the 2016 season. A score of 2.5 was considered as the cut-off value for acceptable retail quality.

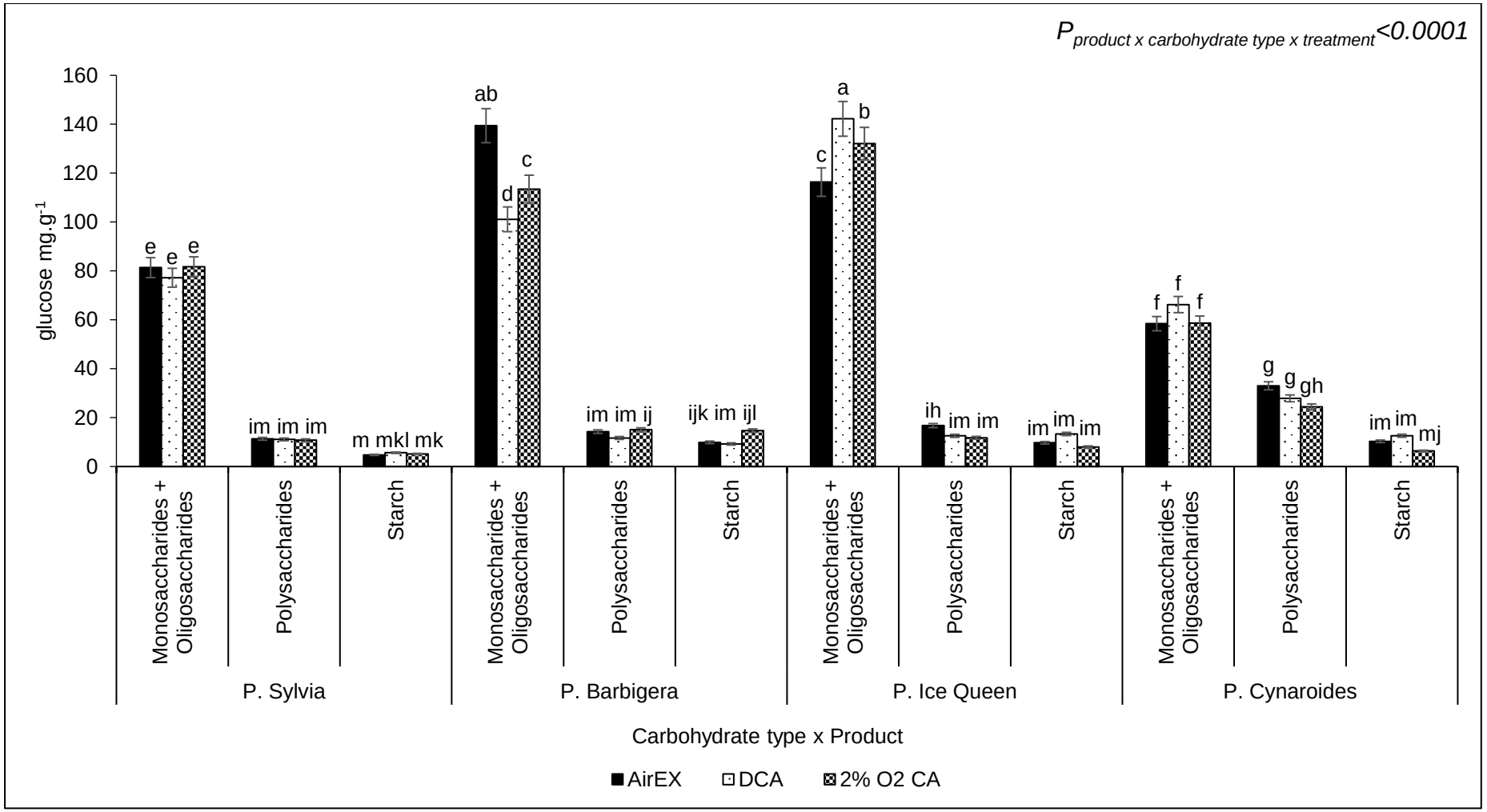


Fig. 13. Soluble carbohydrates in leaves of *Protea* products ('Sylvia', 'Barbigera', 'Ice Queen' and 'Cynaroides') after 21 d of storage at 1 °C in AirEx, dynamic controlled atmosphere (DCA) and 2 % O_2 in the 2016 season.

GENERAL DISCUSSION AND CONCLUSION

Although advances in reefer technology have led to considerable improvements in shipping quality of fresh products over the past decades, the Cape Flora industry has failed to utilise latest reefer technologies due to the associated cost and lack of adequate knowledge of the storage requirements of *Proteaceae* cut flower products pertaining to low O_2 limit and high CO_2 tolerance. Additionally, there are no standardised product handling protocols and quality assessment guides for the major Australian and South African floral products, especially regarding to indigenous species (Gollnow et al., 2014). As a result, the suitability of sea freight shipping of *Proteaceae* has been debatable due to variability in quality of products upon arrival at the markets. The invention of automated reefer technologies like automatic ventilation device (AV+) and advanced fresh air management (AFAM+) has resulted from the global trend to advance sea freight technology to minimise carbon emissions. However, this global move to digitise reefers comes with the challenge of addressing previously overlooked physiological limits of the *Proteaceae* cut flower products from South Africa which are vital to the use of automatic ventilation technology. A study to understand the respiration rate and lower O_2 limits and CO_2 toxicity tolerance limits was conducted to investigate these parameters.

In paper 2, we assessed the feasibility of using automatic ventilation and closed ventilation during shipping of *Leucadendron* cut flower stems. A closed ventilation system was used, which contained a mixed load of *Proteaceae* products and 45-55 % free air and where the HarvestWatch™ DCA system was used for sampling. It was observed that the O_2 level fell to about 8.5 % and the CO_2 level rose approximately 10 %, which shows that a passive MA existed inside the Janny MT boxes. When a handheld gas analyser was used as an alternative sampling method, O_2 concentrations fell below 2 %, whilst CO_2 levels rose to above 17 %. Although the O_2 dropped drastically, by day 21 it was still above the LOL, which ranged from 0.08-0.48 % O_2 in both seasons. Despite the gas dynamics during storage being variable among the trials and being likely dependent on respiration rate of the products, the amount of free air and gas sampling method used, there were no signs of low O_2 and/or or high CO_2 damage on the products stored under closed ventilation. The Q_{10} values were determined to be within the 1.49 to 3.84 range in both seasons. The data for the *Leucadendron* products showed that the respiration rates were within the 15.11-48.07 $mL.kg^{-1}.h^{-1}$ range at 5 °C. Higher respiration rates will deplete the carbohydrate reserves and decrease longevity of the cut stems. Products with

higher respiration rates tend to have lower longevity due to depletion of sugar reserves (Kuc and Workman, 1964).

The flower head and leaf colour changes in *Leucadendron* products stored in closed ventilation low O_2 and high CO_2 treatments were all commercially non-significant and did not compromise the visual quality. Storage in the closed ventilation and high CO_2 atmospheres generally lowered mass loss compared to AirEx. The high RH and reduced air movement that exists in a sealed storage environment (closed ventilation) lowers moisture loss through reduced VPD, lower disturbance of boundary layer around the product, and elevated CO_2 atmosphere promotes closing of stoma (Taiz and Zeiger, 2016).

The AirEx and 5-10-15 % CO_2 treatments had similar flower head visual quality, but exposure to 15 % CO_2 for a three week storage period greatly reduced longevity of some products. The passive MA created by 5-10-15 % CO_2 did not damage the products, however, the quality of 'Discolor' and 'Safari Sunset' was considerably reduced by the 15 % CO_2 treatment which shows product variability. This shows the importance of gradually raising CO_2 so as to avoid CO_2 toxicity. Response to AirEx, DCA and 2 % O_2 on flower head visual quality was variable and inconclusive in some products, with minor quality differences being observed among the treatments in some products.

Generally, AirEx, DCA and 2 % O_2 treatments were equally effective in maintaining leaf visual quality although the longevity of 'Rosette Conebush' leaves was shortened by 2 % O_2 in the 2015 season. This could have been due to differences in harvesting maturity. Product responses differ due to variation in genetics, growing conditions, harvesting maturity, among other factors. The amount of carbohydrates was comparable between AirEx and closed ventilation and also between AirEx low O_2 storage regimes, thus there were no treatment related benefits on carbohydrate content. On the other hand, inconsistent results were obtained from comparison of total carbohydrates resulting from storage in AirEx atmosphere and high CO_2 atmospheres. In terms of carbohydrate classes, monosaccharides plus oligosaccharides were the most abundant sugars followed by polysaccharides, with starch being the least abundant. The MDA quantities due to AirEx and high CO_2 treatments were generally similar. While AirEx atmosphere resulted in higher total phenolic content in the 2015 season, the treatment effect was non-significant in the 2016 season, with differences only existing between products.

In paper 3, we assessed the feasibility of closed ventilation and automatic ventilation for sea freight of *Leucospermum* cut flower stems. The gas dynamics in a Janny MT box with a mixed load of *Proteaceae* products with 45-55 % free air were the same as in paper 2 and the

O_2 did not drop below the LOL values, which were between 0.08-0.41 % O_2. *Leucospermum* cut flowers have high respiration rates and strict maintenance of the cold chain is vital during sea freight shipping. The Q_{10} values ranged between and 2.61 and 3.86 and the respiration rates of the *Leucospermum* products are generally between 19.06-45.44 mL $CO_2.kg^{-1}.h^{-1}$ at 5 °C. Storage in AirEx significantly increased mass loss compared to closed ventilation, whilst moisture loss was comparable between AirEx, 5-10-15 % CO_2 and 15 % CO_2 treatments, and there were slight inconsistencies between the seasons. Moisture loss comparison between AirEx, DCA and 2 % CO_2 treatments revealed inconsistencies between seasons, among treatments and among products. According to Aqüero et al. (2011), mass loss of fresh produce is principally attributed to vaporization of a moisture layer that exists on the surface after harvest and dehydration due to a vapour pressure gradient between the product and surrounding atmosphere.

The changes in leaf colour were visually insignificant between AirEx and low O_2 or high CO_2 storage treatments. *Leucospermum* products stored under AirEx had inferior quality compared to those stored under closed ventilation, which had better inflorescence and leaf longevity. Alternatively, storage under low O_2 treatments did not result in significant longevity benefits over AirEx and product responses to the treatments were variable both within and between seasons. Differences between seasons were likely due to different harvesting maturity and growing conditions. Mixed findings were obtained from effect of storage in AirEx and high CO_2 and although the treatment was non-significant in the first season, leaf visual quality was similar between the AirEx and low O_2 treatments in the second season.

The monosaccharide plus oligosaccharide quantities in the products stored in AirEx were similar to those in products stored in low O_2 and in high CO_2 in the first season. The monosaccharides plus oligosaccharides were the most abundant sugars followed by polysaccharides and starch, and differences in polysaccharide and starch content were minor, between AirEx and low O_2 treatments, and also between AirEx and high CO_2 treatments. Storage in AirEx and 15 % CO_2 resulted in higher MDA content than storage in 5-10-15 % CO_2, which is possibly due to high peroxidation activity under AirEx and 15 % CO_2. Differences in total phenols in the leaves were likely due to product variability since differences among treatments were predominantly minor.

In paper 4, we investigated the viability of closed ventilation and automatic ventilation for sea freight of *Protea* cut flower stems. The results from chapter 3 and 4 indicated that the gas dynamics in a closed ventilation system vary depending on respiration rate of the products, volume of free air and gas sampling method. The respiration rates of the *Protea* products ranged

from 10.76-27.24 mL $CO_2.kg^{-1}.h^{-1}$ at 5 °C. The Q_{10} values for the 5 to 15 °C range were between 1.7 and 3.84. Closed ventilation and high CO_2 atmosphere resulted in lower mass loss than AirEx and there were insignificant differences in mass loss between AirEx and low O_2 atmospheres.

The colour of inflorescences and leaves was visually indistinguishable between the AirEx and closed ventilation treatments, AirEx and high CO_2 and AirEx and low O_2 atmospheres. However, the products stored in closed ventilation had better inflorescence and leaf visual quality than products stored in AirEx. This may have been due to reduced moisture stress due to the high RH atmosphere that is created in a passive MA, such as under closed ventilation. On the other hand, storage in AirEx and high CO_2 atmospheres resulted in similar inflorescence and leaf quality among the products and there were inconsistencies among the products in their leaf quality responses to the high CO_2 treatments. 'Cynaroides' and 'Ice Queen' showed rapid decline in inflorescence and leaf deterioration quality due to the 15 % CO_2 treatment. This may have been due to high CO_2 damage due to the consistently high CO_2 concentration under 15 % CO_2 treatment.

The quality and longevity of products stored in AirEx and the low O_2 was comparable and the AirEx, high CO_2 and low O_2 treatments had insignificant variability in quantity of soluble carbohydrates. Similar to *Leucadendron* and *Leucospermum*, monosaccharides plus oligosaccharides were the most abundant sugars, followed by polysaccharides, and starch was the least abundant. The differences in quantity of polysaccharides and starch were minor between products stored in AirEx and in low O_2 and between AirEx and in high CO_2 treatments. The MDA content was comparable between AirEx and high CO_2 atmospheres, however, 'Barbigera' and 'Ice Queen' had the highest MDA and they are both susceptible to leaf blackening compared to the other products. This may be a sign of oxidative stress which is often triggered by stresses like chilling or freezing, high light levels, ultraviolet radiation, hypoxia or anoxia (Hodges et al, 2004). Generally, the AirEx, DCA and 2 % O_2 treatments had insignificant effect on total phenolic content of the products. The concentration of total phenolics was variable among the products.

The information on the respiration rates, gas dynamics during long-term shipping, and lower O_2 limits was successfully determined in this study. Furthermore, closed ventilation, high CO_2 and low O_2 led to improved visual quality and longevity compared to AirEx. This proves that closed ventilation, lowering O_2 and allowing CO_2 to rise above the normal atmospheric concentration is possible during long-term sea freight of *Proteaceae* cut flowers. The O_2 level should be maintained above the lower O_2 limits of the products, which ranged from

0.08-0.48 % O_2. Thus, in a commercial trial, automatic ventilation may be set to maintain a minimum of 2 % O_2 and maximum of 15 % CO_2 concentration in the reefers during long-term sea freight shipping of *Proteaceae* cut flower products. There is need to assess the effect of harvesting maturity and temperature on the responses of the products to low O_2 and high CO_2 since tolerance to hypoxia and anoxia differs with temperature. The study revealed that an extensive product range is needed when developing a post-harvest protocol for storage since product responses to the treatments varied between seasons and treatments.

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