Postharvest insect pest disinfestation in export Proteaceae cut flowers - the potential of new disinfestation strategies

by

Makhosazana Shantelle Ngwenya

Thesis presented in partial fulfilment of the requirements for the degree of Master of Agricultural Sciences

at

Stellenbosch University

Department of Conservation Ecology and Entomology, Faculty of AgriSciences

Supervisor: Dr Shelley Johnson

Co-Supervisor: Dr Lynn Hoffman

December 2021

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.

Date: December 2021

SUMMARY

The Proteacea*e* family are associated with a wide range of insect pests which feed both externally and internally. International agricultural trade of fresh plant material increases the risk of introducing invasive, polyphagous insects into new areas. The risk of introducing invasive insect species puts pressure on producers, as production costs increase with each required control program, and decreases the total value of the crop, due to damage and quarantine restrictions. Therefore, preharvest and postharvest control measures are required to prevent the introduction of regulatory pests on traded commodities.

The feasibility of the Controlled Atmosphere Temperature Treatment System (CATTS) was assessed as a potential alternative disinfestation technique on export quality *Protea magnifica* ('Barbi'), *Leucospermum lineare x cordifolium* ('Succession'), *Leucospermum patersonii x cordifolium* ('High Gold') and *Leucadendron salignum* ('Goldstrike') cut flower stems. CATTS target temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C with a 15 min soaking period at the target temperature. The atmospheric composition for CATTS treatments was 1% O_2 and 15% CO_2 in N_2, with relative humidity maintained at 80%. CATTS-treated stems underwent vase life studies after treatment, or following freight simulation periods, namely storage at 2°C under normal atmosphere for 3 days or 21 days to simulate air and sea freight, respectively. Additionally, the potential of pretreatment pulsing and/or hydrating to prevent CATTS-induced leaf blackening was investigated. Pretreatment pulsing treatments were conducted by holding the flower stems in 10 ml/L Prof 3 (Chrysal Professional 3 vase and foam solution) for 1 hour, while the hydrating solution contained 5 ml/L Prof 2 (Chrysal Professional 2 transport and display solution).

Protea 'Barbi' marketability was significantly reduced by CATTS treatment. Phytotoxic damage on this cultivar manifested as premature leaf blackening and wilting and intense discoloration of the inflorescence. Pulsing effectively inhibited the resultant phytotoxic damage when *Protea* 'Barbi' stems were evaluated immediately post CATTS treatment and after air freight simulation however, had no significant impact when the stems were evaluated post sea freight simulation. Similarly, hydrating was effective in preventing phytotoxic damage during immediate evaluation however, had no significant impact when the stems were subjected to freight simulation. *Leucadendron* 'Goldstrike' withstood treatments and maintained marketable quality following treatment and air freight simulations. However, subjecting CATTS-treated *Leucadendron* 'Goldstrike' stems to simulated sea-freight storage resulted in severe phytotoxic damage. Post sea freight, neither pulsing nor hydrating during CATTS

treatment had a positive impact on the foliage quality post storage. *Leucospermum* cultivars, 'Succession' and 'High Gold' withstood CATTS treatments and maintained good quality. Pulsing and/or hydration of *Leucospermum* cultivars, 'Succession' and 'High Gold', prior to CATTS treatment, reduced the vase life of the cultivars by causing premature style reflexion. CATTS treatments were ineffective in controlling mixed thrips and insects from the Coleoptera family, which were incidentally included in the treatments. Post CATTS treatment morphological analysis of the mixed thrips identified four different species of thrips on *Leucospermum* 'High Gold' as the western flower thrips, (*Frankliniella occidentalis*), common blossom thrips (*Frankliniella schultzei*), onion thrips (*Thrips tabaci*) and predatory thrips (*Aeolothrips* species). *Leucospermum* 'Jelena' only displayed the western flower thrips, of those that could be identified morphologically and many immature thrips, which could not be identified easily on a morphological basis.

To assess a potential technique to improve the quality of CATTS-treated *Protea* 'Barbi' and *Protea* 'Sylvia', stems were dipped in a 2% thiabendazole solution and then CATTS treated at a target temperature of 40°C. Thiabendazole (TBZ) dipping significantly reduced the incidence of leaf blackening in *Protea* 'Barbi' stems which were assessed immediately posttreatment, as well as post freight simulation. However, TBZ did not inhibit the foliage discoloration and leaf blackening on *Protea* 'Sylvia' stored at low temperatures (2°C) for prolonged duration (21 days).

Lastly, the potential use of ethyl formate (EF) fumigation to control insects on *Serruria florida* ('Blushing Brides') was assessed. The treatments used were: 10.00 g/m^3 for 2 hours, 18.53 g/m^3 for 1.75 hours, 20.00 g/m^3 for 1 hour and 20.00 g/m^3 for 2 hours. Additionally, the impact of pre-fumigation pulsing with 10 ml/L Prof 3 and/or 4 ml/L Chrysal Viva dipping was investigated. The study found that prolonged fumigations were not suitable for 'Blushing Brides'. The phytotoxic damage manifested as discoloration and browning of the foliage. The phytotoxic damage was more evident on mature florets which had already opened during fumigation. Pulsing and dipping florets and foliage did not prevent phytotoxic damage induced by EF fumigation. Effective insect control (100% mortality) was achieved during fumigation at EF concentration of 18. 53 g/m^3 for 1.75 h and 20.00 g/m^3 for 1 and 2 h treatment time.

The current study proved that CATTS technology can potentially be established as a viable disinfestation method for *Leucospermum* cultivars 'Succession' and 'High Gold', and *Leucadendron* 'Goldstrike'. CATTS treatments for *Protea* 'Barbi' stems are the least promising. However, the use of thiabendazole as a pre-CATTS treatment, reduces CATTS induced phytotoxic damage and *Protea* leaf

blackening. With further research, treatment protocols can be developed for export fynbos cut flowers with minimal quality loss. More research, which will incorporate a diverse range of Proteaceae cut flowers and methods for ensuring and improving marketable flower quality after treatment and storage, are still required.

OPSOMMING

Die Proteaceae-familie word geassosieer met 'n wye reeks insekplae wat beide uitwendig en inwendig voed. Internasionale landbouhandel van vars plantmateriaal verhoog die risiko om indringer, polifagotiese insekte na nuwe gebiede in te voer. Die risiko van indringer-insekspesies plaas druk op produsente, aangesien produksiekoste met elke vereiste beheerprogram styg, en die totale waarde van die oes verlaag, as gevolg van skade en kwarantynbeperkings. Daarom word voor- en na-oes beheermaatreëls vereis om die bekendstelling van regulatoriese plae op verhandelde kommoditeite te voorkom.

Die uitvoerbaarheid van die Beheerde Atmosfeer Temperatuur Behandeling Sisteem (CATTS) is beoordeel as 'n potensiële alternatiewe ontsmettingstegniek op uitvoerkwaliteit *Protea magnifica* ('Barbi'), *Leucospermum lineare x cordifolium* ('Opvolging'), *Leucospermum patersonii x cordifolium* ('High Gold')) en *Leucadendron salignum* ('Goldstrike') gesnyde blomstingels. CATTS-teikentemperatuur is bereik deur 35°C/uur verhogingsskaal van 23°C tot 40°C te gebruik met 'n 15 min deurweekperiode by die teikentemperatuur. Die atmosferiese samestelling vir CATTS-behandelings was 1% O_2 en 15% CO_2 in N_2, met relatiewe humiditeit gehandhaaf op 80%. CATTS-behandelde stingels het vaaslewestudies ondergaan na behandeling, of na vragsimulasieperiodes, naamlik berging by 2°C onder normale atmosfeer vir 3 dae of 21 dae om lug- en seevrag onderskeidelik te simuleer. Boonop is die potensiaal van voorbehandeling pulsering en/of hidrering om CATTS-geïnduseerde blaarverswarting te voorkom, ondersoek. Voorbehandeling polsbehandelings is uitgevoer deur die blomstingels vir 1 uur in 10 ml/L Prof 3 (Chrysal Professional 3 vaas en skuimoplossing) te hou, terwyl die hidrerende oplossing 5 ml/L Prof 2 (Chrysal Professional 2 vervoer- en vertoonoplossing) bevat het.

Protea 'Barbi' bemarkbaarheid is aansienlik verminder deur CATTS behandeling. Fitotoksiese skade op hierdie kultivar het gemanifesteer as voortydige blaarverswarting en verwelking en intense verkleuring van die bloeiwyse. Pulsering het die gevolglike fitotoksiese skade effektief geïnhibeer toe *Protea* 'Barbi'-stingels onmiddellik na CATTS-behandeling geëvalueer is en na lugvrag-simulasie het dit egter geen noemenswaardige impak gehad wanneer die stamme na seevrag-simulasie geëvalueer is nie. Net so was hidrering effektief in die voorkoming van fitotoksiese skade tydens onmiddellike evaluering, maar het geen noemenswaardige impak gehad wanneer die stamme aan vrag-simulasie onderwerp is nie. *Leucadendron* 'Goldstrike' het behandelings deurstaan en bemarkbare kwaliteit gehandhaaf na behandeling en lugvrag-simulasies. Die onderwerping van CATTS-behandelde *Leucadendron* 'Goldstrike'-stingels aan gesimuleerde seevragberging het egter ernstige fitotoksiese

skade tot gevolg gehad. Na seevrag, het nie polsing of hidrasie tydens CATTS behandeling 'n positiewe impak op die loof kwaliteit na berging gehad nie. *Leucospermum* kultivars, 'Succession' en 'High Gold' het CATTS-behandelings deurstaan en goeie kwaliteit gehandhaaf. Pulsering en/of hidrasie van *Leucospermum* kultivars, 'Succession' en 'High Gold', voor CATTS-behandeling, het die vaaslewe van die kultivars verminder deur voortydige stylrefleksie te veroorsaak. CATTS-behandelings was ondoeltreffend in die beheer van gemengde blaaspootjies en insekte van die Coleoptera-familie, wat terloops by die behandelings ingesluit is. Post-CATTS-behandeling morfologiese ontleding van die gemengde blaaspootjies het vier verskillende spesies blaaspootjies op *Leucospermum* 'High Gold' geïdentifiseer as die westelike blomblaaspootjies, (*Frankliniella occidentalis*), gewone blomblaaspootjies (*Frankliniella schultzei*), uie blaaspootjies (*Thrips tabaci*) en roofblaaspootjies (*Aeolothrips* spesies). *Leucospermum* 'Jelena' het slegs die westelike blomblaaspootjies vertoon, van dié wat morfologies geïdentifiseer kon word en baie onvolwasse blaaspootjies, wat nie maklik op 'n morfologiese basis uitgeken kon word nie.

Om 'n potensiële tegniek te bepaal om die kwaliteit van CATTS-behandelde *Protea* 'Barbi' en *Protea* 'Sylvia' te verbeter, is stingels in 'n 2% tiabendasooloplossing (TBZ) gedoop en dan CATTS behandel teen 'n teikentemperatuur van 40°C. Tiabendasool-dip het die voorkoms van blaarverswarting in *Protea* 'Barbi'-stingels aansienlik verminder wat onmiddellik na-behandeling, sowel as na-vrag-simulasie beoordeel is. TBZ het egter nie die blareverkleuring en blaarverkleuring op *Protea* 'Sylvia' wat by lae temperature (2°C) gestoor is vir 'n lang tydperk (21 dae) belemmer nie.

Laastens is die potensiële gebruik van etielformaat (EF) beroking om insekte op *Serruria florida* ('Blushing Brides' ook bekend as Bergbruidjies) te beheer, geassesseer. Die behandelings wat gebruik is was: 10.00 g/m^3 vir 2 uur, 18.53 g/m^3 vir 1.75 uur, 20.00 g/m^3 vir 1 uur en 20.00 g/m^3 vir 2 uur. Daarbenewens is die impak van preberokingspulsering met 10 ml/L Prof 3 en/of 4 ml/L Chrysal Viva-dip ondersoek. Die studie het bevind dat langdurige berokings nie geskik was vir Bergbruidjies nie. Die fitotoksiese skade het gemanifesteer as verkleuring en verbruining van die blare. Die fitotoksiese skade was duideliker op volwasse blomme wat reeds tydens beroking oopgemaak het. Polsende en doop blommetjies en blare het nie fitotoksiese skade wat deur EF beroking veroorsaak is, verhoed nie. Effektiewe insekbeheer (100% mortaliteit) is bereik tydens beroking by EF konsentrasie van 18.53 g/m^3 vir 1.75 uur en 20.00 g/m^3 vir 1 en 2 uur behandelingstyd.

Die huidige studie het bewys dat CATTS-tegnologie moontlik gevestig kan word as 'n lewensvatbare ontsmettingsmetode vir *Leucospermum* kultivars 'Succession' en 'High Gold', en *Leucadendron*

'Goldstrike'. CATTS-behandelings vir *Protea* 'Barbi'-stingels is die minste belowend. Die gebruik van tiabendasool as 'n pre-CATTS-behandeling verminder egter CATTS-geïnduseerde fitotoksisiteit en *Protea*-blaarverswarting. Met verdere navorsing kan behandelingsprotokolle ontwikkel word vir uitvoer fynbos snyblomme met minimale kwaliteit verlies. Meer navorsing, wat 'n diverse reeks *Proteaceae*-snyblomme sal insluit en metodes om bemarkbare blomkwaliteit na behandeling en berging te verseker en te verbeter, is steeds nodig.

This thesis is dedicated to my young brother Mlungisi Ngwenya and myself, Makhosazana Ngwenya. May this thesis serve as a reminder that as long as we have each other, we can endure any challenges, and may it forever affirm our belief in God, who sustains us.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

The Post-Harvest Innovation programme and CapeFlora SA for funding this research.

My supervisors, Dr Shelley Johnson and Dr Lynn Hoffman for trusting me with this project, guidance and their unwavering support.

Prof M Kidd for his help with the statical analysis of this research.

Dr Renate Smit for her assistance with the technical aspects of this research.

Caro Kapp and Elleunorah Allsopp for their help with insect identification.

Bryan Michell, Carlo Pieterse, Caroline O'Brien, and Eugenie-Lien Louw, for their willingness to share their knowledge of the South African export cut flower industry and its challenges and guidance with cultivar selections.

Arnelia Farm, Berghoff Fynbos, Cape Mountain Flora and Future Fynbos for supplying me with the flowers.

Abdul Jacobs and Susana Das Neves for their assistance with flower and insect collection and driving me to the different farms.

The anchors of my life; Karin Schonauer, Monika Bosson, Peggy Gilbert, Xola Nondlazi and Zodwa Ngwenya, your relentless trust in my capabilities helped me endure the challenges I faced throughout this research.

My best friends; Annel Bokodisa, Joseph Makhafola and Mlungisi Ngwenya for being my greatest cheerleaders and supporting all my endeavours.

My flatmates; Lillies Ratshidi and Zimbili Sibiya and postgraduates' officemates; Abongile Nxitywa and Thabu Mugala. Thank you for your companionship throughout this journey, beneficial discussions and ensuring that I eat proper food and not cereal all the time.

PREFACE

This thesis is a compilation of 5 chapters, starting with a literature review, followed by three research papers and concluding with a general discussion and conclusion chapter. Each chapter is introduced separately and thus there might be repletion of information.

Chapter 1 General introduction and literature review

Chapter 2 Controlled Atmosphere Temperature Treatment System (CATTS) as a postharvest disinfestation technique against Proteaceae phytosanitary pests

Chapter 3 Evaluating the use thiabendazole (TBZ) to mitigate phytotoxic damage induced by Controlled Atmosphere Temperature Treatment System (CATTS) treatments on export grade *Protea* cut flowers

Chapter 4 Postharvest application of carbohydrate pulse and 6-benzyladenine (cytokinin) to mitigate phytotoxic impact of ethyl formate fumigation on the vase life of export *Serruria florida* ('Blushing Brides')

Chapter 5 General discussion

Table of Contents

LIST OF FIGURES

LIST OF TABLES

1. General introduction and literature review

1.1 The South African cut flower industry

1.1.1 The Proteaceae family

The South African Proteaceae is indigenous to the Cape Floristic Region (CFR), located in the southwestern cape of South Africa, and occupies an area of approximately 90 000 km^2 (Higgins *et al.,* 1997; Hawkins *et al.,* 2007). The region is remarkably species rich containing about 9 383 plant species within 997 genera and 220 flowering plant families, of which approximately 69% are endemic (Giliomee, 2003; Reinten and van Wyk, 2018). The CFR is in a Mediterranean climate, which is characterized by annual precipitation falling in the winter months, along with scorching hot and dry summers (Higgins *et al.,* 1997). Mean annual rainfall ranges from 445 mm in the east to 540 mm along the West Coast region but can exceed 2000 mm on the highest mountain summits located within the CFR (Cowling, 1990).

The highly speciose family Proteaceae is reported to cover 60 genera and made up of approximately 1700 species (Barker *et al.,* 2004). Globally, Australia is the most species rich, with 57% of the total species within 45 genera of the Proteaceae family, followed by South Africa, with 330 species in 14 genera, while the rest are distributed throughout Africa, Polynesia and South America (Malan, 2012). Johnson and Briggs (1975) reconstructed the phylogeny and classification of the Proteaceae family, detailing their characteristics and adaptations, and elaborated on the growth habits and habitat preferences. The family Proteaceae is characterized by a woody shrub nature, sclerophyllous leaves with trichomes and proteoid roots, the latter are considered adaptations to periodic drought and to nutrient-deficient soils (Hernandez *et al.,* 2008; Malan, 2012). The differentiating factor within the genera of the Proteaceae family is the inflorescence, which ranges from the large colourful inflorescence of *Protea* species with long hairy bract-tips, to the cone-like inflorescence of *Leucadendron* species and lastly, to the more dense inflorescences of *Banksia*, *Telopea* and *Leucospermum* species, which display “compositaceous-type clusters with short, thick receptacles subtended by involucral bracts” which resemble pincushions (Johnson and Briggs, 1975; Criley, 1998; Barker *et al.,* 2004).

1.1.2 Proteaceae cultivation and development of an industry

Historically, the Proteaceae flower industry was entirely dependent on wild harvesting. According to Parvin *et al.* (2003), the movement towards commercial *Protea* cultivation was initiated by Frank C. Batchelor who published a guide on commercial cultivation during the 1940s to 1970s and Marie Vogts is credited for pioneering the movement to promote cultivation methods as a means of preventing over-exploitation of wild populations from the CFR. In addition, the demand for true to type and good quality common Proteaceae has steadily increased over the years, statistics shows an increase from the 1009 ha reported in 2015 (Gollnow and Gerber, 2015) to an estimated 1111 ha that is currently under cultivation (N. Viljoen, personal communication). However, dried products and filler foliage of Cape Flora are still mainly veld harvested from approximately 200 000 ha in the Western and Eastern Cape provinces of South Africa (N. Viljoen, personal communication).

The leading cultivated genera in the Proteaceae family are *Protea, Leucospermum* and *Leucadendron*. According to Viljoen (2019) the top exported *Protea* species, selections and cultivars for the 2018/2019 season, in descending order were: 'Pink Ice' (*P. neriifolia* x *P. susannae*), 'King Protea' (*P. cynaroides),* 'Sylvia' *(P. eximia* x *P. susannae),* 'Venus' (*P. repens* x *P. aristata*), and 'Carnival' (*P. compacta* x *P. neriifolia*). In 2018, 'Blushing Brides' (*Serruria florida*) dominated the South African export market when approximately 1 198 317 stems were exported and an increase of 37% was predicted for this niche cut flower for the 2018/2019 season. In this season, 7 280 201 stems of *Leucadendron* were exported. The main exported cultivars, in descending order, were; 'Safari Sunset' (*L. laureolum* x *L. salignum* F), 'Jade Pearl' (*L. linifolium hybrid*), 'Plumosum Female' (*L. rubrum*) and 'Rosette' (*L. laureolum* x *L. elimense*). A total of 10 692 087 *Leucospermum*, in lose stems and bouquets, were exported by the end of the 2018/2019 season. The leading commercial cultivated *Leucospermum* varieties were; 'Succession' (*L. lineare* x *L. cordifolium*), 'Soleil' (*L. glabrum* x *L. cordifolium F*), 'Jelena' (*L. cuneiforme* x *L. cordifolium*) and 'Tango' (*L. linare 'Diadem'* x *L. glabrum 'Helderfontein'*). The volume of 'Queen Juliana' (*L. glabrum* x *L. cordifolium*) stems increased by 26% for the 2018/2019 season, compared to the previous seasons, while the number of stems exported from other varieties decreased with approximately 6% (Viljoen, 2019). Historically, international demand for floriculture products was mainly influenced by availability, price and consumer trends, however sustainability and ethical production preferences are expected to have a significant impact on international floriculture markets (Reinten and van Wyk, 2018).

The domestic demand for Proteaceae cut flowers is relatively low compared to the volume of stems being exported (Huysamer, 2018). It is estimated that about 90% of the Cape Flora products produced in South Africa are being exported, contributing to an estimated R500 million value to the South African cut flower industry (Reinten and van Wyk, 2018). The European Union remains the leading importer of Cape Flora cut flowers (Matsikidze, 2018), even though there has been a substantially decrease in market share over the years. For the 2018/2019 season a 2% decrease in the UK market share was reported from the previous season, while the Middle East, as the first point of arrival by air freight, remained stable at 27%, whereas the Far East market share increased to 10% (Viljoen, 2019). The South African cut flower export industry has a potential to further extend to emerging markets in Asia and Latin America.

1.2 Factors limiting Proteaceae cut flower export

1.2.1 Competitive ability

One of the major strengths of the South African floriculture industry is the availability of diverse indigenous floral products. In the genus *Protea* alone approximately 40% of all species exhibit distinct involucral bracts, ranging from green or cream to increasingly bright pink. It is these inherent exotic characteristics that motivated countries with suitable climates, such as Australia, New Zealand and Zimbabwe, to invest in the cultivation of these South African indigenous floral products towards the end of the twentieth century (Coetzee and Middelmann, 1997; Huysamer, 2018). Countries and regions with Mediterranean-type climates similar to the Western Cape, such as the Azores, California, Canary Islands, Chile, Hawaii, Israel and Portugal are suitable for the successful cultivation of Proteaceae cut flowers (Gerber and Hoffman, 2014). This presents a threat to the South African market share because international competitors are in a more favourable position to support functional selection and breeding programmes to develop new cultivars. In addition, these countries may be better positioned for the commercial production of significant quantities of high-quality export products. This may result in these South African products losing their uniqueness and endemism value. Portugal and Australia may have an advantage over South Africa, because of their proximity to export markets. The distance from South Africa to their main export market remains a significant challenge, especially when production (electricity and labour) and transportation (fuel) costs rise at a rate higher than inflation (Gerber and Hoffman, 2014). An option to reduce transportation costs may include making use of sea freight shipping (Gerber and Hoffman, 2014; Matsikidze, 2018), however, there will be a need to manage long-term storage conditions and their associated risks. These may

include monitoring and avoiding possible anaerobic conditions and possibly ethylene accumulation, while maintaining shipping temperatures between 1 – 4 °C to reduce respiration rates, and limit the development of chilling injury (Matsikidze, 2018).

A major disadvantage faced by South African growers when producing indigenous Proteaceae cut flower products is the occurrence of phytosanitary insect pests on export cut flowers. Since these products are cultivated within their natural habitat, they are more susceptible to their natural occurring insect enemies, along with indigenous pathogens causing fungal diseases. These natural enemies are of quarantine concern to trading partners, making it challenging for the products to pass international phytosanitary requirements on export (Coetzee and Middelmann, 1997). Phyto-sanitation barriers imposed by importing countries aim to prevent the entry and spread of invasive pests into importing countries' territories (Hara *et al.,* 2002), especially when these recipient countries have similar climates. Additionally, South Africa has a shortage of environmentally friendly disinfestation techniques, which maintains the quality of the cut flower, along with poor infrastructure for packaging and cooling units in transit to and at points of export (Coetzee and Middelmann, 1997; Huysamer, 2018).

To improve the profitability of the industry, more research on indigenous Proteaceae cut flower is required (Reinten *et al.,* 2011), in particular with regard to the development of novel floral products which will ensure year around availability (Gerber and Hoffman, 2014). A key area for research as suggested by Reinten and van Wyk (2018) is for horticulturists and plant pathologists to develop new propagation and cultivation protocols that would allow for the production of healthier planting material along with protection measures against natural enemies, as identified and studied by entomologists. If this can be achieved, together with further collaborating with marketing sectors to introduce new products to consumers, a continuous demand for Proteaceae products will be ensured.

1.2.2 Quality and postharvest quality

1.2.2.1 Leaf blackening

Leaf blackening is a major postharvest disorder which manifests as brown or black discolorations of *Protea* foliage and to a lesser extent on *Leucadendron* (Dai and Paull, 1997; van Doorn, 2001). The disorder is prevalent on *P. neriifolia, P. compacta, P. coronata* and *P. eximia*, along with highly susceptible hybrids such as 'Sylvia' and 'Pink Ice' (Stephens *et al.,* 2001; van Doorn, 2001; Matsikidze, 2018). Postharvest stresses have been attributed to the development of the disorder on susceptible

species. These include water stress, heat stress, mechanical damage, disease, low light and food/carbohydrate stress (Hoffman *et al.,* 2018). Masike *et al.,* (2020) noted that the stress factors trigger metabolites such as benzenetriol, anthocyanins and hydroquinon which are associated with leaf blackening on *Protea* cultivars.

The exact biochemical mechanism on how these stressors induce leaf blackening continues to be disputed. According to Windell (2012), water stress occurs on cut stems because of vascular blockage resulting in water uptake impairment. Dehydration results in cell membrane damage, consequently inducing leaf blackening. Heat stress has been attributed to the onset of leaf blackening because of its logarithmic relationship with respiration rates of cut flowers. The increase of respiration rate negatively impacts the functionality of cell membranes, consequently causing increased membrane permeability which leads to oxidation and the development of brown-coloured pigments on the leaf surface, of *Protea* cut flower stems (Jones and Clayton-Greene, 1992).

Carbohydrate depletion induced by inflorescence growth and associated increased respiration rates is generally accepted as the main cause of leaf blackening (Stephens *et al.,* 2001; Hoffman *et al.,* 2014; Hoffman *et al.,* 2018). The accepted hypothesis suggests that carbohydrate depletion results in hydrolysis of intracellular membranes consequently causing polyphenol oxidase and peroxidase which are normally located in the chloroplast to come in contact with phenols which are situated in the vacuoles. Whitehead and de Swardt (1982) reported that polyphenol oxidase and peroxidase activities increased during postharvest inducing phenol oxidation which causes leaf blackening in *P. neriifolia* leaves. In accordance, Dai and Paull (1997) reported that maximum polyphenol oxidase activity occurred six hours after the enzyme had been extracted from senescing leaves of *P. neriifoiia*. Perold (1993) reported that *Protea* species contain *O*-glycocides and cutting *Protea* stems results in disturbance of the chemical equilibriums which are maintained in living plants. As a result, free *O*-glycocides are hydrolysed causing leaf blackening. Further, Perold (1993) speculated that the disturbance of the chemical equilibrium results in free phenols, sugars, phenolic aglycone and esterifying acid components in addition to parent glycoside esters being released in the cells. These compounds are reported to be very reactive, for example the phenolic aglycone found in *P. neriifolia,* is a very reactive trisphenol 1, 2, 4-trihydroxybenzene which stains proteins into a strong black colour and is speculated to be the cause of leaf blackening in this species (Perold, 1993). Recently, Masike *et al.,* (2020) identified benzenetriol derivatives, such as, phlorin ((benzene-1,3,5-triol)-O-hexoside), hydroxyquinol ((benzene-1,2,4-triol)-O-hexoside) derivatives and benzoyl-hexoside of hydroxyquinol (benzoyl-O-hexoside-O-hydroxyquinol) in leaf blackening susceptible cultivars. These derivatives are

known for their proneness to oxidation reactions which result in production of black insoluble products. Additionally, the researchers reported that they identified glycosylated hydroquinone (p-diphenol), arbutin and its derivatives which produce the black insoluble products through both enzymatic and non-enzymatic reactions which takes place during oxidation (Masike *et al.,* 2020).

To delay leaf blackening, Jones and Clayton-Greene (1992) recommended storing cut *Protea* in well-lit environments because it stimulates continuous carbohydrate supply therefore preventing the initial hydrolysis of cell organelles and metabolites. Furthermore, placing *P. neriifolia* stems under a controlled atmosphere of 1% O_2 and 5% CO_2 for 14 days under constant light of approximately 5 µmol m^{-2} s^{-1} at 25°C and 90% RH resulted in negligible blackening (0.3%) compared to 73.8% leaf blackening incidence which was observed on stems which were placed under normal atmospheric conditions (Jones and Clayton-Greene, 1992).

1.2.2.2 Thiabendazole

Thiabendazole (4-(1H -1, 3-benzodiazol-2-yl)-1, 3-thiazole) (TBZ), is a benzimidazole systemic fungicide which controls postharvest diseases caused by *Penicillium digitatum, Colletotrichum gleosporioides* and *Diplodia natalensis* (Kellerman *et al.,* 2014). Of interest here is that it is reported to be effective in controlling chilling injury incidence on citrus fruits (Schirra and Mulas, 1995; Schirra *et al.,* 2000; Hordijk *et al.,* 2013; Kellerman *et al.,* 2014). Leaf blackening in Proteaceae may in some cases result following chilling injury (E.W, Hoffman, personal communication). Chilling injury in both ornamentals and citrus fruits is induced by environmental stresses, which cause cell membrane disintegration (Jones and Clayton-Greene, 1992). On citrus, a TBZ wax coating is effective in preventing surface transpiration and water loss. The biochemical mechanism by which TBZ reduces chilling injury is poorly understood. However, Schirra *et al.*, (1998) attributed it to the suppression of microbial activities and prevention of rapid peel senescing. No studies have been done on the impact of TBZ on ornamentals. The potential for TBZ to provide protection to flowers undergoing an atmospheric and thermal stress treatment needs to be considered. Application of TBZ may improve flower quality by protecting blackening-prone cultivars during normal postharvest practices as described above, as well as after certain postharvest treatments that may induce leaf blackening as was observed in a study by Huysamer (2018), which is discussed later in this chapter.

1.2.2.3 Insects associated with Proteaceae and phytosanitary implications

The taxonomic and structural diversity of Proteaceae shrubs creates heterogeneous microhabitats which allow diverse groups of arthropod assemblages to flourish (Wright and Samways, 2000; Sasa and Samways, 2015; Simaika *et al.,* 2018). Additionally, the genus *Protea* produces abundant pollen, for example, *P. caffra* and *P. simplex* emit volatile compounds, which attract the common pollinator Cetoniine beetle, *Atrichelaphinis tigrina* (Steenhuisen *et al.,* 2013) and nectar, which attract insects.

In a study conducted in the Grabouw area, in the Western Cape province of South Africa, Sasa and Samways (2015) identified economically important pests associated with the family Proteaceae. These included Lepidoptera species such as *Capys alphaeus, Orophia ammopleura, Argyroploce* spp*., Epichoristodes acerbella* and *Phyllocnistis* spp*.* The reported Coleoptera species were *Genuchus hottentottus*, *Diaplochelus longipes* and reported Hemiptera species was *Delottococcus* spp. Lee *et al.* (2017) intercepted a total of 31 samples (11 species belonging to four families: Apionidae, Curculionidae, Dryophthoridae, and Nanophyidae) from South African cut flowers (*Berzelia* spp*., Brunia* spp*., Erica* spp*., Leucadendron* spp.) at the Korean quarantine border (Incheon International Airport). In a quantitative survey conducted on Cape Flora products prior to export at the Cape Town International airport, Huysamer (2018) collected a total of 82 insects, representing eight orders and 26 families. The highly encountered insect families in a descending order were: Thripidae, Curculionidae, and Pseudococcidae. According to Huysamer (2018), the various veld-harvested Cape foliage and filler material were the most infested product types, followed by *Leucospermum, Protea, Leucadendron*, while bouquets hosted a limited number of insects, with only one insect found on *Bank*sia.

This close association with insects can be detrimental because it risks the introduction of these insects into countries which import South African cut flowers. Accidental introduction of insect pests threatens the importing country's biodiversity. Since there is no clear indication that points towards an evolutionary association between fynbos plant species and their associated insects (Wright and Samways, 2000), these pests may potentially find alternative hosts and successfully invade a new area. Sasa and Samways (2015) verified that most of the Proteaceae arthropods such as *Phoracantha semipunctata*, *Epichoristodes acerbella*, *Macrosiphum euphorbiae* and *Pemphigus* spp*.* are also considered economic pests of other plant species. Introduced pests commonly do not have natural enemies, therefore their populations increase at exponential rates and may become pests in other

Protea growing areas. For example, an invasive mealybug (*Delottococcus confuses)* which is native in South Africa was first recorded in 2003 at a *Protea* nursery in southern California before being intercepted on a shipment of cut flowers imported from Hawaii (Miller and Giliomee, 2011). Furthermore, exotic insects destabilise natural assemblages, for example alien coccinellid *Harmonia axyridis* outcompetes native species, and the alien ant *Linepithema humile* interrupts parasitoid wasps in the biocontrol of mealybugs (Sasa and Samways 2015).

1.3 Insect disinfestation treatments

The risk of introducing invasive insect species necessitates preharvest and postharvest quarantine treatments to actively remove and prevent the introduction of regulatory pests. The cost of registering new effective chemicals and the increasing demand for environmentally friendly control techniques for insects has led to lower use of traditional pesticides. Commonly, producers spray insecticides in the field, however these preharvest insecticide applications rarely meet quarantine requirements. Therefore, there is a need to investigate and introduce phytosanitary treatments which will potentially control a wide range of insect taxa to meet the international quarantine requirements, without decreasing crop productivity and marketability (Roberts, 2016). In this review, physical and chemical approaches to disinfestation are discussed.

1.3.1 Physical disinfestation treatments

Hand removal of all insects from flowers and foliage is only practical for small scale production, because it is time consuming and labour intensive (Hansen and Hara, 1994).

Irradiation treatment as a quarantine control measure rarely causes mortality of the insects. This technique stops pest introduction and establishment in new areas by preventing further insect development or by resulting in insect sterility (Bustos-Griffin *et al.*, 2012; Hallman, 2012). This is achieved by damaging the chemical bonds of the nucleic acids in DNA, which then results in cellular dysfunctionality (Farkas, 2006; Yun *et al.*, 2016). The minimum doses that are required for a 100% mortality of the various economic pests differ, as well as the reaction of cut flowers to irradiation. For example, the generic dose for *Tetranychus urticae* (two-spotted spider mite) is 0.3 kGy (Nicholas *et al.*, 2019), whereas it is approximately 0.22 kGy for the polyphagous pest, *Helicoverpa armigera.* Generally, cut flowers are less tolerant to high irradiation doses compared to insects. At minimal doses of 0.1, 0.3 and 0.9 kGy, which are necessary to sterilize and cause insect mortality, Haasbroek *et al.*

(1973) as cited by Seaton and Joyce (1992) reported that the rate which *P. compacta* and *Protea longiflora* inflorescence opens was decreased significantly after treatment. Maughan (1986) as cited by Seaton and Joyce (1992) reported that Gamma irradiation induced leaf blackening on *P. neriifolia, P. compacta* and *P. cynaroides.* Similarly, Sangwanangkul *et al.* (2008) reported that subjecting *Protea* 'Pink Ice' to 0.25 kGy irradiation resulted in leaf blackening within a day of treatment, however pulsing the stems with 2% glucose prior to irradiation and holding the stems in 2% glucose vase solution reduced irradiation injury.

Heat treatment of fresh produce has been explored as a pesticide-free alternative treatment for insect disinfestation (Lurie, 1998). With this treatment, heated air or water is used to increase the host temperature beyond the thermal limits of the insect (Hansen and Hara, 1994). For successful heat treatment without damaging the fresh produce commodities, fresh plant material must be more thermo-tolerant compared to its pest (Hara *et al.*, 2002). The advantage of using heat treatment is the short period of time that is required for either killing or weakening of the pests. However, not all fresh produces are tolerant to extreme temperatures. Hara *et al.* (2002) reported that *Leucadendron* 'Safari Sunset', along with hybrids of *Banksia* and *Leucospermum* are highly susceptible to heat treatments and exhibit phytotoxic damage when immersed in hot water (49°C) for 10 minutes. Conditioning *Protea* flowers to hot air or water prior to treatment is reported to minimise the detrimental impact of heat on flower quality. Additionally, decreasing humidity during treatment has been reported to elevate heat damage to insects and therefore reduce the required treatment time (Hara *et al.*, 2002). Hansen *et al.* (1992) achieved quarantine security by using a one hour hot-air treatment at 44.4°C and 60% relative humidity to eliminate western flower thrips, (*Frankliniella occidentalis)* from chrysanthemum. Banana aphids *(Pentalonia nigronervosa)* in red ginger flowers and magnolia white scale (*Pseudaulacaspic cockerelli*) on bird-of-paradise flowers were killed by subjecting the flowers to 46.6°C for one hour. However, the effectiveness of the treatment depends on the stage of flower opening at the time of treatment (Lurie, 1998).

Controlled atmosphere (CA) involves the manipulation of atmospheric gasses, through increasing the concentration of CO_2 or N_2 while ensuring low concentration of O_2 gas. The most significant advantages of this technique include the preservation of commodities quality through decreasing respiration rate and ethylene production, a natural gas released by maturing fresh produce that accelerates its ripening process. However, CA can also be effective to suppress postharvest pathogens and in suffocating insects and mites (Shelton *et al.*, 1996). Yet, for complete insect mortality a relatively long exposure time is required which might result in commodity damage.

Controlled atmospheric temperature treatment system (CATTS) can potentially control insect pests in cut flowers. The technique employs the effects of a short exposure to high temperature ≥ 40°C and atmospheric stress, in the form of a low O_2 (1%) and high CO_2 (15%) environment allowing both internal and surface pests to be controlled (Neven and Mitcham, 1996). CATTS treatments successfully disinfested several agricultural product pests such as codling moth (*Cydia pomonella*) and oriental fruit moth (*Grapholita molesta*) from apples (Neven and Rehfield-Ray, 2006b) and western cherry fruit fly (*Rhagoletis indifferens*) in sweet cherries, (Neven *et al.*, 2006), and was found to control the peach fruit moth (*Carposina sasakii*) in apples (Son *et al.*, 2012). In addition to controlling insects, CATTS treatment effectively controlled tarsonemid mites and root knot nematodes in strawberry root stock and runners (van Kruistum *et al.*, 2014), as well as protea itch mite (*Proctolaelaps vandenbergii)* on Proteaceae cut flowers (Huysamer, 2018).

The mode of action of CATTS on insect physiology is not well understood, however the combination of atmospheric stress and high temperature have a detrimental insecticidal effect. Neven and Mitcham (1996) and Zhou *et al.* (2000) postulated that at low O_2 levels and high CO_2, insect mortality is caused by either low metabolism or acidification. Hypoxic conditions cause insects to increase their ventilation rate and at high atmospheric CO_2, insects' spiracles remain open, therefore impairing oxidative respiration and inducing anaerobic metabolism which has toxic end products. Furthermore, nicotinamide adenine dinucleotide phosphate (NADPH) formation and adenosine triphosphate (ATP) assimilation are reduced, while the biosynthesis of glutathione is inhibited, in addition to the regeneration of acetylcholine from choline. Secondly, elevated endotracheal CO_2 increases the accumulation of intracellular Ca^{2+} leading to haemolymph acidification, consequently inhibiting biosynthetic activities and activation of phospholipid hydrolysis, resulting in a loss of membrane permeability, eventually causing cell death and insect mortality. However, exposure to controlled atmospheres may require a relatively long time to result in complete disinfestation, during which product quality deterioration may occur. The addition of high temperature to controlled atmosphere may potentially decrease treatment time. Furthermore, the combination of anaerobic metabolism induced by low O_2 and high CO_2 in addition to the increased metabolic rate and low pH caused by high temperature exposure is known to synergistically impair the cellular functionality at a higher rate, resulting in more rapid insect death (Zhou *et al.*, 2000).

A study in the Netherlands reported that subjecting cut flowers such as alstroemeria, carnation, chrysanthemums, gerbera and rose to CATTS treatment (1% O_2, 15% CO_2, at a set air temperature of

46°C) resulted in variable phytotoxic reactions across the cut flowers (Slootweg, 2007). The study reported that chrysanthemums withstood the treatment while other treated cut flowers exhibited leaf and inflorescence discoloration and limping of stems. In South Africa, Huysamer (2018) subjected various Proteaceae cut flowers to CATTS treatment where set air temperatures ranged from 40-50°C with a ramping rate of 30 and 35°C/hr. The study conveyed that *Leucospermum* 'Veldfire' flowers were unmarketable immediately after being subjected to both set air temperatures (i.e., 40 and 50°C). However, *Protea magnifica* 'Barbi', *Leucadendron* 'Safari Sunset' and Geraldton Wax 'Ofir' withstood CATTS treatment where the temperature was ramped from 30 or 35°C/hr to set air temperature of 40°C and exhibited leaf blackening, wilting and desiccation of leaf tips when the temperature was ramped at 35°C/hr to 50°C. Therefore, it is important to identify an optimum time–temperature regime which can disinfest commodities without reducing marketability.

1.3.2 Chemical treatments

Insecticidal dipping and fumigants containing insecticides are widely used and are considered a highly effective technique for postharvest insect disinfestation (Hansen and Hara, 1994). According to Rigby (2016) most insecticidal dips used for insect disinfestation of Australian Proteaceae cut flowers contain 10 g/L^1 deltamethrin and 500 g/L^1 iprodione. Generally, selective insecticides are used to control borers, while leaf feeding insects are commonly eliminated by application of organophosphates, pyrethroids and fumigation with dichlorvos (Wright, 2003). The highly toxic products of hydrogen cyanide, phosphine and methyl bromide have been employed intensively to disinfest floral commodities. However, they are known to cause phytotoxicity on fresh produce. Fumigating *Protea* 'Pink Ice' with 1 and 2 g/m^3 phosphine for five hours had no impact on product quality, however at increased dosage and treatment duration vase life was decreased (Weller and Graver, 1998). Weller and Graver (1998) recommended combining phosphine with other pest management procedures like cold treatment and insecticides to reduce the required treatment time, thereby increasing insect mortality, while reducing possible phytotoxic damage due to phosphine. Excessive residues of phosphates and phosphites can be carcinogenic (Cheng *et al.*, 2012). About 50 mg/L^1 phosphine in air is adequate to kill a human and is highly explosive (Karunaratne *et al*., 1997). In addition, at least 11 insect species have been reported to have developed resistance towards phosphine in more than 45 countries (Cheng *et al.,* 2012). Hydrogen cyanide concentration of 2.8 g/m^3 was reported to be phytotoxic to *Leucospermum* flowers (Hansen and Hara, 1994).

Ethyl formate (EF) is classified as a “generally recognised as safe" (GRAS) compound, as it is a natural plant volatile product which is a liquid at normal ambient temperatures, but vaporises at high concentrations (van Epenhuijsen *et al.*, 2007). Compared to other fumigates it has rapid disinfestation properties. In the presence of water, EF hydrolyses into toxic formic acid and ethanol (Ren and Mahon, 2006). According to Kim *et al.* (2015), the presence of formic acid produces CO_2 and results in the reduction of O_2, which leads to an impairment of O_2 transfer and consequently causing insect suffocation. Further, EF fumigation causes mitochondrial cytochrome *c* oxidase inhibition, therefore blocking O_2 transfer (Damcevski and Annis, 2006). Generally, EF is combined with other compounds to reduce its flammability and enhance its toxicity. VAPORMATE™ is a registered fumigant containing 16.7% by weight ethyl formate, with the remaining 83.3% as liquid CO_2 (Ryan and Bishop, 2003; van Epenhuijsen *et al.*, 2007; Pupin *et al.*, 2013). The addition of CO_2 eliminates the flammability properties of EF whilst also acting as a propellant. (Simpson *et al.*, 2004).

Ethyl formate has been used to fumigate pests associated with dried and stored products (Pupin *et al.,* 2013). EF has been reported to effectively control western flower thrips (Simpson *et al.*, 2004), and third-instar of California red scale (*Aonidiella aurantia),* onion thrips (*Thrips tabaci)* and grape mealybug (*Pseudococcus maritimus)* (Pupin *et al.,* 2013). However, eggs are reported to be resistant to EF fumigation, leading to hatching after treatment (Simpson *et al.*, 2007; Kim *et al.,* 2015). EF fumigation has been reported to induce phytotoxic damage on grapes, sweet pepper, while causing calyx damage in strawberries (Simpson *et al*., 2004). However, van Epenhuijsen *et al.* (2007) observed no phytotoxic damage and/or shelf-life reduction in onions, while Simpson *et al.* (2004) reported that fumigating strawberry with 0.5% EF had no noticeable impact on berry quality. The phytotoxic effect associated with EF is attributed to how strongly it is absorbed by plant material. The damage is exacerbated by high moisture content and warm conditions (van Epenhuijsen *et al.,* 2007; Ren and Mahon, 2006). After subjecting floriculture products such as *Protea* ‘Pink Ice ‘, *P. cynaroides, Banksia coccinea* and Geraldton wax (*Chamelaucium uncinatum*), Weller and Graver (1998) concluded that EF was not suitable for phytosanitary disinfestation. Similarly, Huysamer (2018) concluded that EF is highly phytotoxic to the evaluated Cape Flora specie. However, Weller and Graver (1998) and Williams (1998) noted that fumigation with EF had no negative impact on quality of *Serruria florida*.

1.4 Thesis structure and objectives

This study aims to investigate innovative technologies and provide data that could be used towards developing postharvest disinfestation protocol for Proteaceae cut flowers. The objectives of the study

are: 1) to assess the efficacy and suitability of CATTS treatment as a postharvest technology against insect pests associated with Proteaceae cut flowers; 2) to evaluate the efficacy of thiabendazole fungicide in controlling phytotoxic disorders induced by CATTS postharvest treatment; and 3) to evaluate the feasibility of EF fumigation on *Serruria florida* 'Blushing Brides' to control phytosanitary pests.

Insect infestations is a serious hindrance to the growth and profitability of the South African export cut flower industry. Currently, CATTS treatments seem to be the more viable option in terms of flower quality maintenance. Yet, EF has shown considerable success in other industries as a postharvest treatment and is, in comparison, easier to apply. Both technologies require further research to be expanded on more export cut flower cultivars and more insect pests as targets. The success of these technologies and the development of a comprehensive protocol and handling manual advising exporters will ensure that Cape Flora SA industry remains the main exporter of Proteaceae cut flowers.

1.5 References

Barker, N.P., van der Poorten, A., Morton, C.M. & Rourke, J.P. (2004). Phylogeny, biogeography, and the evolution of life-history traits in *Leucadendron* (Proteaceae). *Molecular Phylogenetics and Evolution*, 33, 845-860.

Bustos-Griffin, E., Hallman, G.J. & Griffin, R.L. (2012). Current and potential trade in horticultural products irradiated for phytosanitary purposes. *Radiation Physics and Chemistry,* 81, 1203-1207.

Cheng, W., Lei, J., Ahn, J-E., Liu, T-X. & Zhu-Salzman, K. (2012). Effects of decreased O_2 and elevated CO_2 on survival, development, and gene expression in cowpea bruchids. *Journal of Insect Physiology,* 58, 792-800.

Coetzee, J.H. & Middelmann, M.C. (1997). Swot analysis of the fynbos industry in South Africa with special reference to research. *Acta Horticulturae*, 453, 145-152.

Cowling, R.M. (1990). Diversity components in a species-rich area of the Cape Floristic Region. *Journal of Vegetation Science*, 1, 699-710.

Criley, R.A. (1998). Leucospermum: Botany and horticulture. *Horticultural Reviews,* 22, 27-90.

Dai, J. & Paull, R.E. (1997). Comparison of leaf susceptibility to enzymatic blackening in *Protea neriifolia* R. Br. and *Leucospermum* 'Rachel'. *Postharvest Biology and Technology*, 11, 101-106.

Damcevski, K.A. & Annis, P.C. (2006). Influence of grain and relative humidity on the mortality of *Sitophilus oryzae* (L.) adults exposed to ethyl formate vapour. *Journal of Stored Products Research*, 42, 61-74.

Farkas, J. (2006). Irradiation for better foods. *Trends in Food Science and Technology*, 17, 148–152.

Gerber, A.I. & Hoffman, E.W. (2014). International Protea Association and current global Proteaceae production: Achievements and challenges. *Acta Horticulturae* 1031, 17-28.

Giliomee, J.H. (2003). Insect diversity in the Cape Floristic Region. *African Journal of Ecology* 41, 237-244.

Gollnow, B. & Gerber, A. (2015). International Protea Association: building on strengths and facing the demands of a dynamic international floricultural trade to secure a successful *Protea* industry. *Acta Horticulturae,* 1097, 73-84.

Hallman, G.J. (2012). Generic phytosanitary irradiation treatments. *Radiation Physics and Chemistry,* 81, 861– 866.

Hansen, J.D. & Hara, A.D. (1994). A review of postharvest disinfestation of cut flowers and foliage with special reference to tropicals. *Postharvest Biology and Technology*, 4, 193–212.

Hansen, J.D., Hara, A.H. & Tenbrink, V.L. (1992). Vapor heat: a potential treatment to disinfest tropical cut flowers and foliage. *HortScience*, 27, 139-143.

Hara, A., Burnham Larish, L. & Tsang, M. (2002). Potential for hot-air disinfestation of *Protea* flowers. *Acta Horticulturae,* 602, 61-65.

Hawkins, H.J., Hettasch, H. & Cramer, M.D. (2007). Putting back what we take out, but how much? Phosphorus and nitrogen additions to farmed *Leucadendron* 'Safari Sunset' and *Leucospermum* 'Succession' (Proteaceae). *Scientia Horticulturae*, 111, 378-388.

Hernandez, M., Fernández-Falcón, M. & Alvarez, C.E. (2008). Study of soil fertility and plant nutrition of proteas cultivated under subtropical conditions. *Communications In Soil Science and Plant Analysis*, 39, 2146-2168.

Higgins, S.I., Turpie, J.K., Costanza, R., Cowling, R.M., Le Maitre, D.C., Marais, C. & Midgley, G.F. (1997). An ecological economic simulation model of mountain fynbos ecosystems: dynamics, valuation and management. *Ecological Economics*, 22, 155-169.

Hoffman, E.W., Vardien, W., Jacobs, G. & Windell, N.E. (2018). Leaf blackening: A serious impediment to long-term cold storage, transport, and extended vase life in *Protea* cut flowers. *Horticultural Reviews*, 45, 73 - 104.

Hoffman, E.W., Windell, N.E. & Jacobs, G. (2014). Optimum time of harvest for the control of leaf blackening in *Protea* 'Sylvia'. *Acta Horticulturae,* 1031, 125-133.

Hordijk, J., Cronjé, P.J.R. & Opara, U.L. (2013). Postharvest application of thiabendazole reduces chilling injury of citrus fruit. *Acta Horticulturae* 1007, 119-125.

Huysamer, A. (2018). An assessment of alternative postharvest technologies for the disinfestation of fresh Cape flora cut flowers for export from South Africa. Unpublished Master's thesis. Stellenbosch: Stellenbosch University [Online]. Available: https://scholar.ac.za.

Johnson, L.A. & Briggs, B.G. (1975). On the Proteaceae—the evolution and classification of a southern family. *Botanical Journal of the Linnean Society*, 70, 83-182.

Jones, R.B. & Clayton-Greene, K.A. (1992). The role of photosynthesis and oxidative reactions in leaf blackening of *Protea neriifolia* R. Br. leaves. *Scientia Horticulturae*, 50, 137-145.

Karunaratne, C., Moore, G.A., Jones, R.B. & Ryan, R.F. (1997). Vase life of some cut flowers following fumigation with phosphine. *HortScience,* 32, 900-902.

Kellerman, M., Erasmus, A., Cronje, P.J.R. & Fourie, P.H. (2014). Thiabendazole residue loading in dip, drench and wax coating applications to control green mould and chilling injury on citrus fruit. *Postharvest Biology and Technology*, 96, 78-87.

Kim, H.K., Lee, S-W., Kim, J-I., Yang, J-O., Koo, H-N. & Kim, G-H. (2015). Synergistic effects of oxygen on phosphine and ethyl formate for the control of *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology,* 108, 2572-2580.

Lee, W., Hwang, J-H., Lee, J-H. & Hong, K-J. (2017). Interception of weevils on cut flowers from South Africa by Korea plant quarantine. *Journal of Asia-Pacific Biodiversity,* 4, 527-531.

Lurie, S. (1998). Postharvest heat treatments. *Postharvest Biology and Technology*, 14, 257-269.

Malan, G. (2012). Protea cultivation: From concept to carton (1st ed.). Stellenbosch: SUN MeDIA.

Masike, K., de Villiers, A., Hoffman, E.W. & Stander, M.A. (2020). Application of metabolomics tools to determine possible biomarker metabolites linked to leaf blackening in *Protea*. *Journal of Agricultural and Food Chemistry*, 68, 12595 - 12605.

Matsikidze, S.N. (2018). Feasibility of closed ventilation and automatic ventilation for sea freight of Proteaceae cut flower stems. Master's thesis. Stellenbosch: Stellenbosch University [Online]. Available: https://scholar.ac.za [2019, May 30]

Miller, D.R. & J.H. Giliomee (2011). Systematic revision of the mealybug genus *Delottococcus* Cox & Ben-Dov (Hemiptera: Pseudococcidae). *African Entomology,* 19, 614 – 640.

Neven, L.G. & Mitcham, J. (1996). CATTS (Controlled Atmosphere/Temperature Treatment System): A novel tool for the development of quarantine treatments. *American Entomologist*, 42, 56-59.

Neven, L.G. & Rehfield–Ray, L.M. (2006). Combined heat and controlled atmosphere quarantine treatment for control of western cherry fruit in sweet cherries. *Journal of Economic Entomology*, 99, 658-663.

Neven, L.G., Rehfield-Ray, L.M. & Obenland, D. (2006). Confirmation and efficacy tests against codling moth and oriental fruit moth in peaches and nectarines using combination heat and controlled atmosphere treatments. *Journal of Economic Entomology*, 99, 1610-1619.

Nicholas, A., Lidbetter, F., Eagleton, N., Spohr, L., Harris, A. & Barchia, I. (2019). Phytosanitary irradiation of three species of spider mites (Trombidiformes: Tetranychidae). *Austral Entomology*, 58, 418-424.

Parvin, P.E., Criley, R.A. & Coetzee, J.H. (2003). Proteas – A Dynamic Industry. *Acta Horticulturae,* 602, 123-126.

Perold, G.W. (1993). Consistency and variation in metabolite patterns of South African Proteaceae: a chemical perspective. *South African Journal of Science*, 89, 90-93.

Pupin, F., Bikoba, V., Biasi, W.B., Pedroso, G.M., Ouyang, Y., Grafton-Cardwell, E.E. & Mitcham, E.J. (2013). Postharvest control of western flower thrips (Thysanoptera: Thripidae) and California red scale (Hemiptera: Diaspididae) with ethyl formate and its impact on citrus fruit quality. *Journal of Economic Entomology*, 106, 2341-2348.

Ren, Y. & Mahon, D. (2006). Fumigation trials on the application of ethyl formate to wheat, split faba beans and sorghum in small metal bins. *Journal of Stored Products Research,* 42, 277-289.

Reinten, E.Y., Coetzee, J.H. & van Wyk, B.E. (2011). The potential of South African indigenous plants for the international cut flower trade. *South African Journal of Botany*, 77, 934-946.

Reinten, E.Y. & van Wyk, B.E. (2018). Floriculture industry benefits from southern African floral biodiversity. *Acta Horticulturae,* 1201, 659-663.

Rigby, E.M. (2016). Disinfestation of floriculture products with ethyl formate fumigant: Vapormate®. In *International Symposia on Tropical and Temperate Horticulture-ISTTH2016, 1205,* 351-358.

Roberts, P.B. (2016). Food irradiation: Standards, regulations and world-wide trade. *Radiation Physics and Chemistry,* 129, 30-34.

Ryan, R. & Bishop, S. (2003). VapormateTM: non-flammable ethyl formate/liquid carbon dioxide fumigant mixture. In Stored Grain in Australia, Proceedings of Australian Postharvest Technical Conference, 25–27 June 2003, Canberra, Australia, ed. by EJ Wright, MC Webb and E Highley, CSIRO, Canberra, 190–192.

Sangwanangkul, P., Saradhuldhat, P. & Paull, R.E. (2008). Survey of tropical cut flower and foliage responses to irradiation. *Postharvest Biology and Technology*, 48, 264-271.

Sasa, A. & Samways, M.J. (2015). Arthropod assemblages associated with wild and cultivated indigenous *Proteas* in the Grabouw area, Cape Floristic Region. *African Entomology,* 23, 19-36.

Schirra, M., D'hallewin, G., Cabras, P., Angioni, A. & Garau, V.L. (1998). Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip treatments. *Journal of Agricultural and Food Chemistry*, 46, 1177-1180.

Schirra, M., D'Hallewin, G., Cabras, P., Angioni, A., Ben-Yehoshua, S. & Lurie, S. (2000). Chilling injury and residue uptake in cold-stored 'Star Ruby' grapefruit following thiabendazole and imazalil dip treatments at 20 and 50°C. *Postharvest Biology and Technology,* 20, 91-98.

Schirra, M. & Mulas, M. (1995). Improving storability of 'Tarocco' oranges by postharvest hot-dip fungicide treatments. *Postharvest Biology and Technology*, 6, 129-138.

Seaton, K.A. & Joyce, D.C. (1992). Gamma irradiation for insect disinfestation damages native Australian cut flowers. *Scientia Horticulturae,* 52,343-355.

Shelton, M., Walter, V., Brandl, D. & Mendez, V. (1996). The effects of refrigerated, controlled-atmosphere storage during marine shipment on insect mortality and cut-flower vase life. *HortTechnology,* 6, 247-250.

Simaika, J.P., Samways, M. & Vrdoljak, S.M. (2018). Species turnover in plants does not predict turnover in flower-visiting insects. *PeerJ*, 6, e6139. https://doi.org/10.7717/peerj.6139

Simpson, T., Bikoba, V., Tipping, C. & Mitcham, E.J. (2004). Effects of ethyl formate on fruit quality and target pest mortality for harvested strawberries. *Postharvest Biology and Technology,* 34, 313–319.

Simpson, T., Bikoba, V., Tipping, C. & Mitcham, E.J. (2007). Ethyl formate as a postharvest fumigant for selected pests of Table Grapes. *Journal of Economic Entomology,* 100, 1084-1090.

Slootweg, G. (2007). EcO2 Freshline voor de ontsmetting van bloemen en planten: verslag fase 1 en 2. PPO Glastuinbouw/Wageningen UR.

Son, Y., Chon, I., Neven, L. & Kim, Y. (2012). Controlled atmosphere and temperature treatments system to disinfest fruit moth, Carposina sasakii (Lepidoptera: Carposinidae) on apples. *Journal of Economic Entomology,* 105, 1540-1547.

Steenhuisen, S-L., Jürgens, A. & Johnson, S.D. (2013). Effects of volatile compounds emitted by *Protea* species (Proteaceae) on antennal electrophysiological responses and attraction of *Cetoniine* Beetles. *Journal of Chemical Ecology,* 39, 438–446.

Stephens, I.A., Jacobs, G. & Holcroft, D.M. (2001). Glucose prevents leaf blackening in 'Sylvia' proteas. *Postharvest Biology and Technology*, 23, 237-240.

van Doorn, W.G. (2001). Leaf blackening in *Protea* flowers: recent developments. *Acta Horticulturae* 545, 197–204.

van Epenhuijsen, C.W., Hedderley, D.I., Somerfield, K.G. & Brash, D.W. (2007). Efficacy of ethyl formate and ethyl acetate for the control of onion thrips (Thrips tabaci). *New Zealand Journal of Crop and Horticultural Science*, 35, 267-274.

van Kruistum, G., Verschoor, J. & Hoek, H. (2014). CATTS as a non-chemical pest and nematode control method in strawberry mother planting stock. *Journal of Berry Research*, 4, 29-35.

Viljoen, N. (2019). CFMA JMF Export, Estimates. Diamant Estate, Paarl. PowerPoint presentation Available: https://www.capeflorasa.co.za/news-publications/minutes-reports/ [2021, May 11].

Weller, G.L. & Graver, J.V.S. (1998). Cut flower disinfestation: assessment of replacement fumigants for methyl bromide. *Postharvest Biology and Technology*, 14, 325-333.

Whitehead, C.S. & de Swardt, G.H. (1982). Extraction and activity of polyphenoloxidase and peroxidase from senescing leaves of *Protea neriifoiia*. *South African Journal of Botany*, 1, 127-130.

Williams, P. (1998). Fumigating post-harvested wildflowers for export. Rural Industries Research and Development Corporation (RIRDC). RIRDC Publication No 98/40. RIRDC Project No DAV-90A. Australia.

Windell, N.E. (2012). Leaf blackening and the control thereof in selected *Protea* species and cultivars. Master's thesis. Stellenbosch: Stellenbosch University [Online]. Available: https://scholar.ac.za [2019, May 30]

Wright, M.G. (2003). Insect pests of Proteaceae: Assessment of predictions of new pests internationally, and management implications. *Acta Horticulturae,* 602, 167-171.

Wright, M.G. & Samways, M.J. (2000). Biogeography and species richness of endophagous insects associated with Proteaceae in South Africa. *African Journal of Ecology,* 38, 16-22.

Yun, S-H., Koo, H-N., Kim, H.K., Yang, J.O. & Kim, G-H. (2016). X-ray irradiation as a quarantine treatment for the control of six insect pests in cut flower boxes. *Journal of Asia-Pacific Entomology,* 19, 31-38.

Zhou, S., Criddle, R.S. & Mitcham, E.J. (2000). Metabolic response of *Platynota stultana* pupae to controlled atmospheres and its relation to insect mortality response. *Journal of Insect Physiology*, 46, 1375–1385.

2. Controlled Atmosphere Temperature Treatment System (CATTS) as a postharvest disinfestation technique against Proteaceae phytosanitary pests

2.1 Introduction

The Proteaceae family is indigenous to the Cape Floristic Region (CFR) of South Africa, with several genera that are endemic to Australia. The CFR includes the Cape Fold Belt Mountains and the southern and south-western coastal strip of the Western Cape Province of South Africa (Richardson *et al.,* 1989). The Proteaceae family comprises over 329 species in 14 genera (Goldblatt and Manning, 2002). The most economically important genera in South Africa are *Protea, Leucospermum* and *Leucadendron*. These genera are highly diverse in size and structural characteristics. However, a persistent characteristic of the fynbos vegetation is the sclerophyllous nature of the leaves. Generally, the leaves have pronounced cyanogenic ability, high concentrations of phenolic compounds and the presence of trichomes (Coetzee and Littlejohn, 2001). These traits have been attributed to minimizing insect infestation and other natural enemies (Wright and Giliomee, 1992; Giliomee, 2003).

The CFR was previously regarded as having low insect diversity, especially of herbivorous insect fauna. Giliomee (2003) further justified this assumption by proclaiming that regular fynbos fires decrease the numbers of insects, proportionally decreasing long-term biodiversity. However, Procheş and Cowling (2006) debated that there is a high insect diversity because of the high structural complexity typical of most *Protea* plants, which forms favourable microhabitats in the absence of enemies, allowing establishment of a wide variety of insect guilds. Among these is the endophagous guild, which is represented by leaf miners such as *Phyllocnistis* spp. (Lepidoptera: Gracillariidae), *Protaephagus capensis* Scoble (Lepidoptera: Incurvariidae) and *Eucosma* spp. (Lepidoptera: Tortricidae)), borers (*Helicoverpa armigera* Hübner; Lepidoptera: Noctuidae) and galling insects (*Psylla* spp.; Hemiptera: Psyllidae) (Malan, 2012). The second insect guild associated with Proteaceae is the sap sucker guild, which is mainly represented by various types of aphids, mealybugs and scale insects. Thirdly, the ectophagous guild insects, that feed specifically on the outward parts of the leaves, include *Afroleptops coetzeei* Oberprieler (Coleoptera: Curculionidae), *Epichoristodes acerbella* Walker (Lepidoptera: Torticidae) and *Bostra conspicualis* Warren (Lepidoptera: Pyralidae) (Malan, 2012*).* Lastly, independent guilds, such as mites and thrips (*Frankliniella occidentalis* Pergande; Thysanoptera: Thripidae) are additional important Proteaceae pests. The presence of insects

compromises overall productivity and product quality. For example, damage caused by the endophagous borer, *H. armigera* results in large holes in the bracts of *Protea* 'Cardinal' (*Protea eximia* x *Protea susannae*) as the plant matures, and in *Serruria florida,* thrips infestation often results in *Alternaria* infestations, leaf discoloration and flower dieback (Malan, 2012).

The presence of insects also results in rejection of inflorescences destined for export, or the application of a postharvest treatment such as methyl bromide fumigation. Fumigation potentially decreases flower quality, and rejections result in loss of consumer confidence (Seaton *et al.,* 1993). Methyl bromide has good penetration ability and minimal impact on flowers; however, this chemical is classified as a Class 1 ozone depleter by the Montreal Protocol (UNEP, 1992). This has led to limited availability and increased cost of methyl bromide. Therefore, sustainable alternative postharvest techniques, which will eliminate quarantine pests, while maintaining fresh produce quality, need to be investigated.

Postharvest treatment technology termed, the Controlled Atmosphere Temperature Treatment System (CATTS), combines the effects of a short exposure to high temperature and atmospheric stress, in the form of a low oxygen (1%) and high carbon dioxide (15%) environment (Neven and Mitcham, 1996). The effect of a reduced O_2 and elevated CO_2 environment on insect metabolism, leads to insect mortality. Both atmospheres negatively affect oxidative phosphorylation, but their target sites are different (Zhou *et al.*, 2000). According to Hochachka (1986), decreased O_2 concentration leads to a shortage of the substrate (O_2) required for respiratory metabolism. The insect responds by metabolic arrest due to limited O_2 consumption, which results in minimal ATP production. This triggers anaerobic metabolism to compensate for insufficient energy (Zhou *et al.,* 2000). Anaerobic metabolism depletes carbohydrate reserves, further decreasing available energy. Insufficient energy causes membrane pumps to fail, leading to K^+ and Na^+ influx and membrane depolarization. As a result, increasing Ca^{2+} influx inside the cytosol is reported. Elevated Ca^{2+} inside the cytosol activates phospholipid hydrolysis. The cell and mitochondrial membrane become more permeable, which lead to cell damage and eventually apoptosis. Elevated CO_2 affects oxidative phosphorylation by inhibiting and decreasing the capacity of respiratory enzymes (e.g., succinic dehydrogenase) (Zhou *et al.,* 2000). Additionally, elevated CO_2 contributes to cell damage by acidifying the cell, causing an accelerated increase in Ca^{2+} (Hochachka, 1986). In addition, increased temperature enhances the detrimental impact of reduced O_2 and elevated CO_2 by increasing metabolism demand, thereby leading to faster insect mortality (Zhou *et al.,* 2000).

CATTS treatments have been developed, in the United States of America, for a number of insect pests on fresh fruit that is produced for export. These include the codling moth, *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae), the Western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae) and the oriental fruit moth, *Grapholita molesta* Busck (Lepidoptera: Tortricidae) on apples, sweet cherries, peaches and nectarines (Neven and Mitcham, 1996; Neven and Rehfield-Ray, 2006a; 2006b; Neven *et al.,* 2006). In South Africa, the technology was simulated using a water-bath system to evaluate its efficacy on fruit phytosanitary pests, including the false codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) larvae, the grain chinch bug, *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae) and the banded fruit weevil, *Phlyctinus callosus* Schoenherr (Coleoptera: Curculionidae*)* (Johnson and Neven, 2010; Johnson and Neven, 2011). Recently, when Smit *et al.* (2018) evaluated the treatments for control of *M. diplopterus*, *P. callosus* and *T. leucotreta* larvae in Japanese plum cultivars, in a laboratory scale CATTS unit, effective control of the pests was achieved, however fruit quality was negatively impacted.

In the Netherlands, CATTS treatments are implemented on a commercial scale on strawberry planting stock, against tarsonemid mites (*Phytonemus pallidus)* and parasitic nematodes (*Meloidogyne hapla* and *Pratylenchus penetrans*) (van Kruistum *et al.,* 2012; 2014). Additionally, Slootweg (2007) in the Netherlands, investigated the treatment's potential on ornamentals, such as chrysanthemum, rose, gerbera, alstroemeria and carnation. The study found that CATTS treatments (1% O_2, 15% CO_2, at a set air temperature of 46°C) resulted in phytotoxic damage that varied across the cut flowers. Gerbera and roses exhibited leaf discoloration limping of stems, while treated freesias resulted in weak combs whilst florets failed to open. In contrast, chrysanthemums withstood the treatment, and treated stems did not differ from controls during vase life studies. Furthermore, Slootweg (2007) reported that elevated CO_2 is not the cause of the observed phytotoxic damage. Similar phytotoxic damage was evident even when CO_2 was not elevated, but O_2 was decreased to 1% and the set air temperature was held at 46°C. Treatments where the set air temperature was decreased to 35°C, and treatment time was increased from 3.5 to 8 hours performed better, and flower quality was maintained. Huysamer (2018) reported that South African Proteaceae cultivars, 'Safari Sunset' (*Leucadendron laureolum* x *salignum F*), 'Barbi' (*Protea magnifica*) and Australian Geraldton Wax (*Chamelaucium uncinatum*), withstood CATTS treatment (1% O_2, 15% CO_2 and temperature ramp from 30 and 35°C to set air temperatures of 40°C). The cultivar, 'Veld Fire' *(Leucospermum conocarpodendron* x *glabrum)* was not able to tolerate this treatment however, exhibiting style wilting and severe leaf discolouration. Increasing set air temperature to 50°C resulted in severe heat damage across all

treated Proteaceae cut flowers. ‘Safari Sunset’ foliage exhibited wilting and desiccation, *Protea* ‘Barbi’ exhibited leaf blackening and discolouration, and ‘Veldfire’ exhibited rapid and severe style wilting.

Although Huysamer (2018) effectively controlled the Western flower thrips, *F. occidentalis* and the protea itch mite, *Proctolaelaps vandenbergi* (Ascidae), using CATTS treatment conditions, with no detrimental impact on flower quality, these are only two of the economically important pests on Proteaceae cut flowers in South Africa. Other pests may require more intense treatments. The observed phytotoxic damage as described above (leaf blackening, style wilting and inflorescence discoloration), can potentially be ameliorated by pretreatments of cut flowers to enable development of this postharvest treatment technique.

Leaf blackening in Proteaceae has been reported to develop due to carbohydrate depletion (Hoffman *et al.,* 2018.) Sugar pulsing is known to either prevent or delay the onset of leaf blackening (Stephens *et al.,* 2001). Slootweg (2007) reported that pretreating cut flowers with sugar based Chrysal products and using Chrysal Clear as a vase agent, reduced damage induced by CATTS treatments. Therefore, incorporating sugar pulsing as a pretreatment could potentially alleviate CATTS-induced phytotoxic damage, and establish CATTS as a postharvest mitigation measure for South African Proteaceae cut flowers. The objective of this chapter is to assess insect (thrips) mortality, as well as, the potential of pretreatment pulsing in preventing CATTS-induced leaf blackening and other phytotoxicity damage, towards developing treatment parameters to disinfest commodities without reducing cut flower marketability.

2.2 Materials and Methods

2.2.1 Sources of research material

2.2.1.1 Insect procurement

Thrips infested flowers of *Leucospermum* cultivars ‘High Gold’ (*Leucospermum patersonii x cordifolium*) and ‘Jelena’ (*Leucospermum cuneiforme x cordifolium*) flowers were sourced from Pomona farms and Burglers Post, respectively, in Piketberg, Western Cape, South Africa. For each cultivar, 25 flower heads were individually placed in 1L ventilated plastic containers. The containers were ventilated by cutting parts of the lid and replacing the plastic with a finely meshed gauze. The bottom of the plastic container was lined with moist tissue paper to prevent stem desiccation during transport to the laboratory for trials.

2.2.1.2 Flower cultivar procurement

Export quality cut flowers were sourced from three farms within the Western Cape, South Africa. *Protea magnifica* 'Barbi', and *Leucospermum* 'Succession' (*Leucospermum lineare x cordifolium*), were sourced from Berghoff, Porterville. 'Goldstrike' (*Leucadendron strobolinum* x *laureloum*), stems were sourced from Arnelia, Hopefield. *Leucospermum* 'High Gold' (*Leucospermum cordifolium* x *patersonii*) was sourced from Pomona, Piketberg. The cultivars were selected based on seasonal availability and increasing demand in the export market (Cape Flora statistics, 2019). Additionally, *Leucospermum* 'High Gold' was selected because of a high western flower thrips, *F. occidentalis* infestation. Flowers were transported from the farms to the Cape Town International airport (DSV Sky Services), at 4 °C, where after it was collected and transported to the laboratory within 24 hours of harvesting.

2.2.2 CATTS treatments

CATTS treatments were done in a laboratory-scale CATTS chamber manufactured by Techni-Systems (USA). The chamber is based in the Department of Conservation of Ecology and Entomology, Stellenbosch University. This forced hot air chamber system has computerised controllers for the regulation of temperature and dew point as described by Neven and Mitcham (1996). The atmospheric conditions inside the chamber are controlled by systemic injection of N_2, O_2 and CO_2 from pressurised containers. The chamber has humidifying micro-misting nozzles and a heater element to increase air temperature (Smit, 2019).

2.2.2.1 Insects

The moist tissue paper in the ventilated containers containing the flower heads, was replaced with a sheet of white paper, and flowers were inspected for living thrips by gently shaking the flower head. Temperature logging iButtons (Thermochron; Maxim Integrated Products) were placed inside the flower heads during the CATTS treatment to record temperature changes inside the containers. The containers where then placed in open crates in the CATTS units and subjected to a CATTS treatment with an atmospheric condition of 1% O_2, 15% CO_2 in N_2, and the temperature was ramped at a rate of 35°C/hr from 23°C to a target temperature of 40°C with a 15 minute soaking period at 40°C, under a relative humidity of 80%. Total CATTS treatment time was 44 minutes.

2.2.2.2 Flowers

Flower stem bottoms were cut at a 45° angle and flowers were randomly divided to five different treatments (T1 – T5), as described in Table 2.1. Each treatment was replicated three times per cultivar, and each replicate consisted of 6 stems. The controls were held in tap water, at room temperature (± 25°C) and not subjected to CATTS treatments (T1). Flowers that received no pretreatment or hydrating during CATTS comprised T2. Pretreatment pulsing treatments were conducted by holding the flower stems in 10 ml/L Prof 3 (Chrysal Professional 3 vase and foam solution) for 1 hour (T3 and T5), and the hydrating solution contained 5 ml/L Prof 2 (Chrysal Professional 2 transport and display solution) (T4 and T5). The atmospheric composition for treatments was 1% O_2 and 15% CO_2 in N_2, with relative humidity maintained at 80%. During CATTS treatments the temperature inside the chamber was ramped up at 35°C/hour from 23°C to 40°C, with a 15 min soaking period at the target temperature. Total CATTS treatment time was 44 minutes.

Table 2.1: Combination of pulsing, hydrate and CATTS treatments applied to Proteaceae stems. Atmospheric composition in the CATTS unit was: 1% O_2 and 15% CO_2 in N_2 and relative humidity of 80% was maintained.

Treatment	Treatment description	
T1	Controls – not pulsed and not subjected to CATTS	
T2	CATTS only	**CATTS treatments** Temperature ramp rate of 35°C/hour from 23°C to 40°C with a 15 min soaking period at 40°C
T3	Pulse and CATTS	
T4	Hydrate during CATTS	
T5	Pulse and hydrate during CATTS	

Post CATTS treatment, stems were immediately assessed for marketability and vase life suitability during a 14-day evaluation period using a 2-point scoring system (Table 2.2). Inflorescence and foliage were evaluated independently and summed to achieve an overall score of 0 to 18. This first trial comprised the "immediately after CATTS" quality evaluation. Trials were also conducted to evaluate flower quality after CATTS-treated stems underwent freight simulation periods for air and sea freight. In these trials' treatments T1 – T5 were repeated, as described above, for all the cultivars, and after removal from the CATTS unit, stored at 2°C under normal atmosphere for 3 days (air freight) or 21 days (sea freight). Post air and sea freight, 'Barbi' stems vase life was evaluated for 12 and 10 days respectively. For 'Succession', 'High Gold' and 'Goldstrike' stems were evaluated for 12 days post both air and sea freight simulation.

2.2.3 Posttreatment evaluations

2.2.3.1 Insect mortality assessment

After treatments, flowers were individually shaken against a white paper to dislodge any thrips present on flowers. Thrips were scored as either alive (moving without being prodded with blunt forceps), moribund (movement when prodded with blunt forceps) or dead. Alive and moribund insects were held for 24 hours at room temperature in ventilated containers, then assessed again. The treated thrips were sent to the ARC INFRUITEC-NIETVOORBIJ for morphological identification.

2.2.3.2 Flower quality evaluation

Flower quality was visually evaluated for phytotoxic damage (discoloration, leaf blackening, style wilting and collapsing) using a 2-point scoring system developed by Huysamer (2018). During the 14-day evaluation period, stems were kept in a vase solution containing 10 ml/L Prof 3, at room temperature (25 °C ±2 °C). The quality scores were recorded every second day of vase life (i.e., Day 2, 4, 6, 8, 10, 12 and 14). Inflorescences and foliage were evaluated independently and summed to achieve an overall score of 0 to 18. An overall flower score of zero meant that there was no discernible damage, a score of 4 was established as the marketability limit (the product is not saleable) and 8 was selected as the vase life limit (the flower is severely damage and no longer in an appreciable state).

Table 2.2: Rating system for evaluation of phytotoxic damage induced by postharvest CATTS treatment on several Proteaceae cultivars (Huysamer, 2018).

Rating	Inflorescence score			Foliage score
	Protea	***Leucospermum***	***Leucadendron***	
0	No damage to bracts	≤25% styles reflexed	No discolouration or damage	No damage to stem and leaves
1	Slight discoloration or very minor flaws	[26-50] % styles reflexed	10% discolouration or damage	Few leaves have slight damage, good appearance
2	Some discolouration, still marketable	[51-75] % styles reflexed	30% discolouration or damage	More leaves with slight damage, generally good appearance, still marketable
3	Discoloration expanding (≈10%); not marketable, still suitable for vase	[76-90] % styles reflexed	40% discolouration or damage	Many leaves with slight damage or few leaves with major damage, still with good appearance
4	More discoloration (10%- 20%); vase life questionable	[91-100] % styles reflexed	50% discolouration or damage	More leaves with major damage, appearance fair; vase life questionable
5	Increased discoloration (≈ 30%); definitely not suitable for vase	≤10% styles collapsed	60% discolouration or damage	Most leaves with some damage, appearance fair to poor; not suitable for vase
6	Some discoloration throughout (≈ 50%)	[11-25] % styles collapsed	70% discolouration or damage	Most leaves with major damage, appearance poor
7	Much discoloration throughout (≈ 70%)	[26-50] % styles collapsed	80% discolouration or damage	Much of foliage damaged or dead (≈ 70%), appearance very poor
8	Major discoloration throughout (≈ 90%)	[51-75] % styles collapsed	90% discolouration or damage	Most foliage dead (≈ 90%), few undamaged areas
9	Entire flower discoloured	[76-100%] styles collapsed	100% discolouration or damage	Foliage dead

2.2.4 Statistical analysis

Mean overall inflorescence scores were statistically analysed using STATISTICA 13.2 software (Dell Statistica data analysis software system, Dell Inc). To evaluate vase life posttreatments, two-way analysis of variance (ANOVA) and mixed model ANOVA tests were performed on mean overall inflorescence scores per evaluation over time (days). Means were separated by the Fisher's least significant difference ($P \leq 0.05$).

2.3 Results

2.3.1 Insect mortality

Assessing insects immediately after CATTS treatment, mortality was very low. Thrips were actively moving inside the containers. Insects from the Coleoptera order which were incidentally included in the treatments were also alive and active inside the containers. Counting the number of alive insects proved that more than 50% of the encountered insects were alive.

2.3.2 Thrips identification

For both *Leucospermum* cultivars, the thrips species in highest abundance was the western flower thrips, (WFT) (*Frankliniella occidentalis*) (Fig 2.1). Four different species of thrips were identified on 'High Gold' and included the WFT, common blossom thrips (*Frankliniella schultzei*), onion thrips (*Thrips tabaci*) and predatory thrips (*Aeolothrips* species), while *Leucospermum* 'Jelena' had only the WFT, of those that could be identified morphologically. 'Jelena' had many immature thrips, which could not be identified easily on a morphological basis.

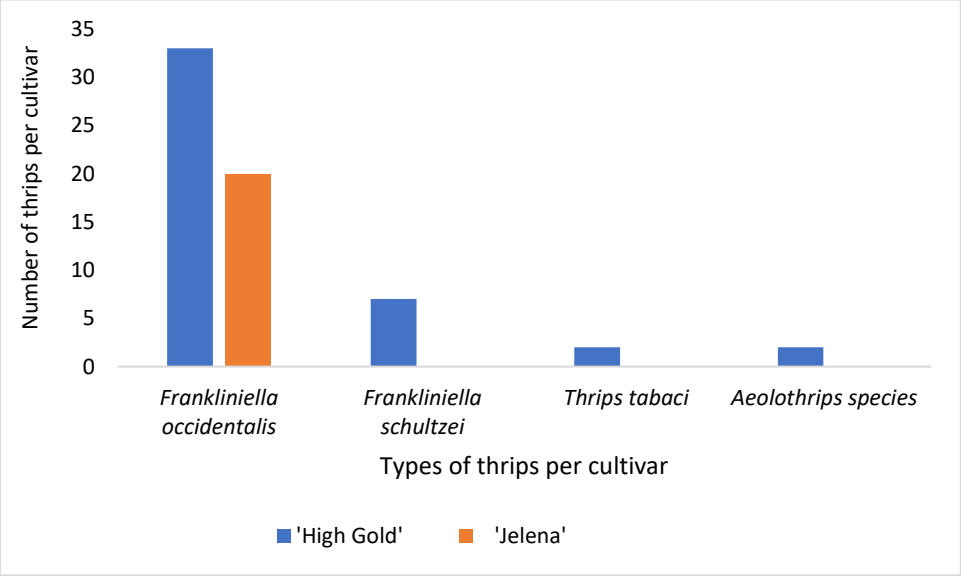


Figure 2:1: Frequency of thrips species morphologically identified on cultivated *Leucospermum patersonii x cordifolium* 'High Gold' and *Leucospermum cuneiforme x cordifolium* 'Jelena' post CATTS treatment.

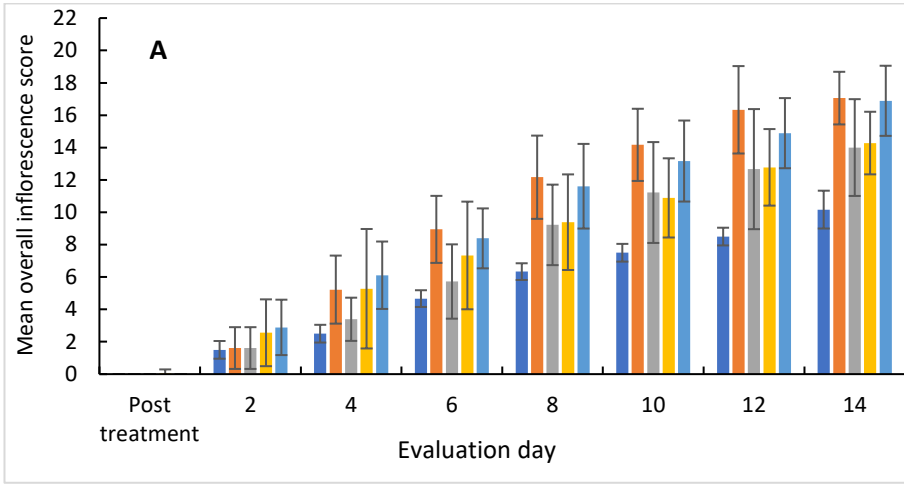
2.3.3 *Protea magnifica* 'Barbi'

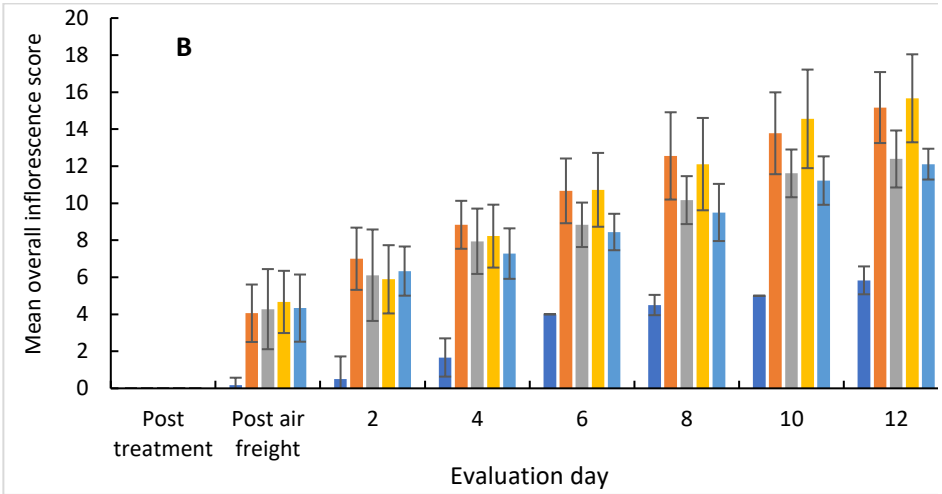
In 'Barbi' stems treated with CATTS at a target temperature of 40°C, evaluated immediately and then for a period of 14 days, pulsing and /or hydrating stems to improve posttreatment quality did significantly reduce the mean overall inflorescence score to less than that of T2. However, was not able to maintain flower quality similar to that of the untreated controls. (Fig 2.2A). Additionally, pulsing and hydrating during CATTS treatment resulted in inflorescence bracts being fully reflexed, causing bracts to collapse prematurely. During the first two evaluations (day 0 and day 2), there were no significant differences between untreated stems (T1) and stems which had been subjected to CATTS treatment without pretreatments (T2) ($p > 0.01$, $p = 1$). However, on day 4, significant differences were observed ($p < 0.01$), and T2 stems breached the limit for marketability with a mean overall inflorescence score of 5.22 ± 2.10 and exceeded the vase life limit by day 6, with mean overall inflorescence score of 8.94 ± 2.10. Up to and including day 4 of the evaluation period there were no statistically significant differences between T2 stems and stems which were pulsed prior to CATTS treatment (T3). This was also true for stems which were hydrated during CATTS treatment (T4). Thereafter, significant differences were observed ($p < 0.01$). In comparison, phytotoxic damage was worse on T2 stems from day 6 to day 12, and it manifested in the form of leaf blackening and inflorescence wilting and intense discoloration. Treatment 5 stems, which were pre-treated with pulsing and hydrated during treatment, also had mean overall inflorescence scores higher than T3 and T4 but still below T2 from day 6 to 14 exhibiting minimal leaf blackening. Overall, pulsing and /or hydrating stems to improve posttreatment quality, did significantly reduce the mean overall inflorescence score to less than that of T2. However, was not able to maintain flower quality like that of the untreated controls.

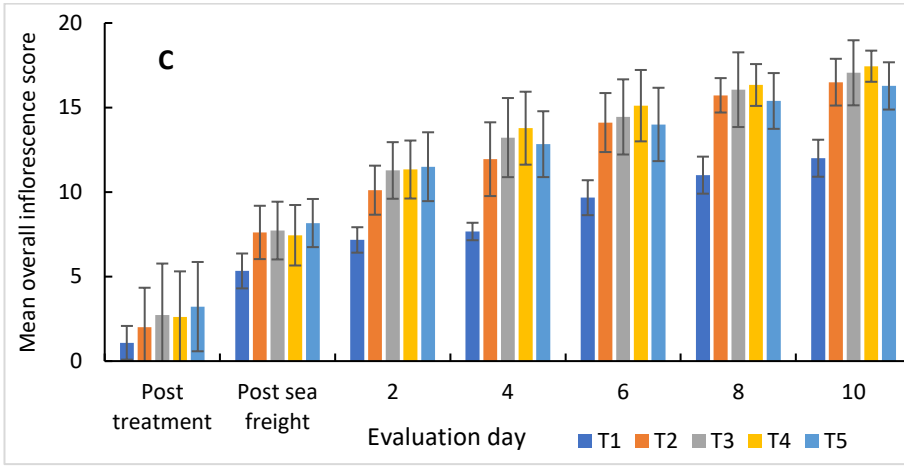
Phytotoxic damage to all CATTS-treated 'Barbi' stems after air freight simulated storage for 3 days at 2°C, was severe, and had breached the marketability limit by the first evaluation post air freight (Fig 2.2B), while untreated controls reached the marketability limit between day 4 and day 6 of vase life evaluation. Pulsing alone and pulsing and hydrating did significantly decrease phytotoxic damage on CATTS treated stems. However, the treated stems were not comparable to untreated controls. Immediately out of storage, stems showed distinct signs of injury, which worsened during vase life studies. Leaf blackening became pronounced on day 4 of vase life studies, especially on T2 and T4 stems. As a result, T2 and T4, on day 4, exhibited mean overall inflorescence score of 8.83 ± 1.29 and 8.22 ± 1.67. Therefore, breaching the vase limit. In comparison, stems subjected to T3 and T5 exhibited minimal leaf blackening and however, breached the vase limit two days later (day 6) with mean overall

inflorescence scores of 8.83 ± 1.20 (T3) and 8.44 ± 0.98 (T5). Inflorescence quality was similar across all the treatments. Pulsing and/or hydrating did not prevent the CATTS-induced phytotoxic damage to inflorescences. This damage was aggravated by air freight simulated storage for 3 days at 2°C.

When stems were evaluated immediately after sea freight simulation (storage at 2°C for 21 days), all the stems, including the untreated controls, had breached the marketability limit. Treated stems, T2, T3 and T4, were already close to the vase life limit, with mean overall flower scores of 7.61 ± 1.58, 7.22 ±1.71 and 7.44 ± 1.78, respectively (Fig. 2.2C), while stems which were subjected to pretreatment pulsing and hydrating during CATTS treatment (T5), had just breached the vase life limit, (8.17 ± 4.42). Phytotoxic damage on the inflorescences were evident, while leaf blackening was pronounced and worsened during vase life studies







	Two-way ANOVA table		
Source	A	B	C
Treatment	0.02	<0.01	<0.01
Time	<0.01	<0.01	<0.01
Treatment x Time	<0.01	<0.01	0.01

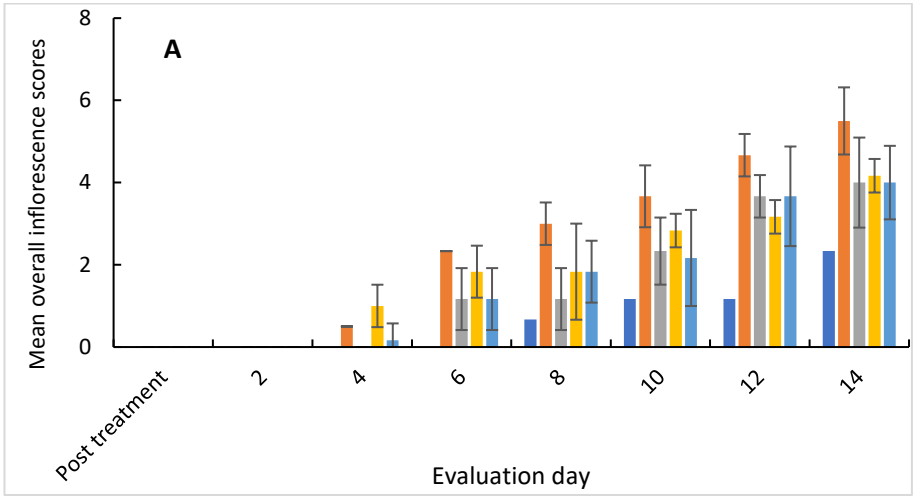
Figure 2:2: Mean overall inflorescence and foliage scores of *Protea magnifica* 'Barbi' stems treated with CATTS at a target temperature of 40°C and assessed for phytotoxicity immediately after CATTS treatment (A), after storage at 2°C for 3 days to simulate air freight (B) and after storage at 2°C for 21 days to simulate sea freight (C). CATTS target temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C. Treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Vertical error bars indicate the standard deviations of the mean for each data point. Treatments were; Control (T1), CATTS only (T2), Pulse & CATTS (T3), Hydrate during CATTS (T4) and Pulse and hydrate during CATTS (T5)

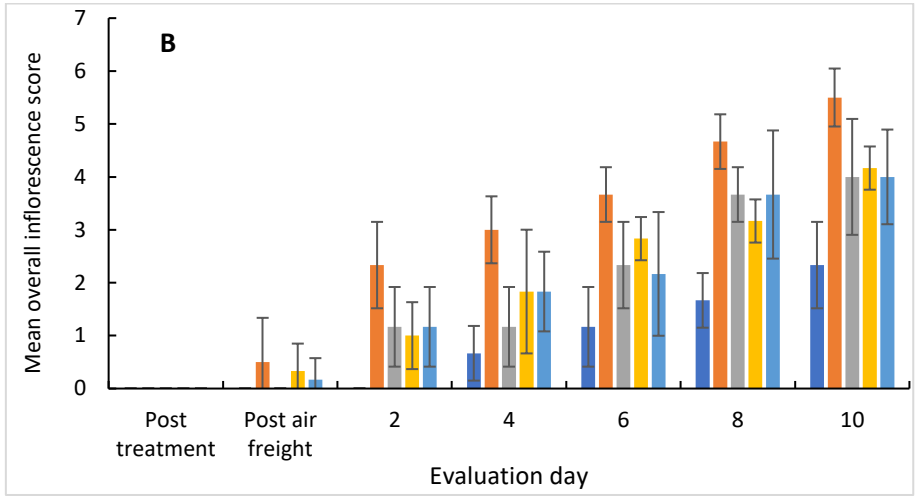
2.3.4 *Leucadendron salignum* 'Goldstrike'

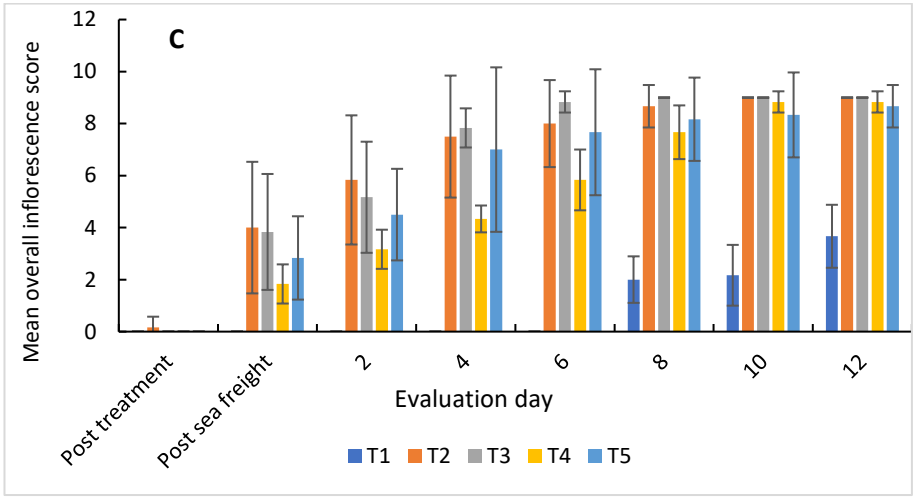
Leucadendron salignum 'Goldstrike' stems which were subjected to CATTS at a target temperature of 40°C and assessed for phytotoxic damage post CATTS treatment for 14 days, showed that pretreatment with pulsing and/or hydrating during CATTS treatment significantly improved the overall quality and the vase life duration (Fig 2.3A). The quality of stems which were neither pulsed nor hydrated during CATTS treatment (T2) deteriorated faster during vase life studies. Although T2 stems maintained quality suitable for both the market and vase until day 10 of evaluations, phytotoxic damage was evident and manifested as foliage wilting. In comparison, pre-treated stems (T3, T4 and T5) maintained marketable quality until the end of vase life studies (day 14), exhibiting mean overall scores of 4.0 ± 1.09 (T3), 4. 17 ± 0.40 (T4) and 4. 0 ± 0.89 (T5).

Evaluations of CATTS-treated 'Goldstrike' stems after air freight simulated storage for 3 days at 2°C, also showed that pretreatment with pulsing and/or hydrating during CATTS treatment does enhance the overall quality of the stems, and inhibits foliage wilting and chilling injuries, as these were more prevalent in T2 stems, as opposed to pre-treated stems. As a result of phytotoxic damage, T2 stems breached the marketability limit on day 10 with a mean overall inflorescence score of 4.67 ± 0.52 (Fig 2.3B). Stems which were pre-treated (T3, T4 and T5) maintained good foliage quality, exhibiting mean overall scores below marketability up until day 10. By the conclusion of vase life evaluations on day 10, the pre-treated stems had just reached the marketability limit with mean overall flower scores of 4.00 ± 1.10 (T3), 4.16 ± 0.41 (T4) and 4.00 ± 0.89 (T5).

Subjecting CATTS-treated 'Goldstrike' stems to simulated sea freight storage (21 days at 2°C), resulted in severe phytotoxic damage, as when stems were evaluated immediately after simulation, T2 stems had already reached the marketability limit with a mean overall score of 4.00 ± 2.53 (Fig 2.3C), followed closely by T3 and T5 stems, which were no longer marketable two days later. Phytotoxic damage on treated 'Goldstrike' manifested as browning of the involucral foliage (Fig. 2.4B). In this trial, the overall foliage damage was exasperated by a fungal mycelium infection (*Trichoderma*) (Fig. 2.4C).







	Two-way ANOVA table		
Source	A	B	C
Treatment	<0.01	<0.01	0.07
Time	<0.01	<0.01	<0.01
Treatment x Time	<0.01	<0.01	<0.01

Figure 2:3: Mean overall inflorescence and foliage scores of *Leucadendron salignum* 'Goldstrike' stems treated with CATTS at a target temperature of 40°C and assessed for phytotoxicity immediately after CATTS treatment (A), after storage at 2°C for 3 days to simulate air freight (B) and after storage at 2°C for 21 days to simulate sea freight (C). CATTS target temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C. Treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Vertical error bars indicate the standard deviations of the mean for each data point. Treatments were; Control (T1); CATTS only (T2); Pulse & CATTS (T3); Hydrate during CATTS (T4); Pulse and hydrate during CATTS (T5).

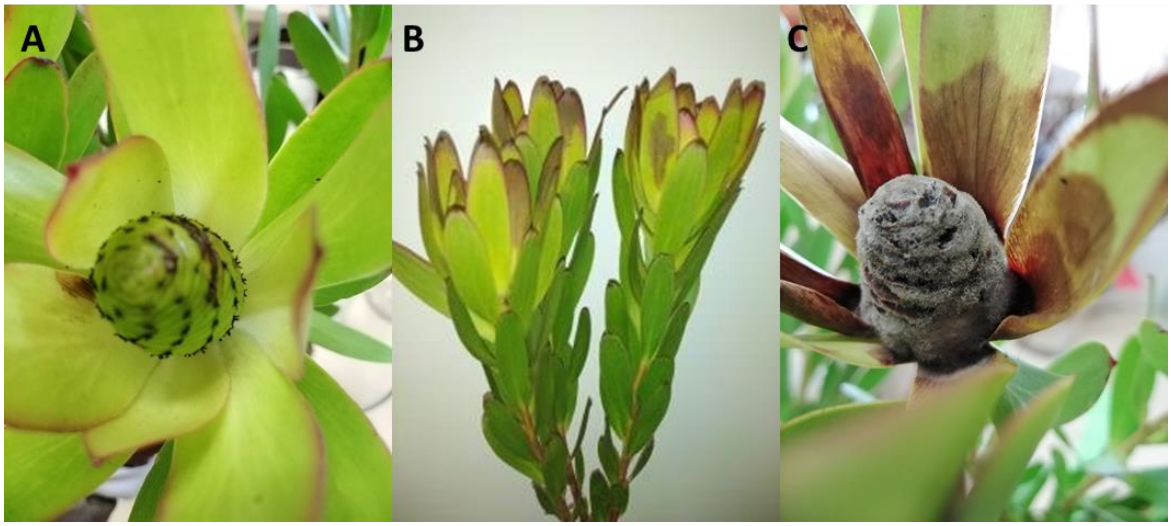


Figure 2:4: *Leucadendron salignum* 'Goldstrike' subjected to CATTS treatment and sea freight simulation for 21 days at 2°C under normal atmospheric conditions. CATTS target temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C. Treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Control stems with minor foliage and cone discolouration (A), involucral foliage discolouration on day 2 of vase life studies (B) and fungal mycelium growth surrounding treated cones during vase life studies (C).

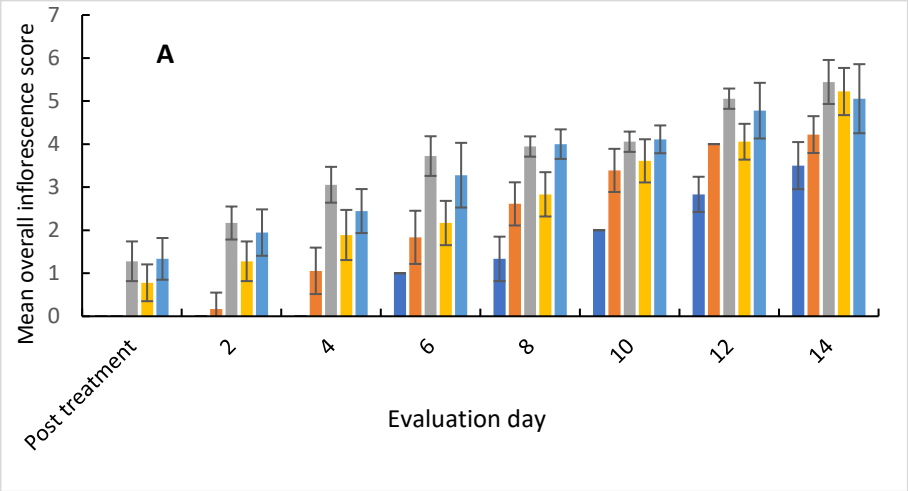
2.3.5 *Leucospermum lineare x cordifolium* 'Succession'

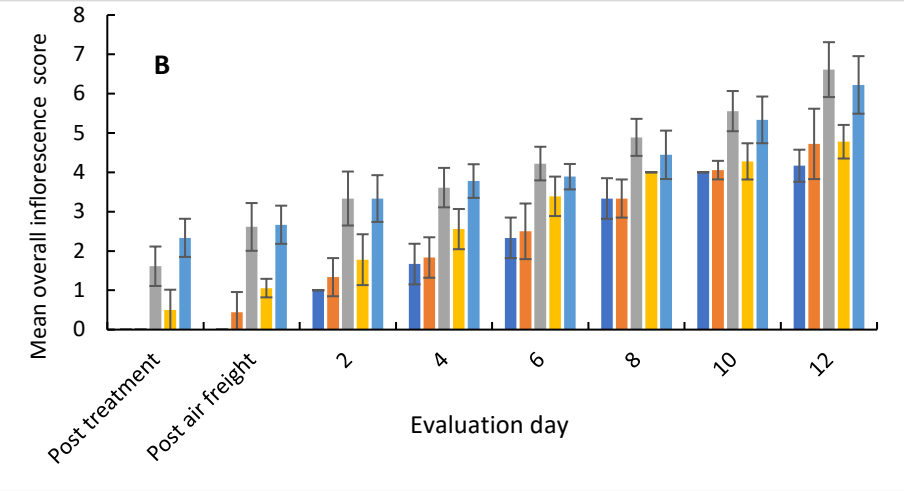
Trials on 'Succession' stems which were subjected to CATTS treatment and evaluated immediately after, indicated that pretreatment with pulsing and/or hydrating during treatment, negatively impacts the quality of the stems. Untreated controls (T1) and CATTS-only treated stems (T2) had comparable flower scores during the first two evaluations (day 0 and day 2), while pre-treated stems (T3, T4, T5) had significantly higher scores posttreatment, and throughout the evaluation period (Fig. 2.5A). The observed higher scores were a result of premature reflexion. Mean overall flower scores of stems subjected to T2 were significantly different from stems which were hydrated during CATTS (T4) until day 6 of vase life studies. However, from day 8 to end of vase life studies, they were not significantly different ($p > 0.01$). Both T2 and T4 breached the marketability limit by day 12, with mean overall inflorescence score of 4.00 ± 0.00 and 4.06 ± 0.42 respectively. Treatment 3 and 5 were not significantly different from each other ($p > 0.01$), from first day of evaluation to the end of vase life studies. The stems subjected to T3 and T5 were unmarketable from day 10, with mean overall inflorescence score of 4.05 ± 0.23, and 4.11 ± 0.32 respectively.

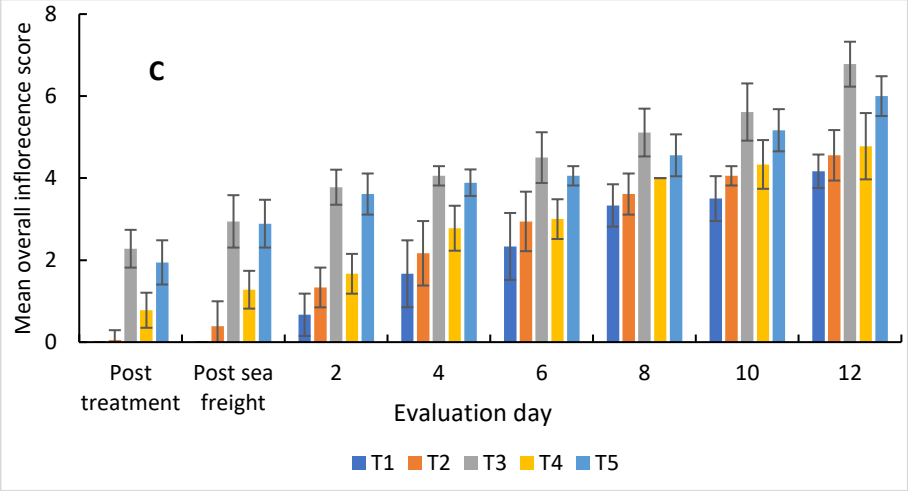
Evaluating 'Succession' stems which were subjected to CATTS treatment and air freight simulations studies also indicated that pretreatment with a pulse and/or hydrating during CATTS aggravated the quality of CATTS-treated stems by causing the styles to reflect prematurely and thus resulting in

mechanical damage during packaging and storage (Fig. 2.5B). Throughout the evaluation period, when treatments were compared to each other, results showed that untreated control stems (T1) were not significantly different ($p > 0.01$) from CATTS-only stems (T2). Similarly, T3 and T5 stems were not significantly different ($p > 0.01$) from each other. Treatment 4 stems (hydrated during CATTS) were significantly different ($p < 0.01$) from all the treatments. The observed differences were not due to phytotoxic damage but the rate which the styles reflexed. T4 stems did not reflex to the same extent to be comparable with T3 and T5. The hydration during treatments resulted in the stems being more reflexed compared to T1 and T2 stems. Stems subjected to pulsing before CATTS treatment (T3), matured earlier, by being fully reflexed by day 6. As a result, the marketability limit was breached on day 6, with average flower scores of 4.2 ± 0.42. Treatment 5 stems (pulsed and hydrated during CATTS treatment) breached the marketability on day 8, with a mean overall inflorescence score of 4.44 ± 0. 61. The ascending order which styles reflexed was T1, T2, T4, T3 and T5.

When 'Succession' stems were evaluated immediately after CATTS treatment and directly after sea freight simulation, there were no significant differences ($p > 0.01$) between untreated control stems (T1) and stems which had been subjected to CATTS only treatment (T2) (Fig. 2.5C). Similarly, there were no significant differences between T3 and T5 stems. Stems which were subjected to T4 significantly differed ($p < 0.01$) from all the other treatments. The observed significant differences as ascribed to 50 – 75% styles of the stems having reflexed after subjection to T4, resulted in a lower mean overall inflorescence score of 1.28 ± 0.46, while in stems which were subjected to T3 and T5, more than 75% styles had reflexed. As a result, immediately after sea freight simulation T3 and T5 had mean overall flower scores of 2.94 ± 0.64 and 2.88 ± 0.58 repetitively. The high mean overall inflorescence score was due to styles being broken during storage. Additionally, by day 2 of vase life studies, styles which were broken were evidently not recovering or reflexing in accordance with other styles (see Fig. 2.6B for example of damage). Treatment 3 stems were unmarketable from day 4 with mean overall inflorescence score of 4.06 ± 0.24. Two days later (day 6), T5 stems breached the marketability limit with a mean overall inflorescence score of 4.06 ± 0.24. Mechanically damaged styles (broken styles) failed to reflex, therefore contributing towards T3 and T5 stems breaching the marketability limit. Additionally, across all the treatments, (including untreated controls) foliage discolouration was evident. However, was more pronounced on all the stems which were subjected to CATTS treatment (as seen in Fig.2.6B)







Two-way ANOVA table			
Source	A	B	C
Treatment	<0.01	<0.01	<0.01
Time	<0.01	<0.01	<0.01
Treatment x Time	<0.01	<0.01	<0.01

Figure 2:5: *Leucospermum lineare x cordifolium* 'Succession' stems treated with CATTS at a target temperature of 40°C and assessed for phytotoxicity immediately after CATTS treatment (A), after 3 days storage at 2°C to simulate air freight (B) and after 21 days storage at 2°C to simulate sea freight (C). CATTS targeted temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C. Treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Vertical error bars indicate the standard deviations of the mean for each data point. Treatments were; Control (T1), CATTS only (T2), Pulse & CATTS (T3), Hydrate during CATTS (T4) and Pulse and hydrate during CATTS (T5).

Figure 2:6: Mechanical damage in *Leucospermum lineare x cordifolium* 'Succession' subjected to CATTS treatment and sea freight simulation for 21 days dry storage at 2°C. Control stem exhibiting no discernible damage post sea freight simulation (A) and irreversible mechanical damage and wilting on stems which had reflexed fully during CATTS treatments (B).

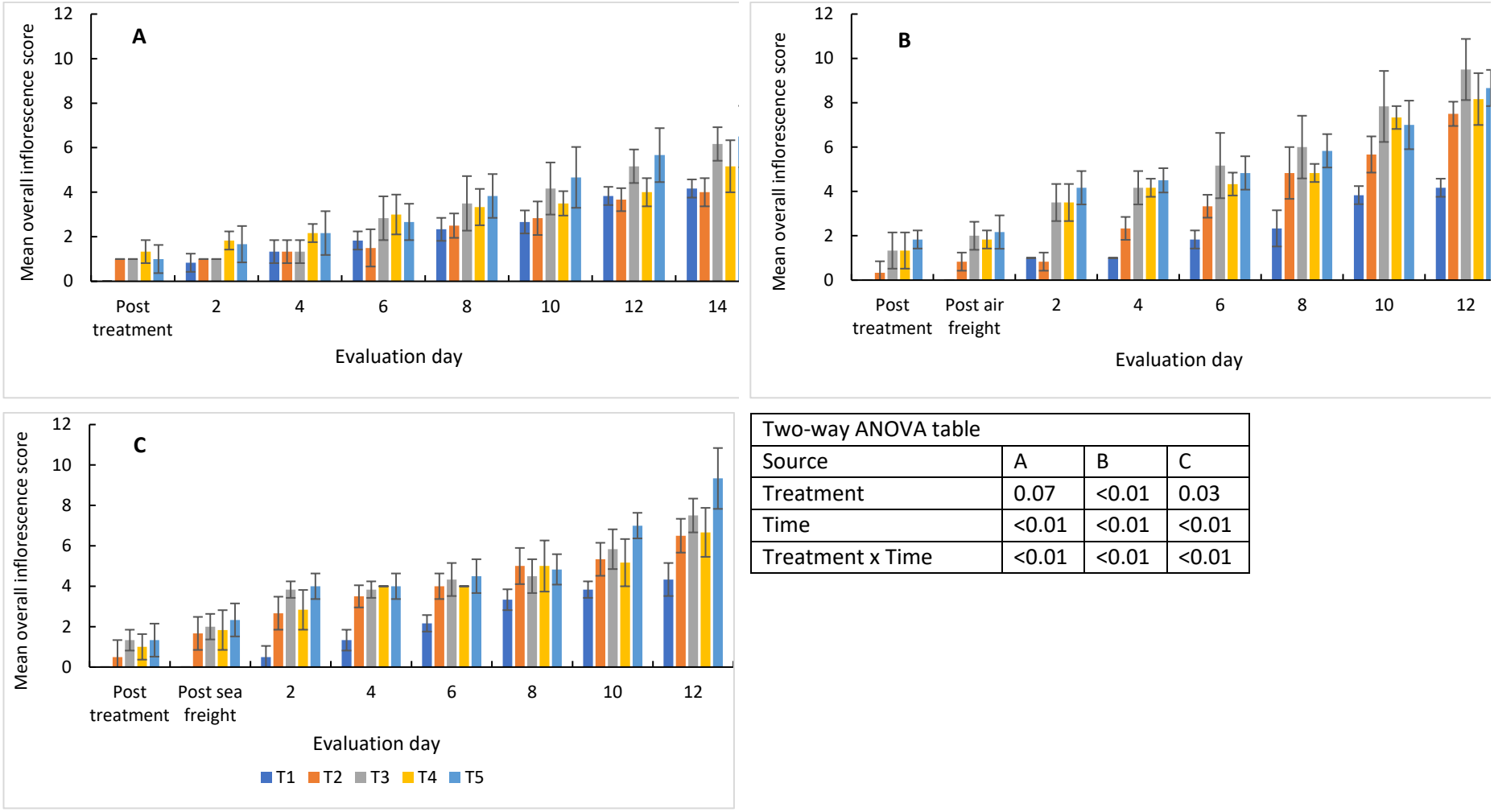
2.3.6 *Leucospermum patersonii x cordifolium* 'High Gold'

The mean overall flower scores of 'High Gold' stems subjected to CATTS treatment and evaluated for 14 days, differed significantly ($p < 0.01$) throughout vase life studies (Fig. 2.7A). Immediately after treatment, stems which were subjected to CATTS without pretreatment (T2) were significantly different ($p < 0.01$) from the untreated control stems, exhibiting mean overall score of 1.00 ± 0.00. The observed differences were because T2 stems exhibited minor wilting, however they recovered during vase life. As a result, from day 2 until the end of vase life studies there were no significant differences ($p > 0.01$) between untreated controls and T2 stems. Within the first 4 days of vase life studies, there were no significant differences ($p > 0.01$) between T2 and stems which were pulsed before CATTS treatments stems (T3). However, differences were observed from day 6 because 76-90% styles of stems which were subjected to T3 had reflexed by then. Similarly, there were no significant differences between stems which were hydrated during CATTS treatment (T4) and stems which were pulsed and hydrated during CATTS treatment (T5) until day 8 of vase life studies. Thereafter, the stems differed significantly ($p <0.01$) from each other. No damage was discernible on 'High Gold' styles, with significant differences that were mainly due to the rate at which styles reflexed. Treatment 3 and 5 stems reflexed prematurely and therefore reached market limit on day 8, exhibiting

mean overall scores of 3.94 ± 0.24 and 4.00 ± 0.34 respectively. However, none of the treated stems breached the vase limit (mean overall score ≥ 8) by conclusion of the study (day 14).

Post air freight simulated storage for 3 days at 2°C, stems which were subjected to CATTS without pre-treatment (T2), were not significantly different from untreated control stems (Fig. 2.7B). Immediately after CATTS treatment, T2 stems were not significantly different ($p > 0.01$) from treatments 3, and 4. After air freight simulated storage, T2 stems differed significantly from 3, 4 and 5 while these treatments (3, 4 and 5) were not significantly different ($p > 0.01$) from each other. The significant difference was a result of mechanical damage which was evident on stems which were pulsed and/or hydrated during treatments (treatments 3, 4 and 5). On day 2, significant differences were observed within the treatments until the end of vase life studies. This led to these T5 stems breaching the marketability limit on day 2 of vase life studies with a mean overall inflorescence score of 4.17 ± 0.75 and subsequently T3 and T4 stems breached the marketability limits on day 4 with mean overall inflorescence score of 4.17 ± 0.75 and 4.17 ± 0.40 respectively. Treatment T2 styles matured and reflexed slowly, and as a result, the stems breached the market limit on day 8 with a mean overall inflorescence score of 4.83 ± 1.17.

When 'High Gold' stems were evaluated directly after simulated sea freight storage, all treatments, T2, T3, T4 and T5 stems differed significantly from the untreated control stems (not subjected to CATTS) (Fig. 2.7C). However, there were no significant differences within the CATTS treated stems ($p > 0.01$). Additionally, post sea freight, both untreated controls and CATTS treated 'High Gold' foliage exhibited foliage discoloration and pronounced tip desiccation. Mechanical damage observed in stems which were subjected to air freight simulation were similarly evident on stems which were subjected to pulsing and/or hydrating during CATTS treatment and sea freight simulation storage. The damage was more pronounced on stems which were subjected to T5 treatments. As a result, T5 stems were unmarketable from day 2 with mean overall inflorescence score of 4.00 ± 0.63, while T4 breached the market limit on day 4 exhibiting mean overall inflorescence score of 4.00 ± 0. 00 and T3 breached the market limit on 6, with a mean overall inflorescence score of 4.33 ± 0.82. On day 12 of vase life studies, T5 treated stems exhibited mean overall inflorescence score of 9.33 ± 1.50 thus breaching the vase limit.



Two-way ANOVA table			
Source	A	B	C
Treatment	0.07	<0.01	0.03
Time	<0.01	<0.01	<0.01
Treatment x Time	<0.01	<0.01	<0.01

Figure 2:7: *Leucospermum patersonii x cordifolium* 'High Gold' stems treated with CATTS at a target temperature of 40°C and assessed for phytotoxicity immediately after CATTS treatment (A), after 3 days storage at 2°C to simulate air freight (B) and after 21 days s storage at 2°C to simulate sea freight (C). CATTS targeted temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C. Treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Vertical error bars indicate the standard deviations of the mean for each data point. Treatments were; Control (T1), CATTS only (T2), Pulse & CATTS (T3), Hydrate during CATTS (T4) and Pulse and hydrate during CATTS (T5).

2.4 Discussion

The present study investigated the mortality of commonly encountered insects (thrips) on *Leucospermum* cultivars 'High Gold' and 'Jelena' which were dominated by the western flower thrips, *Frankliniella occidentalis* (WFT). The mortality percentage was relatively low, and thus CATTS treatments were not considered effective. These results were contrary to Huysamer (2018) who reported that 100% mortality was achieved within 24 hours of subjecting the WFT and protea itch mite, to the same CATTS treatments used in the current study. Li *et al.* (2011) also reported to have achieved 100% mortality after subjecting WFT to 41°C under normal atmospheric conditions for 2 hours. In the current study, the temperature recorded by temperature logging iButtons, which were placed inside the *Protea* inflorescence during CATTS treatment, did not rise above 25°C (results not shown). To achieve high mortality at a such low temperature, longer treatment time and higher CO_2 concentrations are required. Seki and Murai (2012) reported that controlled atmosphere treatment using 60% $C0_2$ at 25°C requires approximately 16 and 20 hours treatment time to achieve 100% mortality of WFT and *T. tabaci* respectively. Additionally, previous studies have shown that thermal stress (> 26°C) induces the activation of heat shock proteins (*Fo-HPS70, Fo-HSP28.9* and *Fo-HSP40*) on WFT (Li *et al.,* 2011; Wang *et al.,* 2014; Jing *et al.,* 2018). The activation of heat shock proteins prevents denaturation of proteins and cell damage in general and therefore, increases thermal tolerance and decreases mortality.

The feasibility of CATTS as a postharvest disinfestation technique is dependent on the marketability of the cut flowers posttreatment. Results from the present study show that when 'Barbi' stems are pulsed with 10 ml/L Prof 3 (Chrysal Professional 3 vase and foam solution) and/or hydrated with 5 ml/L Prof 2 (Chrysal Professional 2 transport and display solution) during CATTS treatment phytotoxic damage is reduced. However, hydration did not significantly reduce phytotoxic damage when 'Barbi' stems were subjected to air freight, the mean overall inflorescence score was not significantly different from stems which were subjected to CATTS treatment without pretreatment. Post sea freight simulation, both pulsing and hydrating was found to be not effective in inhibiting damage. Additionally, combining pretreatment pulsing and hydrating during treatment aggravated the quality of 'Barbi' significantly. Posttreatment inflorescence bracts were fully reflexed resulting in bracts collapsing prematurely. Carbohydrate supplementations are commonly used to effectively suppress the development of the leaf blackening physiological disorder (Stephens *et al.,* 2003). Huysamer (2018) reported that 'Barbi' stems which were pulsed with a 6% sucrose solution withstood CATTS treatment when temperature was ramped from 23°C to 40°C. According to Stephens *et al.* (2003)

pulsing 'Susara' (*P. magnifica × P. susannae*) and 'Sylvia' (*P. eximia × P. susannae*) with 10% glucose extended vase life to 14 and 12 days respectively.

CATTS treatment heats up the inflorescence, and the large and enclosed *Protea* inflorescence requires time to cool down post exposure to CATTS treatments, resulting in continuous accelerated respiration rate, consequently accelerating carbohydrate depletion and general phytotoxicity. Hydrating 'Barbi' during CATTS treatment promotes high humidity and condensation of water inside the inflorescence, resulting in inflorescence decay during storage. Stephens *et al.* (2003) reported that 'Sylvia' cut flowers that were vacuum cooled (1°C) and then shipped for 21 days at 1°C had minimal leaf blackening. Therefore, cooling *Protea* post CATTS treatment might possibly prevent the observed phytotoxic damage on both the inflorescence and foliage.

Results from the present study show that CATTS treatment decreases the vase life of 'Goldstrike' stems, particularly when the stems are subjected to CATTS treatment without any prior pretreatment. The impact was worsened by sea freight simulation. Vase life of the cultivar decreased significantly and inflicted phytotoxic damage on these stems which manifested as foliage wilt. Similar results were obtained by Hara *et al.* (2003), when investigating the potential of hot-air (44°C for 0, 60, 120 or 180 minutes) as an alternative disinfestation technique. The study reported that phytotoxic damage on 'Safari Sunset' manifested as chlorotic spotting and browning of foliage. Generally, pulsing the stems with 10 ml/L Prof 3 and/or hydrating with 5 ml/L Prof 2 during CATTS treatment effectively decreased phytotoxic damage, however the treated stems had a significantly short vase life compared to untreated control stems. In contrast, Huysamer (2018) observed that post CATTS treatments at a target temperature of 40°C, 'Safari Sunset' and 'Jade Pearl' (*Leucadendron linifolium*) stems, which were pulsed with 5% glucose, exhibited overall quality which was not significantly different from untreated control stems. The efficacy of a sugar based pulsing solution, in terms of preventing phytotoxicity and leaf blackening, depends on the harvesting period, type of sugar and concentration used, cultivar, stem characteristic, osmotic potential and transpiration rate which is influenced by vapour pressure differential between the flowering stem (Hoffman *et al.,* 2014; Vardien *et al.,* 2018). The observed differences between this study's results and Huysamer (2018) can potentially be attributed to such factors.

In the current study, foliage discolouration which was observed in 'Goldstrike' suggests that a combination of CATTS treatment and sea freight storage is not feasible for this cultivar. Phytotoxic damage in 'Goldstrike' can be attributed to chilling injuries. According to Graham (2005) prolonged

storage of *Leucadendron* cultivars at chilling conditions (e.g., 21 days storage at 2.5°C) results in hydrolysis of intracellular membranes which increase membrane permeability. Dysfunctional membrane results in flavonoids, leu-anthocyanins and phenols, which are normally vacuole compartmentalised, to come in contact with chloroplast bound polyphenol oxidase therefore resulting in oxidation. Oxidised flavonoids and leu-anthocyanins turn brown, hence the observed browning/discolouration (Crick and McConchie, 1999; Graham, 2005). Graham (2005) observed that storing 'Laurel Yellow' (*Leucadendron laureolum x discolor*) and 'Safari Sunset' for 21 days at 1°C and 2.5 °C respectively, resulted in severe damage which manifested specifically on the inner bracts as brown water-soaked patches before desiccation. In the present study, neither pulsing nor hydrating during CATTS treatment had a positive impact on the foliage quality post storage at 2°C for 21 days. Results here are in accordance with research conducted by Graham (2005) in which the effectiveness of pulsing with 1% solutions of lactulose, sucrose, glucose, fructose or mannose on inhibiting chilling injuries was investigated, when *Leucadendron* cultivars are cold stored for extended periods (stored at 1°C for 14, 21 and 28 days). Graham (2005) reported that the different sugars tested did not prevent chilling injuries on involucral bracts of 'Laurel Yellow' and 'Safari Sunset'. Similarly, Philosoph-Hadas *et al.* (2010) reported that 'Safari Sunset' which had been pulsed with either 5% sucrose or 5% glucose prior to sea shipment at 2°C under darkness and 80% RH, for 8 days, exhibited leaf blackening and tip desiccation.

The observed damage in 'Goldstrike' was exacerbated by *Trichoderma* spp. infections. 'Goldstrike' stems used in the current study had been sprayed preharvest with *Trichoderma* spp. fungus (conidia suspensions) to control *Botrytis cinerea* and *Fusarium* (E. Harmse and E.L Louw, personal communication) which are known pathogens of *Leucadendron* and other Proteaceae species (Herrera *et al.,* 2006; Bezuidenhout *et al.,* 2010; Malan, 2012). In the same way, Philosoph-Hadas *et al.*, (2010). reported that *Alternaria* and *Fusarium* pathogens aggravated phytotoxic damage on 'Safari Sunset' following 21 days storage at 2°C under darkness and 80% RH. Fungal infection on cut flowers during vase life is promoted by high relative humidity micro-environments, especially when short stem lengths are used, resulting in the foliage being very close to water (Hara *et al.,* 2003).

For both immediate evaluation and freight simulations, *Leucospermum* cultivars, 'Succession' and 'High Gold' withstood CATTS treatments and maintained good quality during vase life studies. However, CATTS treated stems were not comparable to untreated controls. These results are in contrast with previous studies, investigating the potential of vapour heat as an alternative disinfestation technique. Hansen *et al*. (1992) reported that an unidentified *Leucospermum spp*. was

highly susceptible to heat treatment at 45.2°C for 2 hours. Hara *et al.* (2003) reported that subjecting 'High Gold' to hot-air treatment at 44°C for 60 minute significantly reduced vase life in comparison to untreated controls. According to Hara *et al.* (2003), phytotoxic damage manifested as collapsing of the styles, which cannot be compared to the damage reported in the current study where styles were broken, not wilting. Huysamer (2018) reported that 'Veldfire' exhibited severe foliage bleaching and rapid style wilting after being subjecting to CATTS treatments where temperature was ramped to 50℃. Decreasing the temperature to 40°C resulted in less phytotoxic damage, however the overall flower quality was still compromised.

Pulsing *Leucospermum* cultivars, 'Succession' and 'High Gold' with Prof 3 prior to CATTS treatment and/or hydration with Prof 2 during CATTS treatment seems to reduce the vase life of the cultivars by causing premature style reflexion. The quality of the treated stems is worsened by packaging for freight. Once the styles are fully reflexed it is difficult to safely package for storage. Additionally, prolonged cold storage of both 'High Gold' and 'Succession' decreases the overall quality of the treated flowers because of foliage bleaching and tip desiccation. Graham (2005) attributed these symptoms to chilling injury and reported that pulsing 'High Gold' with a 2% solution of lactulose, sucrose, glucose, mannose or fructose significantly reduced chilling injury, with lactulose being the most effective (Graham *et al.,* 2010).

2.5 Conclusion

The results reported in the current study indicate that the susceptibility to heated modified atmosphere is genus and even cultivar dependent. Among the treated Proteaceae cultivars, 'Barbi' was the most susceptible to CATTS treatments. Additionally, prolonged storage at low temperatures was detrimental to the product quality of this cultivar. Therefore, the combination of CATTS treatment and sea freight will not be a viable option for 'Barbi'. CATTS treatments have a potential to effectively disinfest *Leucospermum* cultivars, 'Succession' and 'High Gold'. However, the results of this study showed that pretreatments will not be necessary for these cultivars. Similarly, CATTS can be utilised to disinfest *Leucadendron* cultivars, however contrary to *Leucospermum* cultivars, pretreatments will be necessary to prevent foliage wilting and desiccation. Additionally, to ensure that *Leucadendron* products withstand prolonged cold storage, the use of closed ventilation during storage under low O_2 can be explored, provided that O_2 percentage is above the lower 0_2 limits of the product and does not exceed 15% CO_2. In conclusion, to develop CATTS treatment as an export disinfestation technique for

Proteaceae, a broad range of cultivars will need to be studied and hydro-cooling as a heat mitigation preventative measure, applied post CATTS treatment should be considered.

2.6 References

Bezuidenhout, C.M., van den Berg, G. & Denman, S. (2010). Post-harvest control of *Botrytis cinerea* on *Leucospermum* 'High Gold' flowers. *Acta Horticulturae*, 869, 49-56.

Cape Flora South Africa (Cape Flora SA) (2019) Statistics [WWW Document]. URL https://www.capeflorasa.co.za/wp-content/uploads/docs/2019/06/CFSA-JMF-14-MAY-2019.pdf

Coetzee, J.H. & Littlejohn, G.M. (2001). *Protea*: a floricultural crop from the Cape Floristic Kingdom. Proteaceous ornamentals: *Banksia, Leucadendron, Leucospermum* and *Protea. Scripta Horticulturae*, 22, 77-105.

Crick, S.G. & McConchie, R. (1999). Ethanol vapour reduces leaf blackening in cut flower *Protea* 'Pink Ice' stems. *Postharvest Biology and Technology*, 17, 227-231.

Giliomee, J.H. (2003). Insect diversity in the cape floristic region. *African Journal of Ecology*, 41, 237-244.

Goldblatt, P. & Manning, J. (2002). Plant diversity of the Cape Region of South Africa. *Annals of the Missouri Botanical Garden*, 89, 281-302

Graham, S. (2005). Cold storage of *Leucospermum* cut flowers and *Leucadendron* greens. (MSc Thesis, Stellenbosch: Stellenbosch University).

Graham, S., Stephens, I.A. & Jacobs, G. (2010). Effect of carbohydrate supplementation on *Leucospermum* chilling injury. *Acta Horticulturae,* 869, 219-222.

Hansen, J.D., Hara, A.H. & Tenbrink, V.L. (1992). Vapor heat: a potential treatment to disinfest tropical cut flowers and foliage. *HortScience*, 27, 139-143.

Hara, A.H., Burnham Larish, L.L. & Tsang, M.M.C. (2003). Potential for hot-air disinfestation of *Protea* flowers. *Acta Horticulturae,* 602, 61-65.

Herrera, R., Obreque, M., Vico, V., Rebolledo, P., Lolas, M., Schiappacasse, F. & Sandoval, C. (2006). Fungi and bacteria affecting protea plantings in Chili. *Acta Horticulturae*, 716, 107-110.

Hochachka, P.W. (1986). Defense strategies against hypoxia and hypothermia. *Science*, 231, 234-241.

Hoffman, E.W., Vardien, W., Jacobs, G. & Windell, N.E. (2018). Leaf Blackening: A serious impediment to long-term cold storage, transport, and extended vase life in *Protea* cut Flowers. *Horticultural Reviews*, 73.

Hoffman, E.W., Windell, N.E. & Jacobs, G. (2014). Optimum time of harvest for the control of leaf blackening in *Protea* 'Sylvia'. *Acta Horticulturae,* 1031, 125-133.

Huysamer, A.J. (2018). An assessment of alternative postharvest technologies for the disinfestation of fresh Cape Flora cut flowers for export from South Africa (MSc Thesis, Stellenbosch: Stellenbosch University).

Jing, Q.I.N., Peng, G.A.O., Zhang, X.X., Lu, M.X. & Du, Y.Z. (2018). Characterization of two novel heat shock protein 70s and their transcriptional expression patterns in response to thermal stress in adult of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Integrative Agriculture*, 17, 1023-1031.

Johnson, S.A. & Neven, L.G. (2010). Potential of heated controlled atmosphere postharvest treatments for the control of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Journal of Economic Entomology*, 103, 265-271.

Johnson, S.A. & Neven, L.G. (2011). Heated-controlled atmosphere postharvest treatments for *Macchiademus diplopterus* (Hemiptera: Lygaeidae) and *Phlyctinus callosus* (Coleoptera: Curculionidae). *Journal of Economic Entomology*, 104, 398-404.

Li, H. B., Shi, L., Lu, M. X., Wang, J. J. & Du, Y.Z. (2011). Thermal tolerance of *Frankliniella occidentalis*: Effects of temperature, exposure time, and gender. *Journal of Thermal Biology*, 36, 437-442.

Malan, G. (2012). *Protea cultivation: from concept to carton*. Stellenbosch: SUN MeDIA.

Neven, L.G. & Mitcham, E.J. (1996). CATTS (Controlled Atmosphere Temperature Treatment System): a novel tool for the development of quarantine treatments. *American Entomologist*, 42, 56-59.

Neven, L.G. & Rehfield-Ray, L. (2006a). Combined heat and controlled atmosphere quarantine treatments for control of western cherry fruit fly in sweet cherries. *Journal of Economic Entomology*, 99, 658-663.

Neven, L.G. & Rehfield-Ray, L. (2006b). Confirmation and efficacy tests against codling moth and oriental fruit moth in apples using combination heat and controlled atmosphere treatments. *Journal of Economic Entomology*, 9, 1620-1627.

Neven, L.G., Rehfield-Ray, L.M. & Obenland, D. (2006). Confirmation and efficacy tests against codling moth and oriental fruit moth in peaches and nectarines using combination heat and controlled atmosphere treatments. *Journal of Economic Entomology*, 99, 1610-1619.

Philosoph-Hadas, S., Perzelan, Y., Rosenberg, I., Droby, S. & Meir, S. (2010). *Leucadendron* 'Safari Sunset': postharvest treatments to improve quality of cut foliage during prolonged sea shipment. *Acta Horticulturae*, 869, 207-218.

Procheş, Ş. & Cowling, R.M. (2006). Insect diversity in Cape fynbos and neighbouring South African vegetation. *Global Ecology and Biogeography*, 15, 445-451.

Richardson, D.M., MacDonald, I.A.W. & Forsyth, G.G. (1989). Reductions in plant species richness under stands of alien trees and shrubs in the fynbos biome. *South African Forestry Journal*, 149, 1-8.

Seaton, K.A., Woods, W.M. & Walsh, P.G. (1993). Postharvest disinfestation of arthropods from field-grown Geraldton wax (*Chamelaucium uncinatum* Schauer). *New Zealand Journal of Crop and Horticultural Science*, 21, 147-151.

Seki, M. & Murai, T. (2012). Responses of five adult thrips species (Thysanoptera; Thripidae) to high-carbon dioxide atmospheres at different temperatures. *Applied Entomology and Zoology*, 47, 125-128.

Slootweg, G. (2007). *EcO2* Freshline voor de ontsmetting van bloemen en planten: verslag fase 1 en 2. PPO Glastuinbouw/Wageningen UR.

Smit, R., Jooste, M.M. & Johnson, S.A. (2018). CATTS technology: phytosanitary control and market expansion of chill sensitive Japanese plums for South Africa. *Acta Horticulturae* 1194, 201-208.

Smit, R. (2019). Postharvest phytosanitary disinfestation strategies using thermal and atmospheric stress: commodity and insect tolerances (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Stephens, I.A., Jacobs, G. & Holcroft, D.M. (2001). Glucose prevents leaf blackening in 'Sylvia' proteas. *Postharvest Biology and Technology*, 23, 237-240.

Stephens, I., Holcroft, D. & Jacobs, G. (2003). Postharvest treatments to extend vase life of selected Proteaceae cut flowers. *Acta Horticulturae,* 602, 155-159.

United Nations Environmental Programme (UNEP). (1992). Methyl bromide: atmospheric science, technology & economics. U.N. Headquarters, Owne Secretariat. Nairobi, Kenya.

Van Kruistum, G., Hoek, H., Verschoor, J. & Molendijk, L. (2012). Controlled atmosphere temperature treatment as sustainable alternative to control strawberry Tarsonemidae mites and plant parasitic nematodes in strawberry plants. *Acta Horticulturae,* 926, 601-608.

Van Kruistum, G., Verschoor, J. & Hoek, H. (2014). CATT as a non-chemical pest and nematode control method in strawberry mother planting stock 1. *Journal of Berry Research*, 4, 29-35.

Vardien, W., Jacobs, G. & Hoffman, E.W. (2018). The efficacy of alternate sugars, trehalose and lactulose, as a postharvest treatment in *Protea* 'Sylvia', to alleviate leaf blackening. *Acta Horticulturae,* 1201, 463-470.

Wang, H.H., Reitz, S.R., Wang, L.X., Wang, S.Y., Xue, L.I. & Lei, Z.R. (2014). The mRNA expression profiles of five heat shock protein genes from *Frankliniella occidentalis* at different stages and

their responses to temperatures and insecticides. *Journal of Integrative Agriculture*, *13*, 2196-2210.

Wright, M.G. & Giliomee, J.H. (1992). Insect herbivory and putative defence mechanisms of *Protea magnifica* and *P. laurifolia* (Proteaceae). *African Journal of Ecology*, *30*, 157-168.

Zhou, S., Criddle, R.S. & Mitcham, E.J. (2000). Metabolic response of *Platynota stultana* pupae to controlled atmospheres and its relation to insect mortality response. *Journal of Insect Physiology*, 46, 1375–1385.

3. Evaluating the use of thiabendazole (TBZ) to mitigate phytotoxic damage induced by Controlled Atmosphere Temperature Treatment System (CATTS) treatments on export grade *Protea* cut flowers

3.1 Introduction

South Africa's Cape Floristic Region (CFR) is renowned for its high plant endemism, with over 16.2% of the genera and four families that are endemic to this region (Goldblatt and Manning, 2002). Among the most economically important families is the Proteaceae, which is used predominantly as cut flowers. Proteaceae cut flowers are favoured in the international market due to their distinctive characteristics (Middelmann, 2012). These include the large and striking inflorescence and foliage of *Protea* species, the colourful leafy foliage of *Leucadendron* species, the pincushion inflorescence of *Leucospermum*, and the delicate pink *Serruria florida*, known as 'Blushing Brides', which is gradually rising to prominence on the international market. Additionally, the off-season supply of cut flowers from South Africa to Europe has contributed to the successful establishment of this niche market (Reinten *et al.,* 2011). To prevent overexploitation of wild populations and ensure consistency in quality, most of the Proteaceae cut flowers are currently commercially cultivated (Reinten *et al.,* 2011). The Cape Flora SA (2020) statistics reported that the total area of Proteaceae under cultivation is 1 133 hectares. *Protea, Leucospermum* and *Leucadendron* remain the main cultivated genera making up 58.52%, 17.65%, and 15.09% respectively.

Of concern is that the export of Proteaceae cut flowers from South Africa is significantly affected by infestation of arthropods. The structural complexity of Proteaceae plants provides diverse niches for a wide variety of entomofauna. These insects inhabit and feed on all the parts of the plant, ranging from inflorescence feeders, leaf feeders and miners, stem and bud borers to root borers (Wright, 2003). The large inflorescence of the *Protea* and high nectar production contribute to the high insect population. A survey by Gess (1968) as cited by Huysamer (2018) revealed that up to 200 insects can be found on *Protea* inflorescence. In a more recent survey on export Proteaceae cut flowers, Huysamer (2018) made 82 insect interceptions, which consisted of 8 orders and 26 families. The most abundant families were Thripidae (specifically *Frankliniella occidentalis* Pergande *(*Thysanoptera: Thripidae*)*, followed by Coleoptera (mainly *Phlyctinus callosus* (Schnonherr) *(*Coleoptera: Curculionidae*)* and *Naupactus godmanni* (Crotch) (Coleoptera: Curculionidae). Arthropod interceptions lead to either consignment rejection or mandatory fumigation (Huysamer, 2018).

Precautionary postharvest disinfestation techniques, which eliminate the pest without damaging the commodity, are a necessity to ensure maintenance and growth of the industry.

Controlled Atmosphere Temperature Treatment System (CATTS) technique combines the effect of high temperatures and a modified atmosphere (low oxygen and high carbon dioxide) to control insect pests (Neven and Mitcham, 1996). However, this technology has not yet been commercially implemented on cut flowers due to the phytotoxic impact on flower quality. Previous studies in the Netherlands on cut flowers reported that there was variable sensitivity within plant species. Slootweg (2007) reported that gerbera (*Gerbera jamesonii*), Alstroemeria (*Alstroemeria spp.),* and carnation (*Dianthus caryophyllus* L) cut flowers were highly susceptible to the treatments, while roses and chrysanthemums withstood these treatments. In South Africa, Huysamer (2018) reported that *Leucadendron* cultivars, *(Leucadendron laureolum x salignum)* and *(Leucadendron linifolium)* and Geraldton Wax *(Chamelaucium uncinatum)* withstood the treatments tested and exhibited no phytotoxic damage until the end of vase life studies. The *Leucospermum* cultivar, 'Veldfire' and *Protea magnifica* 'Barbi' were susceptible to treatments, exhibiting wilting and collapsing of the inflorescence styles and leaf blackening and discolouration of involucral bracts within 2 days of vase life studies (Huysamer, 2018).

Leaf blackening is a well-known disorder of the *Protea* genus. The symptoms of the disorder primarily develop within three to seven days postharvest on susceptible cultivars (McConchie and Lang, 1993), appearing as brown/black discoloration of the leaves, mainly on the tips and margins. The presence of blackened foliage during quality assessment can potentially lead to consignment rejection (Hoffman *et al.,* 2014). Additionally, it decreases the market value and vase life of cut flower products. The onset of the disorder has been attributed to carbohydrate depletion from the source (leaves) due to high sink (inflorescence) demand. The carbohydrate demand is reported to be higher when the inflorescence is harvested before bracts retract (pre-soft tip stage) (Paull and Dai, 1990; McConchie *et al.,* 1991) and the flower head is producing nectar (Rodríguez-Pérez *et al.,* 2012). Higher temperatures significantly contribute to carbohydrate depletion (Stephens *et al.,* 2001). The sequence of events that leads to leaf blackening is an on-going debate in the literature. Jones and Clayton-Greene (1992) speculated that depletion of carbohydrates leads to membrane hydrolysis allowing vacuole sequestrated phenols to be oxidised. Historically, polyphenol oxidase and peroxidase were reported to be responsible for oxidising phenols (Whitehead and de Swardt, 1982). However, McConchie *et al.,* (1994) reported that there is no direct link between polyphenol oxidase and peroxide activity with *Protea* leaf blackening, because they observed that the chloroplast does not degrade during leaf

blackening. Therefore, enzymes remain separated from phenolic substrates. Additionally, the researchers reported that continued leaf respiration and lack of lipid peroxidation in blackening *Protea* indicates that the onset of the disorder starts before membrane degradation. Phenolic concentration is reported to increase on blackened *P. susannae x P. compacta* because of glycosylated compounds which are cleaved under carbohydrate stress. The high concentration of available phenolics potentially modifies enzymes and proteins causing metabolic dysfunction therefore resulting in leaf blackening.

Techniques recommended to delay the onset of leaf blackening focus on increasing the supply of the available carbohydrates and supplying sufficient water. Transferring cut stems immediately into water after harvesting contributes towards delaying leaf blackening (Malan, 2012). Harvesting in the afternoon is recommended, rather than mornings when stems have low sucrose and reduced sugar levels (Paull and Dai 1990). Furthermore, it is important to harvest varieties which are prone to leaf blackening in the afternoon, especially during winter and early spring, however temperatures should be below 30°C (Malan, 2012). Rodríguez-Pérez *et al.* (2012) recommended that to prevent leaf blackening incidence on *Protea* 'Susara' (*P. magnifica × P. susannae*), the stems should be harvested on cool autumn mornings, when temperatures are about 18 °C and under low vapour pressure deficit. Storing cut *Protea neriifolia* in light conditions (light levels exceeding 25 µmol.ms^{-1}) has been reported to be better than storing them in the dark, because it encourages continuous photosynthesis, and therefore increases the available carbohydrates and inhibits phenol oxidation (McConchie *et al.,* (1991; Jones and Clayton-Greene, 1992). The use of sucrose and glucose as pulsing treatments and supplementary vase solution significantly delays the onset of leaf blackening and has been reported to prolong vase life of *Protea* (Stephens *et al.,* 2005). However, varieties respond differently to both sucrose and glucose. According to Rodríguez-Pérez *at al.* (2012), factors such as growth condition and soil humidity contribute to the varying response. Additionally, the concentration of the supplied exogenous sugar contributes to the success in delaying leaf blackening. Jones (1991) as cited by Stephens *et al. (*2005) reported that higher sucrose concentrations may aggravate leaf blackening incidences in *P. neriifolia.*

To establish CATTS treatments as a viable disinfestation method and develop treatment protocols for export fynbos cut flowers with minimal quality loss, methods for ensuring flower quality need to be investigated. Thiabendazole (4-(1H -1, 3-benzodiazol-2-yl)-1, 3-thiazole), a benzimidazole systemic fungicide, has been shown to control certain postharvest diseases caused by *Penicillium digitatum, Colletotrichum gleosporioides* and *Diplodia natalensis* (Chien *et al.,* 2007; Kellerman *et al.*, 2014), as well as reduce chilling injury (a physiological rind disorder) in citrus fruits (Schirra and Mulas, 1995;

Schirra *et al.,* 1998; 2000). Ehlers (2016) found that thiabendazole (TBZ) provided some protection to fruit that underwent a dehydration/rehydration stress treatment. However, the mechanism by which TBZ provides such protective functions is currently unknown, and no research has been done on the use of TBZ on flowers to maintain postharvest quality.

The potential for TBZ to provide protection to flowers undergoing an atmospheric and thermal stress treatment, such as CATTS, needs to be considered. Application of TBZ may improve flower quality after CATTS or may even prove to be beneficial in protecting untreated blackening-prone cultivars during normal postharvest practices (EW Hoffman, personal communication). Therefore, the objective of this study was to assess the potential of TBZ in preventing CATTS-induced leaf blackening through evaluating posttreatment quality, storage capacity and shelf life of the treated *Protea magnifica* 'Barbi' and *Protea eximia x susannae* 'Sylvia'.

3.2 Materials and Methods

3.2.1 Product variety and procurement

Export quality, *Protea magnifica* 'Barbi' stems were sourced from Berghoff farm, Piketberg and *Protea eximia x susannae* 'Sylvia', from Cape Mountain Flora, Stellenbosch, both in the Western Cape, South Africa. All stems were treated within 24 hours of harvesting. The cultivars were chosen because of their proneness to leaf blackening, and Huysamer (2018) reported that CATTS treated 'Barbi' exhibited leaf blackening.

3.2.2 Thiabendazole treatment, CATTS treatment and freight storage simulations

To access the impact of TBZ, only foliage was treated with TBZ, without wetting the inflorescence. TBZ treatments were applied by dipping the cut stems in a 2% TBZ [ICA-TBZ, ICA International Chemicals (Pty) Ltd.] in water solution, as per the industry recommendation. Post TBZ-dipping, the flowers were stored at room temperature for approximately 3 hours to allow the foliage to dry. CATTS treatments were performed in a laboratory-scale CATTS unit (Techni- Systems, Chelan, WA, USA). The temperature was ramped from 23°C to 40°C at 35°C/hr with 15 minutes soaking period at the targeted temperature. Total CATTS treatment time was 44 minutes. The atmospheric composition for treatments was 1% O_2 and 15% CO_2 in N_2. The treatment conditions were selected based on the Huysamer (2018) results.

Four treatments were executed as follows, to assess the effect of TBZ dipping and CATTS treatments on cut stems:

1. Untreated control stems - neither dipped in TBZ nor subjected to CATTS treatment
2. Stems not dipped in TBZ, but subjected to CATTS treatment (CATTS only)
3. Stems dipped in TBZ, however not subjected to CATTS treatment (TBZ only)
4. Stems dipped in TBZ and subjected to CATTS treatment (TBZ plus CATTS)

Each treatment was repeated 3 times with replicates of 6 flower stems in each.

Trial 1 was conducted as described above, and treated stems were evaluated immediately after CATTS treatment, for a period of 12 days (evaluation procedure described in section 3.2.3). For Trial 1, visual quality scores were evaluated for 12 days - day 0 (post CATTS treatment), day 2, 4, 6, 8, 10 and 12. Another two sets of stems were subjected to treatments as described above, however, after the post-CATTS treatment evaluation, stems were packed into standard S22 boxes (100 x 28 x 22 cm) and stored at 2°C under normal atmospheric conditions, for 3 days (Trial 2) and 21 days (Trial 3), to simulate the storage duration required for commercial air and sea freighting, respectively. Following freight simulations, stems were recut at 45^{o} angle and were evaluated for phytotoxic damage. For Trial 2 (air freight simulation), stems were evaluated post CATTS treatment, post 3-days storage and then every second day for 12 days. For Trial 3 (sea freight simulation), 'Barbi' stems were evaluated post CATTS treatment, post 21-days storage and then every second day for 12 days. 'Sylvia' stems were evaluated for 6 days, instead of 12, as evaluations had to be stopped due to the South African National lockdown restrictions. During vase life studies, flower stems were held in a solution containing 10ml Professional 3 (Chrysal International B.V, The Netherlands). All the evaluations were done at room temperature (20 °C ±2 °C) under normal atmospheric conditions.

3.2.3 Visual quality evaluation

Phytotoxic damage (e.g., discoloration, leaf blackening) was evaluated based on the flower quality assessment system developed by Huysamer (2018) (Table 3. 1.). A score of zero was assigned to indicate no discernible damage (Fig. 3.1A), a score of 4 was established as the marketability limit, that is, the product is no longer suitable for sale (Fig. 3.1B), while a score of 8 depicted the end of vase life (Fig. 3.1B), where the flower is severely damage and no longer in an appreciable state and will therefore result in rejection by consumers. Although both inflorescence and foliage were evaluated, the inflorescence scores were not used to evaluate the effectiveness of TBZ because the study

specifically focused on the impact of TBZ on the quality of treated foliage. Therefore, all the results presented are based on the foliage scores.

Table 3.1: Rating scores that correspond with descriptions of foliage quality parameters used to calculate an overall product score for genus *Protea* (Huysamer 2018)

Rating	Damage description
0	No damage to stem and leaves
1	Few leaves have slight damage, good appearance
2	More leaves with slight damage, generally good appearance, still marketable
3	Many leaves with slight damage or few leaves with major damage. Still with good appearance
4	More leaves with major damage, appearance fair but vase life questionable
5	More leaves with major damage, appearance fair to poor,
6	Most leaves with major damage, appearance poor, not suitable for vase
7	Much of the foliage (≥ 70%) damaged or dead
8	Much of the foliage (≥ 90%) damaged or dead
9	Foliage dead

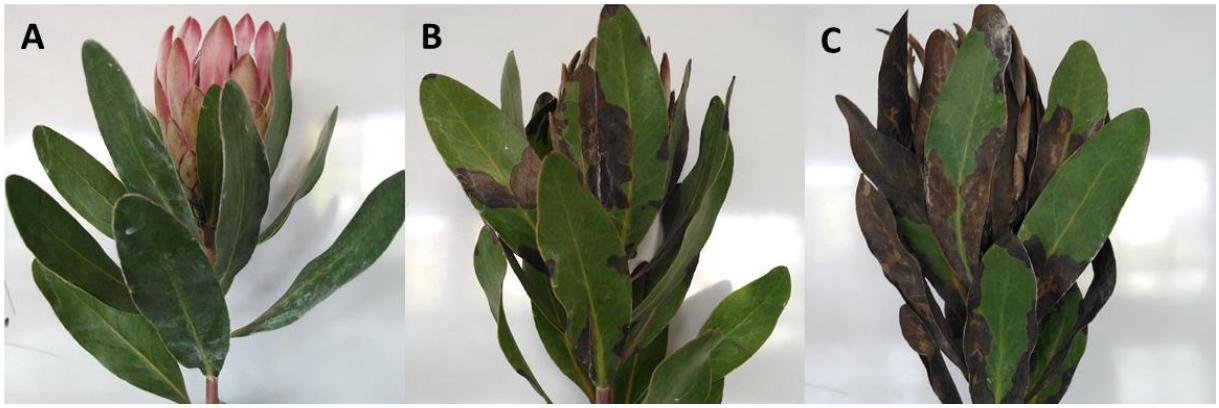


Figure 3:1: *Protea eximia x susannae* 'Sylvia' visual representation of the evaluation scale. No discernible damage indicating rating score 0 (A), many leaves with slight damage indicating the marketability limit rating score of 4 (B), and many leaves with severe leaf blackening indicating the vase life limit rating score of 8 (C).

3.2.4 Statistical analysis

Mean overall foliage scores were statistically analysed using STATISTICA 13.2 software (Dell Statistica data analysis software system, Dell Inc). To evaluate vase life posttreatments, two-way analysis of variance (ANOVA) and mixed model ANOVA tests were performed on mean overall foliage scores per evaluation over time (days). Means were separated by the Fisher's least significant difference ($P \leq 0.05$).

3.3 Results

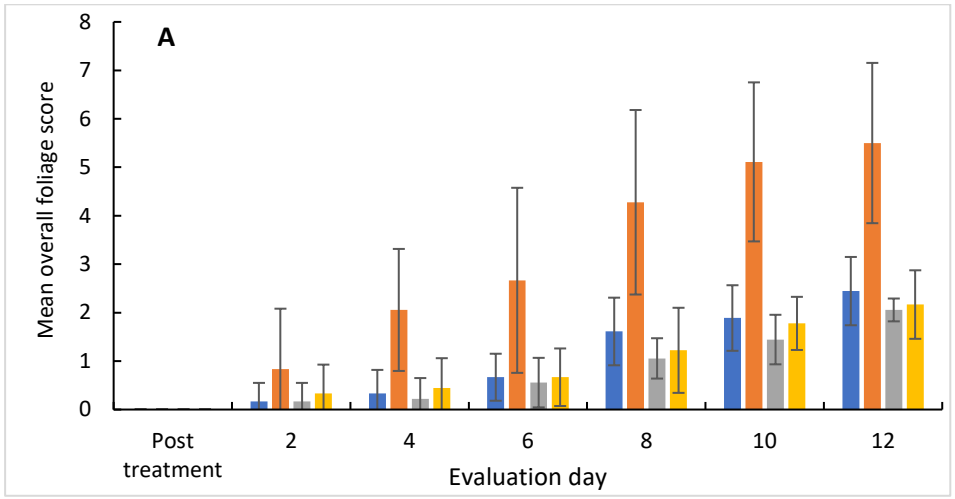
3.3.1 *Protea magnifica* 'Barbi'

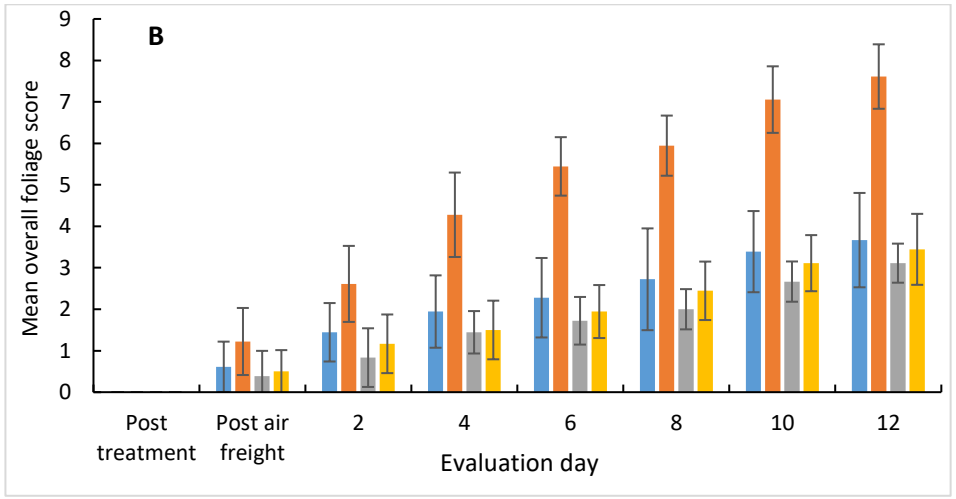
From the second day of evaluation (day 2) (Fig. 3.2A), phytotoxic damage manifested as wilting on stems which were subjected to CATTS treatment, but without TBZ dipping (T2). Thereafter, more leaves exhibited slight damage, however they were still marketable until day 8, when the stems exhibited a mean overall foliage score of 4.28 ± 1.90. Throughout vase life studies, stems which were dipped in TBZ but not CATTS treated (T3) and which had been dipped in TBZ prior to CATTS treatment (T4) did not differ from untreated control stems ($p > 0.01$). Although, there was evident negligible leaf blackening symptoms observed on T1, T3 and T4 stems towards the end of the vase life studies, the stems did not breach the marketability limit. By the conclusion of vase life studies (day 12), the mean overall foliage scores of T3 and T4 were 2.06 ± 0.27 and 2.17 ± 0.71. In comparison, stems subjected to T2 exhibited mean overall foliage score of 5.50 ± 1.65.

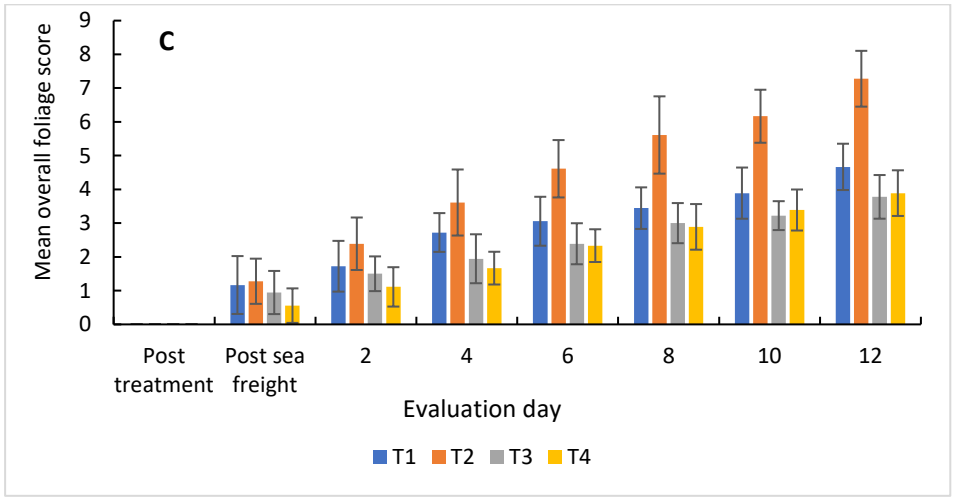
Immediately after CATTS treatments and post simulated air freight storage, there were no significant differences ($p > 0.01$) between all the treatments (Fig. 3.2B). However, 4 days after stems were removed from storage, significant differences ($p < 0.01$) were observed between untreated control stems (T1) and CATTS-only treated stems (T2). Untreated control stems exhibited mean overall foliage score of 1.94 ± 0.87, while T2 exhibited a mean overall score of 4.28 ± 1.02, consequently breaching the marketability limit. Leaf blackening was the reason foliage quality of T2 foliage deteriorated. Thiabendazole dipping before CATTS treatment (T4), and only dipping the stems without CATTS treatment (T3), delayed the onset of leaf blackening post air freight simulation. As a result, mean overall foliage scores of T3 and T4 stems did not differ significantly from each other, or from those measured in the untreated control stems.

Post sea freight simulation, untreated control stems (T1) did not differ significantly ($p > 0.01$) from stems subjected to CATTS-only treated stems (T2), as well as stems which were only dipped in TBZ (T3) (Fig. 3.2C). However, T1 stems differed significantly from stems which were dipped in TBZ and subjected to CATTS treatment (T4). Phytotoxic damage post sea freight simulation was seen as yellowing (Fig. 3.3A) and sporadic blackening predominately on T1 and T2 stems. Although leaf blackening was evident on T1, T4 and stems which were only dipped in TBZ (T3), it did not expand at the same rate as on T2 stems. By the conclusion of vase life assessment (Day 12), CATTS-only treated stems had a mean overall foliage score of 7.78 ± 1.52 exhibiting severe leaf blackening (Fig. 3.3B). In

comparison, untreated control stems breached the marketability limit, exhibiting mean foliage score of 4.61 ± 0.85, whereas the overall foliage quality was better in T3 and T4 stems which were not significantly different from each other ($p > 0.01$) and maintained mean overall foliage scores below the marketability limit and leaf quality remained in good condition (Fig 3.3C).







	Two-way ANOVA table		
Source	A	B	C
Treatment	<0.01	<0.01	<0.01
Time	<0.01	<0.01	<0.01
Treatment x Time	<0.01	<0.01	<0.01

Figure 3:2: Mean overall foliage scores of *Protea magnifica,* 'Barbi' assessed post dipping in a 2% thiabendazole solution and CATTS treated at a target temperature of 40°C. Phytotoxicity damage was assessed immediately after CATTS treatment (A), after storage at 2°C for 3 days to simulate air freight (B) and after storage at 2°C for 21 days to simulate sea freight (C). CATTS target temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C. Treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Vertical error bars indicate the standard deviations of the mean for each data point. Treatments were; Untreated control (T1), CATTS only (T2), TBZ only (T3) and TBZ plus CATTS (T4).

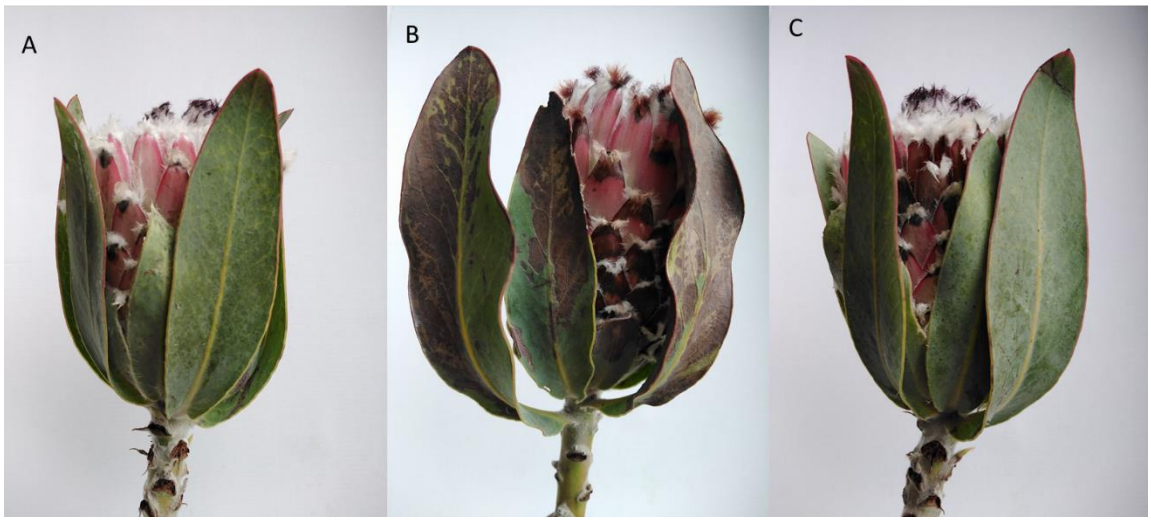


Figure 3:3: Phytotoxic damage on *Protea magnifica, '*Barbi' assessed post dipping in a 2% thiabendazole solution, CATTS treated at a target temperature of 40°C and after storage at 2°C for 21 days to simulate sea freight. Control exhibiting yellowing post storage(A). Stem subjected to CATTS only exhibiting severe leaf blackening at the end of vase life studies (B). TBZ dipped and CATTS stem with no discernible foliage damage at the end of vase life studies (C). CATTS target temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C.

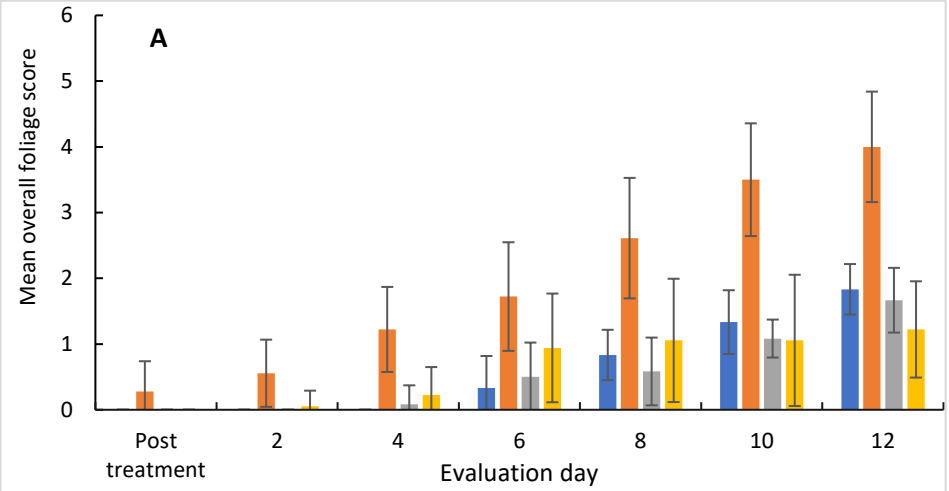
3.3.2 *Protea eximia x susannae,* 'Sylvia'

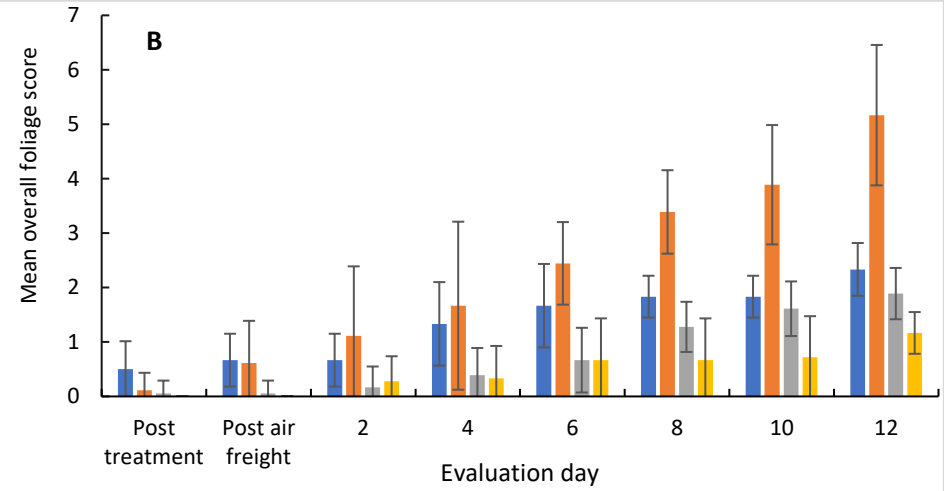
Evaluating 'Sylvia' stems immediately after CATTS treatment and then for a period of 12 days confirms that TBZ pretreatment significantly inhibits phytotoxic damage caused by CATTS treatment (Fig. 3.4A). The results presented indicate that there were no significant differences ($p > 0.01$) between untreated stems (T1), stems which had been dipped in TBZ without CATTS treatment (T3) and stems which were dipped in TBZ and subjected to CATTS treatment (T4). This was true until the end of vase life studies (day 12) and when the three treatments (T1, T3 and T4) stems exhibited mean overall foliage scores of 1.83 ± 0.38 (T1), 1. 67 ± 0.49 (T3) and 1.22 ± 0.73 (T4), without breaching the marketability limit. Stems which were subjected to CATTS treatment without TBZ dipping (T2) differed significantly from other treatments ($p < 0.01$). The differences were readily observed by day 4 and phytotoxic damage manifested specifically on leaves subtending the inflorescence as brown patches along the leaf margin which quickly spread throughout the leaf during vase life studies. As a result, on day 4, T2 stems exhibited mean foliage score of 1.22 ± 0.65. Consequently, T2 stems breached the marketability limit on day 12 exhibiting a mean foliage score of 4 ± 0.84.

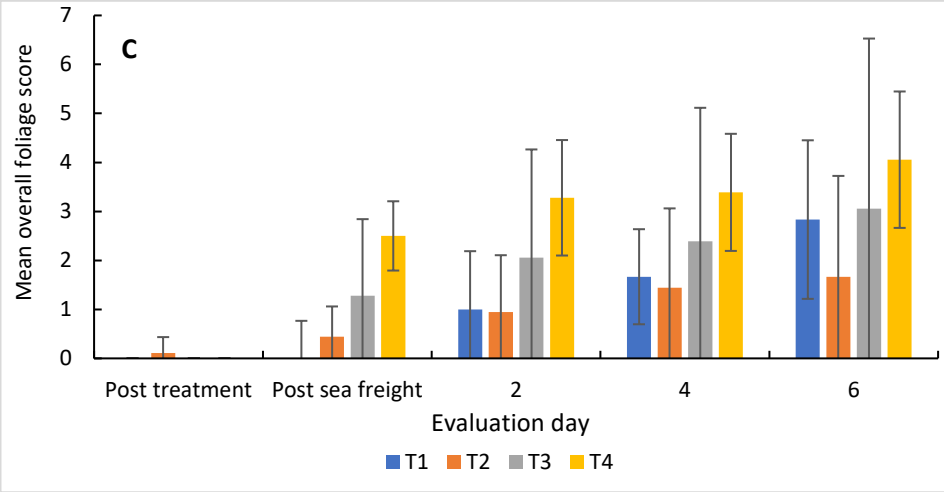
Immediately after CATTS treatment, post air freight simulation at day 4 (i.e., second evaluation post air freight), no significant differences ($p > 0.01$) were observed between T1 and stems which were subjected to CATTS treatment without TBZ dipping (T2) (Fig. 3.4B). Similar observations were true

between T3 and T4 stems. On day 6 and thereafter, T1 and T2 stems differed significantly from each other ($p < 0.01$). Phytotoxic damage was observed on T2 stems, initially manifesting as wilting and minor leaf blackening spots which gradually developed during vase life studies. Treatment 2 stems breached the marketability limit on day 12, exhibiting mean overall foliage score of 5.17 ± 1.54. Thiabendazole dipping significantly reduced phytotoxic damage induced by CATTS treatment and storage at 2°C for 3 days. Therefore, there were no significant differences ($p > 0.01$) between T3 and T4 throughout the evaluations. Treatment 3 and 4 mean overall foliage scores at the end of vase life studies were 1.89 ± 0.47 and 1.17 ± 0.38 respectively.

Post sea freight simulation, T1 and T2 differed significantly ($p < 0.01$) from T3 and T4 stems (Fig. 3.4C). However, phytotoxic damage was evident across all the treatments and manifested as foliage discoloration (yellowing) and blackening (Fig. 3.5). Evidently, 'Sylvia' visual quality was compromised because of storage at low temperatures (2°C) for a prolonged duration (21 days). Leaf blackening was more pronounced on TBZ and CATTS treated stems (T4). As a result, these stems breached the marketability limit on day 6 (i.e., third evaluation post sea freight simulation), exhibiting a mean overall foliage score of 4.06 ± 1.39. Based on these results thiabendazole dipping (T3 and T4) did not prevent phytotoxicity damage evident after prolonged storage.







	Two-way ANOVA table		
Source	A	B	C
Treatment	<0.01	<0.01	0.05
Time	<0.01	<0.01	<0.01
Treatment x Time	<0.01	<0.01	0.04

Figure 3:4: Mean overall foliage score of *Protea eximia x susannae,* 'Sylvia' assessed post dipping in a 2% thiabendazole solution and CATTS treated at a target temperature of 40°C. Phytotoxicity was assessed immediately after CATTS treatment (A), after storage at 2°C for 3 days to simulate air freight (B) and after storage at 2°C for 21 days to simulate sea freight (C). CATTS target temperature was reached by using 35°C/hr ramp rate from 23°C to 40°C. Treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Vertical error bars indicate the standard deviations of the mean for each data point. Treatments were; Control (T1), CATTS only (T2), TBZ only (T3), and TBZ plus CATTS (T4).

Figure 3:5: Phytotoxic damage manifesting as leaf blackening and discoloration on *Protea eximia x susannae,* 'Sylvia' subjected to CATTS treatment and 21 days storage at 2℃. Untreated stems (A), CATTS only stems (B) and Thiabendazole dipped stem (C).

3.4 Discussion

The feasibility of a disinfestation technique is determined by its ability to effectively cause insect mortality while maintaining flower quality. Utilising CATTS treatments of 30°C/hr or 35°C/hr to 40°C Huysamer (2018) effectively controlled western flower thrips, and protea itch mite and successfully maintained flower quality of *Protea* 'Barbi'*, Leucadendron* 'Safari Sunset' (*L. laureolum × L. salignum*) and Geraldton Wax 'Ofir'. However, increasing temperatures had detrimental impact on the overall quality, inducing leaf blackening. The current study results indicate that the application of thiabendazole would aid in the establishment of CATTS technology by inhibiting the incidence of phytotoxic damage. Thiabendazole dipping significantly reduced the incidence of leaf blackening in *Protea* 'Barbi' stems which were assessed immediately posttreatment, as well as post freight simulation.

The effectiveness of thiabendazole in inhibiting phytotoxic damage on *Protea* 'Barbi' subjected to CATTS treatments, in combination with sea freight simulation, will contribute towards successful establishment of the CATTS technology, and could potentially contribute towards increasing the amount of consignments which can be shipped. According to Matsikidze (2018), adoption of sea freighting of South African cut flowers has been hindered by the chilling injury and leaf blackening which is triggered by lengthy storage and low temperature imposed by sea freighting.

However, TBZ did not inhibit the foliage discoloration and leaf blackening on *Protea* 'Sylvia' stored at low temperatures (2°C) or prolonged duration (21 days). These results are in accordance with those obtained by Philosoph-Hadas *et al.* (2010), who reported that pre-treating *Leucadendron* 'Safari Sunset' with TOG-3 (composed of thiabendazole, 8-hydroxyquinoline citrate and benzolconium chloride) did not prevent the occurrence of phytotoxic damage which manifested as leaf blackening and tip desiccation following 21 days shipment. The onset of leaf blackening on these stems can be attributed to substantial decline of carbohydrates associated with shipping conditions (McConchie and Lang, 1993; Stephens *et al.,* 2001; Hoffman *et al.,* 2014). Harvesting time potentially contributed to high leaf blackening incidence. The stems which were used in the present study were harvested midsummer (late February), in the morning and according to Hoffman *et al.* (2014), there is an interaction between morning harvesting, cold storage and leaf blackening incidence on Proteaceae cut flowers. Hoffman *et al.* (2014), reported that leaf blackening progressed at an accelerated rate on stems which had been harvested early in the morning (08h00) and then subjected to cold storage, compared to stems which had been harvested in the afternoon. Furthermore, yellowing of stored *Protea* 'Sylvia' is a common condition of stored *Protea* cultivars due to chlorophyll degradation in the absence of light. Matsikidze (2018) reported a decrease in hue angle measurements post cold storage, indicating a decrease in chlorophyll. Additionally, storing *Protea* in the dark results in depletion of $NADPH^+$ H^+ which are required for glutathione synthesis and therefore, the ascorbate-glutathione antioxidant system is compromised possibly resulting in free radicals attacking macromolecules leading to cell membrane death (McConchie and Lang, 1993) and subsequently, leaf blackening.

Although TBZ dipping had a positive impact on the foliage of treated stems, inflorescences, whether on stems that were TBZ treated or not, exhibited severe discoloration and desiccation after CATTS treatment. Conditioning the product to heat prior to CATTS treatments can potentially contribute towards withstanding the treatments (Hara *et al*., 2003), and thus help maintain good quality of the inflorescence. Decay of the inflorescence can possibly be attributed to high condensation accumulation and failure to cool down post CATTS treatment. Therefore, cooling the stems prior to packaging, as suggested by van Doorn (2001), can be effective in inhibiting phytotoxic damage observed posttreatment. Furthermore, instead of storing the treated stems at normal atmospheric conditions, adoption of modified atmosphere in the form of low oxygen and high carbon dioxide will be beneficial. Mastikidze (2018) reported to significantly reduced moisture loss, which has been attributed to chilling injury, and maintained *Protea* 'Barbi' quality during sea freight simulation by storing the stems in a modified atmosphere condition where 2% oxygen and high CO_2 (15%) was maintained.

3.5 Conclusion

To effectively disinfest cut flowers and ensure that high quality is being maintained posttreatment, different postharvest techniques will need to be combined. The current study has proved that pretreatment TBZ dipping can reduce the adverse impacts of CATTS treatments, specifically on foliage, however more studies should be done to investigate post CATTS treatment handling techniques which will ensure that both the inflorescence and foliage quality are not compromised. Lastly, cultivation practises prior to harvesting in a commercial orchard might aid less insect infestation and potentially reduce the need for postharvest disinfestation.

3.6 References

Cape Flora South Africa (Cape Flora SA), 2020. Statistics [WWW Document]. https://www.capeflorasa.co.za/wp-content/uploads/docs/2021/01/CFSA-JMF-JUNE-2020.pdf (Accessed July 2021).

Chien, P.J., Sheu, F. & Lin, H.R. (2007). Coating citrus (*Murcott tangor*) fruit with low molecular weight chitosan increases postharvest quality and shelf life. *Food Chemistry*, 100, 1160-1164.

Ehlers, J.L. (2016). Post-harvest rind pitting studies on 'Valencia' orange. MSc thesis, University of Stellenbosch, Stellenbosch, South Africa.

Goldblatt, P. & Manning, J.C. (2002). Plant diversity of the Cape region of southern Africa. *Annals of the Missouri Botanical Garden*, 89, 281-302.

Hara, A.H., Burnham Larish, L.L. & Tsang, M.M.C. (2003). Potential for hot-air disinfestation of Protea flowers. *Acta Horticulturae,* 602, 61–65.

Hoffman, E.W., Windell, N.E. & Jacobs, G. (2014). Optimum time of harvest for the control of leaf blackening in *Protea* 'Sylvia'. *Acta Horticulturae,* 1031, 125-133.

Huysamer, A.J. (2018). An assessment of alternative postharvest technologies for the disinfestation of fresh Cape Flora cut flowers for export from South Africa (MSc Thesis, Stellenbosch: Stellenbosch University).

Jones, R.B. & Clayton-Greene, K.A. (1992). The role of photosynthesis and oxidative reactions in leaf blackening of *Protea neriifolia R. Br.* leaves. *Scientia Horticulturae*, 50, 137-145.

Kellerman, M., Erasmus, A., Cronjé, P.J. & Fourie, P.H. (2014). Thiabendazole residue loading in dip, drench and wax coating applications to control green mould and chilling injury on citrus fruit. *Postharvest Biology and Technology,* 96, 78-87.

Malan, G. (2012). Protea cultivation: From concept to carton (1st ed.). Stellenbosch: SUN MeDIA.

Matsikidze, S.N. (2018). Feasibility of closed ventilation and automatic ventilation for sea freight of Proteaceae cut flower stems (MSc Thesis, Stellenbosch: Stellenbosch University).

McConchie, R. & Lang, N.S. (1993). Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. *Journal of the American Society for Horticultural Science,* 118, 355-361.

McConchie, R., Lang, N.S. & Gross, K.C. (1991). Carbohydrate depletion and leaf blackening in Protea neriifolia. *Journal of the American Society for Horticultural Science,* 116, 1019-1024.

McConchie, R., Lang, N.S., Lax, A.R. & Lang, G.A. (1994). Re-examining polyphenol oxidase, peroxidase, and leaf-blackening activity in Protea. *Journal of the American Society for Horticultural Science,* 119, 1248-1254.

Middelmann, M. (2012). Proteas: the birth of a worldwide industry. Xlibris Corporation.

Neven, L.G. & Mitcham, E.J. (1996). CATTS (Controlled Atmosphere Temperature Treatment System): A novel tool for the development of quarantine treatments. *American Entomologist*, 42, 56-59.

Paull, R.E. & Dai, J. (1990). Protea postharvest black leaf a problem in search of a solution. *Acta Horticulturae,* 264, 93-102.

Philosoph-Hadas, S., Perzelan, Y., Rosenberg, I., Droby, S. & Meir, S. (2010). Leucadendron 'Safari Sunset': Postharvest treatments to improve quality of cut foliage during prolonged sea shipment. *Acta Horticulturae,* 869, 207-218.

Reinten, E.Y., Coetzee, J.H. & Van Wyk, B.E. (2011). The potential of South African indigenous plants for the international cut flower trade. *South African Journal of Botany,* 77, 934-946.

Rodríguez-Pérez, J.A., Vera-Batista, M.C., de León-Hernández, A.M. & Rodríguez-Hernández, I. (2012). Effect of sucrose pulsing and postharvest rehydration delay on vase life of Protea 'Susara'. *Acta Hhorticulturae,* 937, 837-842.

Schirra, M. & Mulas, M. (1995). Improving storability of 'Tarocco' oranges by postharvest hot-dip fungicide treatments. *Postharvest Biology and Technology*, 6, 129-138.

Schirra, M., D'hallewin, G., Cabras, P., Angioni, A., Ben-Yehoshua, S. & Lurie, S. (2000). Chilling injury and residue uptake in cold-stored 'Star Ruby' grapefruit following thiabendazole and imazalil dip treatments at 20 and 50 °C. *Postharvest Biology and Technology*, 20, 91-98.

Schirra, M., D'hallewin, G., Cabras, P., Angioni, A. & Garau, V.L. (1998). Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip treatments. *Journal of Agricultural and Food Chemistry,* 46, 1177-1180.

Slootweg, G.C. (2007). EcO2 Freshline voor de ontsmetting van bloemen en planten. Report of phase1 and 2. (In Dutch) Praktijkonderzoek Plant & Omgeving B.V. Wageningen, The Netherlands.

Stephens, I.A., Jacobs, G. & Holcroft, D.M. (2001). Glucose prevents leaf blackening in 'Sylvia' proteas. *Postharvest Biology and Technology,* 23, 237-240.

Stephens, I.A., Meyer, C., Holcroft, D.M. & Jacobs, G. (2005). Carbohydrates and postharvest leaf blackening of proteas. *HortScience*, 40, 181-184.

van Doorn, W.G. (2001). Leaf blackening in Protea flowers: recent developments. *Acta Horticulturae,* 545, 197–204.

Whitehead, C.S. & de Swardt, G.H. (1982). Extraction and activity of polyphenoloxidase and peroxidase from senescing leaves of Protea neriifoiia. South African Journal of Botany, 1, 127-130.

Wright, M.G. (2003). Insect pests of Proteaceae: Assessment of predictions of new pests internationally, and management implications. Acta Horticulturae, 602, 167–171.

4. Evaluating the potential of using ethyl formate fumigation as a phytosanitary treatment for *Serruria florida* ('Blushing Bride'), with prefumigation carbohydrate pulsing and 6-benzyladenine (cytokinin) to mitigate posttreatment phytotoxicity

4.1 Introduction

The genus *Serruria* Salisb. (Proteaceae) is comprised of approximately 65 species (Rourke, 1990), making it the largest Proteaceae genus of the Cape Floristic Region (de Villiers, 2004). *Serruria* is generally a small shrub characterised by pedunculate capitulum inflorescences (e.g., *Serruria reflexa*), lanceolate-acuminate involucral bracts (e.g., *Serruria deluvialis*), sprawling growth habits (eg. *Serruria effuse*) or can be identified by its prostrate, procumbent growth habit as seen in *Serruria viridifolia* (Rourke, 1990). With the exception of *Serruria triternata*, which has large and stout leaves, a consistent characteristic within *Serruria* is the thin and fine leaves (de Villiers, 2004). *Serruria florida*, commonly known as 'Blushing Brides', is the main cultivated species, while the less common species are *S. rosea* and *S. foeniculacea* (Criley, 2001). According to Cape Flora SA (2019), a non-profit company that monitors and promotes sustainable exporting of proteas from South Africa, there has been a significant increase in the amount of *S. florida* exported. In the 2018/2019 season, 21. 3% of the total exported Proteaceae were *S. florida,* in comparison to 16. 7% which was exported in the previous season.

Export quality flowers need to be free of living arthropods as when present it might result in consignment rejection, leading to financial and farmer integrity loss (Huysamer *et al.,* 2019). To reduce overall rates of infestations, growers spray field insecticides, and to further meet quarantine requirements, exporters apply postharvest disinfestation treatments, such as gamma irradiation, insecticidal dips and spray, cold storage, hot water baths, controlled atmosphere and fumigation (Hansen and Hara, 1994). Fumigation is generally preferred because it eliminates the potential health risk associated with insecticidal dips and costs of having to dispose of used chemical dipping solutions. Additionally, fumigants readily penetrate cryptic habitats and insects' protective structures, as posed during different growth stages (Rigby, 2018). The most commonly used fumigant is methyl bromide, relating to the fumigant's efficiency and effectiveness across a wide range of insects associated with ornamentals and fresh produce. Due to the ozone depleting properties of methyl bromide, the Montreal protocol mandate was created to restrict and phase out its use for quarantine and pre-shipment purposes, resulting in its reduced availability and increased costs (Simpson *et al.,* 2004). A

challenge is now to find alternatives to methyl bromide, with comparable effectiveness against a wide range of agricultural pests and maintain product quality.

Ethyl formate (EF) has been investigated and adopted as a methyl bromide alternative because of its rapid insecticidal properties. The effectiveness of EF is attributed to how it hydrolyses into an aliphatic alcohol component (ethanol) and active metabolite formic acid in an organism because of its carboxylic acid esterases (Haritos and Dojchinov, 2003; Song and Scharf, 2008). Haritos and Dojchinov (2003) attributed the insecticidal action of EF in stored-product beetle, *Sitophilus oryzae* (L.) to the inhibition of cytochrome *c* oxidase, therefore, impairing oxidative phosphorylation leading to depletion of cellular energy stores. Song and Scharf (2009) studying the impact of insecticidal formate esters on *Drosophila melanogaster* reported this chemical disrupts the mitochondria, respiratory inhibition, apoptotic effects and neurological disruption. In addition to causing rapid insect mortality, EF is advantageous as firstly, its toxicity is dependent on the concentration of formic acid, therefore decreasing the potential for development of insect resistance following widespread use of the fumigant (Haritos and Dojchinov, 2003). Secondly, ethanol and formic acid are imperceptible residues; as a result, EF is exempted from the Maximum Residue Limits (MRL) (Jamieson *et al.,* 2014). Lastly, it is enlisted as a GRAS compound (generally recognised as safe) with a Threshold Limit Value (TLV) reported to be 100 ppm, compared to that of methyl bromide, which is 3 ppm (Agarwal *et al.,* 2015).

This rapid effectiveness of EF has been observed against a wide range of floriculture pests. Research conducted by Ryan *et al.* (2005) effectively controlled onion thrips (*Thrips tabaci* Lind.) (Thysanoptera: Thripidae) and Western flower thrips (WFT) (*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) with a single 2 h exposure of 1.0 % (35 g/m^3) and 0.15% (5 g/m^3) EF respectively. The study reported that WFT were the least susceptible to low EF concentrations and mortality was achieved at a dose rate of 2.4% (80 g/m^3). Simpson *et al.* (2007) achieved high mortality of the grape mealybug (*Pseudococcus maritimus* (Ehrhorn) (Hemiptera: Pseudococcidae)) and *F. occidentalis*, using between 0.04 and 4.7% concentration of reagent grade liquid EF (99.5% purity) at 2°C. Van Epenhuijsen *et al.* (2007) effectively controlled both adult and juvenile *T. tabaci* using a low EF concentration of 2.7 g/m^3 for 2h; however, eggs withstood the low concentration and did not pupate after being fumigated at 27.00 g/m^3 for 2h.

Investigating the suitability of EF at concentrations of 90.00 and 60.00 g/m^3 and a treatment time of 2 h, and concentrations of 60.00 and 30.00 g/m^3 at a treatment time of 1 h on 16 Australian cut flowers including King protea, Rigby (2018) reported that the majority of the cut flowers maintained an

acceptable 7-day vase life. The only exceptions were Umbrella fern (*Sticherus flabellatus)* and Sea star fern™ (*Gleichenia dicarpa)* when treated at the highest dosage (90.00 g/m^3 for 2 h), phytotoxic damage manifested as browning and drying of leaf margins. At lower dosages (18.53 g/m^3 for 1.75 h), Huysamer (2018) reported that *Leucadendron* 'Safari Sunset' exhibited severe deterioration and darkening of foliage by day 1 of vase life studies whereas *Leucospermum* 'Veldfire' exhibited unacceptable quality by day 2 of vase life studies, with phytotoxic damage manifesting as discolouration and brittleness of leaves and desiccation of styles. In contrast, *Protea magnifica* treated at similar dosages maintained overall flower quality comparable to untreated stems. These recent studies are in accordance with Weller and Graver (1998) and Williams and Muhunthan (1998) who observed severe phytotoxic damage on several ornamentals while 'Blushing Bridess and roses withstood EF fumigation at dosage of a 20.00 g/m^3 for 3 hours.

Considering that *S. florida* is a prominent cut flower in South Africa, further investigating the potential of EF as an alternative fumigate is warranted. Concerns regarding the phytotoxic damage caused by the fumigant can potentially be ameliorated. Currently, the commercial practise in the cut flower industry is to pulse commodities with sugar solutions to supplement available carbohydrates to extend vase life and delay the onset of leaf blackening on prone *Protea* species such as *P. neriifolia, P. compacta, P. coronata* and *P. eximia* (Stephens *et al.,* 2001; van Doorn, 2001; Matsikidze, 2018; Vardien *et al.,* 2018). Additionally, application of a synthetic cytokinin, benzyladenine, has been reported to delay cut flower senescence. According to Paull and Chantrachit (2001) dipping or spraying anthurium (*Anthurium andraeanum*), Heliconia (*Heliconia psittacorum* cv. 'Andromeda', *H. chartacea cv*. 'Sexy Pink'), red and pink ginger inflorescence (*Alpinia purpurata*) with 100 mg/L benzyladenine, increased their vase life. Similarly, Fukui *et al.,* (2005) observed vase life extension after spraying cut anthurium with 200 µg/mL benzyladenine post importation. Application of both exogenous benzyladenine and sugar might provide protection against phytotoxic damage induced by EF fumigation (EW Hoffman, personal communication).

The aim of this chapter is to assess the feasibility of EF fumigation as a disinfestation treatment for *Serruria florida* cut flowers destined for export. The cultivar was selected because of its significance to the export market as the leading export Proteaceae cut flower (Cape Flora SA, 2019). Additionally, Weller and Graver (1998) and Williams and Muhunthan (1998) reported that 'Blushing Brides' withstood EF fumigation. The aim was achieved by the following objectives:

i. Determining feasibility through assessment of phytotoxic damage post fumigation at different dosage rates and treatment time, and insect mortality at those dosage rates.

ii. Determining the impact of sugar pulsing and benzyladenine (cytokinin) dipping in preventing phytotoxic damage caused by EF fumigation

4.2 Materials and methods

4.2.1 Plant material

Freshly harvested, export quality *Serruria florida* (Blushing Bride cv 'Rose') stems were sourced from New Caledon Flora, Piketberg, in the Western Cape, South Africa. Flowers were transported from the farms to the Cape Town International airport (DSV Sky Services), held at 4°C, collected and transported to the laboratory within 24 hours of harvesting.

4.2.2 Pretreatment and ethyl formate fumigation regimes

Prior to treatment, the bottom 1 cm of each stem was trimmed off at a 45° angle. Stems were randomly selected and assigned to a treatment (T0, T1, T2 and T3). One set of control stems received no pretreatment solutions or fumigation (T0), and another set was not pre-treated before fumigation (T1). T2 stems were pulsed before fumigation and T3 stems were subjected to dipping and pulsing as pretreatments. The dipping solution was a 4 ml/L Chrysal Viva solution (Chrysal International BV, Netherlands), in which stems were dipped for 3 minutes. The pulsing solution was a 10 ml/L Professional 3 (Prof 3) solution (Chrysal International BV, Netherlands), in which stems were pulsed for 1 h. Pretreatments were made up of stems that were either only pulsed or dipped and pulsed before fumigation. See Table 4.1 for a summary of treatments. After pretreatment flowers were placed in empty buckets for drying at room temperatures (± 25 ℃) for approximately 3 hours before fumigation. Three replicates for each treatment were performed and each replicate consisted of seven stems.

Ethyl formate fumigations were carried out in 14 L glass desiccators at room temperature (25°C ± 2°C) under normal atmospheric conditions. After the drying period stems were placed inside the desiccators and fumigated at the following concentrations and exposure times: 1) 10.00 g/m^3 for 2 hours, 2) 18.53 g/m^3 for 1.75 hours, 3) 20.00 g/m^3 for 1 hour, and 4) 20.00 g/m^3 for 2 hours. Desiccators were left open for an hour at room temperature (± 25°C) allow ventilation between each repeat (Simpson *et al.*, 2004). The concentrations were selected based on previous studies which reported that *S. florida* withstood the EF concentration of 20.00 g/m^3 (Weller and Graver, 1998) and Huysamer

(2018) who achieved 100% insect mortality at a fumigation dosage rate of 18.53 g/m^3 for 1.75 h treatment time.

Table 4.1: Summary of pretreatments and ethyl formate fumigation applied to *Serruria florida* stems

Treatments	**Dip** 4 ml/L Chrysal Viva solution for 3 min	**Pulse** 10 ml/L Prof 3 solution for 1h	**Ethyl formate fumigation regimes**
T0 (controls)	no	no	none
T1 (pre-treated controls)	no	no	10.00 g/m^3 for 2 hours 18.53 g/m^3 for 1.75 hours 20. 00 g/m^3 for 1 hour 20.00 g/m^3 for 2 hours
T2	no	yes	
T3	yes	yes	

To obtain the volume of liquid EF (reagent grade, 97%, Sigma-Aldrich) required for dosing, the free volume inside a desiccator was determined using a flower volume: mass ratio. Then the ratio was used to calculate the dose of liquid EF (µL) necessary to achieve the desired concentration. The liquid EF was pipetted into the glass desiccators through an opening which was immediately sealed for the duration of the treatment. The control stems (T0) were held in the glass desiccators for the duration of the treatments as applied to its fumigated counterparts (T1, T2 and T3).

4.2.3 Post fumigation vase life quality evaluation

Directly after treatment, stem bottoms were recut at 45^0 angles and placed in vases with a solution of 10 ml/L Prof 3 for vase life evaluation at room temperature (25°C ± 2°C). Evaluations were done immediately after fumigation and then every second day for a period of two-weeks (Day 0, 2, 4, 6, 8, 10 and 12). Each inflorescence and foliage were evaluated separately and given a score from 1 to 5, based on a flower evaluation scoring system (see Table 4.2) adapted from the system used by Huysamer (2018), and then summed up to obtain an overall flower score between 0 and 10 for each stem. The overall flower score of 0 indicates that there is no damage. A score of 4 was selected as the marketability limit, indicating that the stems are no longer suitable for commercial sale, while 6 was selected as the vase life limit, that is, the overall flower quality has deteriorated and no longer suitable for vase. Huysamer (2018) vase life limit of 8 was lowered to a mean overall flower score of 6, to suit the damage-prone 'Blushing Brides' florets and the cultivar's short vase life. Figure 4.1 gives a visual representation of the scoring system in Table 4.2.

Table 4.2: Phytotoxicity scoring system used for evaluation of flower quality during vase life studies. Each stem was given a foliage and an inflorescence score, ranging from 0 to 5.

Rating	Inflorescence score	Foliage score
0	No damage to bracts	No damage to stem and leaves
1	≤10% of the leaves with slight discoloration	Slight damage evident; good appearance
2	Some discolouration, still marketable	Many leaves with slight damage or few leaves with major damage, still with good appearance
3	Discoloration expanded; not marketable, still suitable for vase	Upper damage limit (significant colour change). Most leaves with some damage, appearance fair to poor; not suitable for market.
4	Majority of the florets failed to reflex, already desiccating and major discoloration throughout (≈ 90%)	End of vase life; much of foliage damaged or dead (>70% dead), appearance very poor
5	Entire flower discoloured	Complete foliage desiccation

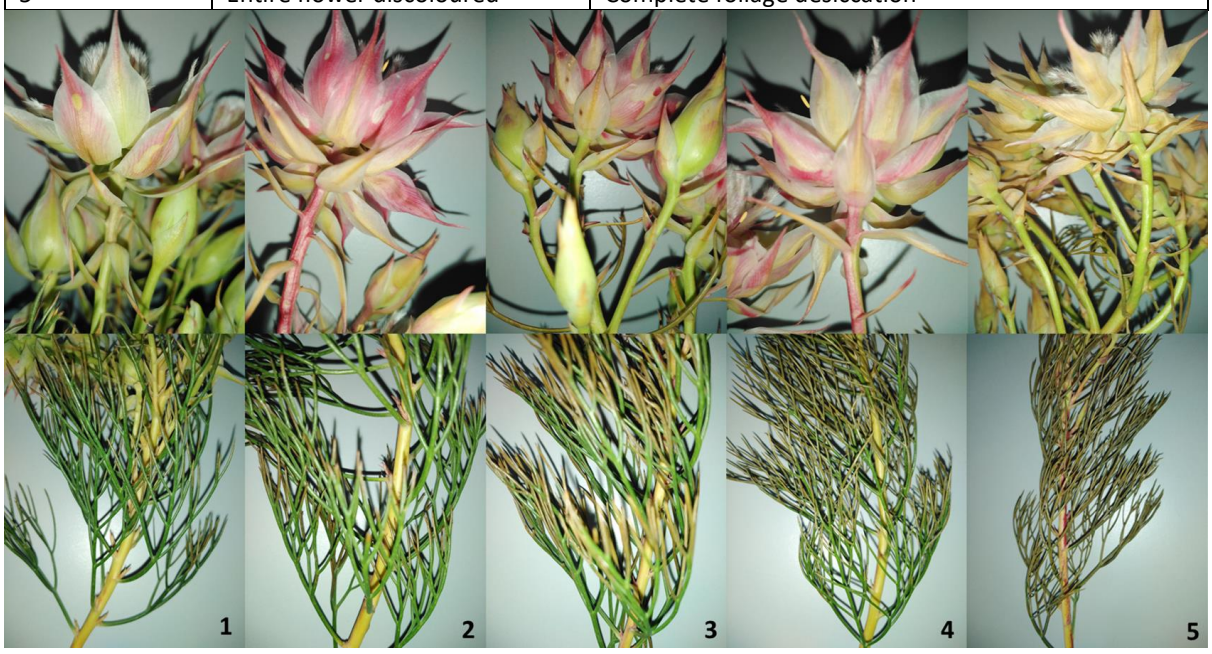


Figure 4:1: Images of *Serruria florida* (cv. Rose) depicting the phytotoxicity scoring system.

4.2.4 Insect mortality assessment

Infested *Protea longifolia* flowers were harvested at the beginning of July 2020 at Coetzenburg Mountain, Stellenbosch in the Western Cape Province, South Africa. *Protea longifolia* was used because its flowering period coincides with 'Blushing Brides' harvesting times and because of its high nectar content it was filled with insects. The harvested flowers were held in zip lock plastic bags during the 30 minutes transport to laboratory facilities. Upon arrival the plant material was transferred into

14 L glass desiccators lined with white paper and subjected to different EF fumigation regimes. The treatments used were 1) 10.00 g/m^3 for 2 hours, 2) 18.53g/m^3 for 1.75 hours, 3) 20.00 g/m^3 for 1 hour, and 4) 20.00 g/m^3 for 2 hours. Two replicates were used per treatment and each replicate consisted of 10 flowers. Posttreatment flowers were removed from desiccators and shaken to dislodge insects over a white paper. Insect mortality was assessed by using a straight teasing needle. All moving and moribund insects were classified as alive, and no movement was classified as dead. Alive insects were collected by a handheld aspirator connected to a sampling vial. The sampling vial was covered with finely meshed gauze to assess the insects again after 24 hours. All samples were preserved in 70% alcohol for morphological identification, which was carried out by the Insect Diagnostic Services in the Department of Conservation Ecology and Entomology at Stellenbosch University.

4.2.5 Statistical analysis

A Mixed model ANOVA in R (lmer package) was performed using STATISTICA version 13.6 (Statistica data analysis software system, Dell Inc). To analyse vase life posttreatment data, two-way analysis of variance (ANOVA) and mixed model ANOVA tests were performed on mean overall flower scores per evaluation over time (days). Means were separated by the Fisher's least significant difference ($P \leq 0.05$).

4.3 Results

4.3.1 Phytotoxicity after EF fumigation, market and vase suitability

At all the EF concentration used, immediately after fumigation treatment, the quality of stems that were fumigated (T1, T2 and T3), did not differ significantly from unfumigated control stems (T0) (Fig. 4.2A-D). Differences between T0 and the fumigated stems were observed on day 2 of the evaluation period. Minor phytotoxic damage on stems which were fumigated with the lowest EF dosage of 10.00 g/m^3 for 2 h (Fig. 4.2A), manifested as discoloration of leaf tips and white spots on florets. This damage was more pronounced on stems that were only fumigated (T1) and stems that were first dipped and pulsed before fumigation (T3). As a result, stems subjected to T1 and T3 breached the marketability limit two days earlier (on day 6) with mean overall flower score of 4.76± 1.09 and 4.71 ± 0.85 respectively. Stems that were not dipped, but only pulsed before fumigation (T2) breached the market limit on day 8 with a mean overall flower score of 4.619 ± 0.9207.

Fumigation with EF at 18.53 g/m^3 for 1.75 hours (Fig. 4.2B) compared to fumigation at 10.00 g/m^3 for 2 h (Fig. 4.2B), decreased the number of days which stems subjected to T1 and T3 were marketable and suitable for vase display by 2 days. On day 4, the stems which were subjected to T1 and T3 exhibited mean overall flower scores of 4.38 ± 0.59 and 4.52 ± 0.81 respectively. However, T1 and T3 were not significantly different ($p > 0.01$) from T2 which exhibited a mean overall inflorescence score of 3.71 ± 0.85. However, in stems which were only fumigated florets failed to reflex (i.e., bracts failed to loosen, remained closed not exposing the central flower mass) and therefore desiccated while still closed, while stems which were first subjected to pretreatment reflexed during pulsing and consequently their florets were open. T2 stems florets failure to reflex was also observed on stems which were subjected 20.00 g/m^3 for 1 h and 20.00 g/m^3 for 2 h.

Increasing EF concentration to 20.00 g/m^3 and decreasing the treatment time to 1 hour resulted in a significant decrease on the observed phytotoxic damage especially for T1 and T2 (Fig. 4.2C). However, stems subjected to T1 and T2 only breached the market limit on day 8, exhibiting mean overall flower scores of 4.57 ± 1.47 and 4.33 ± 2.22 respectively and only breaching the vase life limit on day 12 (i.e., the end of vase life studies) when mean overall flower scores were 6.19 ± 1.47 and 6.29 ± 1.35 respectively. However, decreasing the treatment time to 1 hour did not have an impact on T3 stems, which were unmarketable by day 4 exhibiting a mean overall inflorescence score of 4.62 ± 1.36. Consequently, exceeding the vase limit on day 6 with a mean overall inflorescence score of 6.14 ± 1.28.

Maintaining EF concentration at 20.00 g/m^3 and increasing the treatment time to for 2 hours was detrimental to the flower quality regardless of the pretreatment regime (Fig. 4.2D). High phytotoxicity was observed on T3 stems, which manifested as intense browning of the foliage and discoloration on the inflorescence, which made the inflorescence look almost yellow. As a result, T3 stems had breached both the marketability and vase life limit by day 4, exhibiting a mean overall inflorescence score of 6.38 ± 1.20. Doubling the treatment time, decreased the marketability days of the stems which were subjected to T1 and T2. Instead of the 8 days which was observed at EF concentration of 20.00 g/m^3 and treatment time of 1 hour, the stems were unmarketable 4 days post fumigation with mean overall flower scores of 4.57 ± 0.75 ± and 3.95 ± 1.07 respectively. Additionally, T1 and T2 stems breached the vase limit by day 8, with stems exhibiting mean overall flower scores of 6.05 ± 0.92 and 6.33 ± 0.91 respectively.

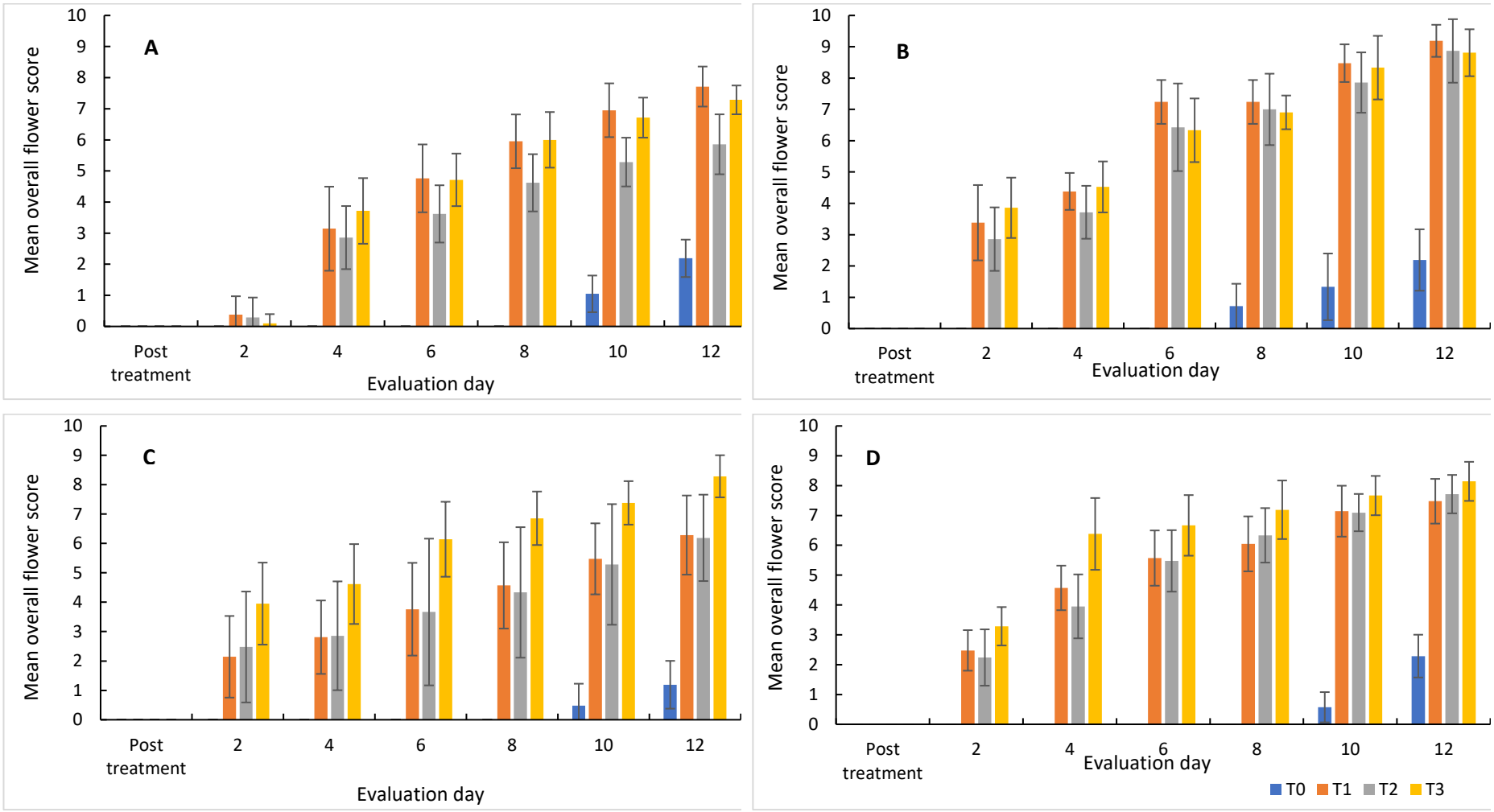


Figure 4:2: Mean overall flower scores of *Serruria florida* 'Blushing Brides' stems that were dipped in 4 ml/L Chrysal Viva solution for 3 min and/or pulsed with 10 ml/L Prof 3 solution for 1h and fumigated using ethyl formate at dosages of 10.00 g/m^3 for 2 h (A), 18. 53 g/m^3 for 1. 75 h (B), 20.00 g/m^3 for 1 h (C), and 20.00 g/m^3 for 2 h (D) and evaluated for phytotoxic damage over a period of 12 days at room temperatures (± 25°C). Treatments were: unfumigated control (T0); fumigated without dip or pulse (T1); pulsed before fumigation (T2); dipped and pulsed before fumigation (T3).

4.3.2 Insect mortality trials

Complete control (100% mortality) was achieved during fumigation at EF concentration of 18. 53 g/m^3 for 1.75 h and 20.00 g/m^3 for 1 and 2 h treatment time. Decreasing EF concentration decreased the mortality rate of the encountered insects resulting in 50% mortality being achieved during fumigation. Although, 100% mortality was recorded after 24 hours for insects from the Diptera and Hymenoptera orders. The Coleoptera order was the least susceptible to EF concentrations of 10.00 g/m^3 for 2h. The species from the Carabidae and Staphylinidae family were either moribund or actively moving post fumigation. Mortality assessment after 24 hours showed that moribund insects had recovered, and no further mortality had been achieved. However, the EF concentration of 10.00 g/m^3 for 2h remained effective for western flower thrips and other unidentified mixed thrips and vinegar flies, *Drosophila melanogaster* (Hemiptera: Drosophilidae). As a result, 100% was achieved for the susceptible species during treatment.

The most encountered insects were from the Coleoptera order specifically the Carabidae family (ground beetles) (Fig. 4.3) followed by the Staphylinidae family (rove beetles). Only a solitary species from the Scarabidae family (scarab beetles) was encountered. The second most abundant order was the Thysanoptera, which was dominated by the western flower thrips, *Frankliniella occidentalis* (Thripidae). The Diptera order consisted of the Drosophilidae family (flies) and Sciaridae (gnats). The least encountered order was the Hymenoptera which consisted of the following in descending order; Anthocaridae (pirate bugs or flower bugs), Lygaeidae (milkweed bugs or seed bugs) and Ichneumonidae (wasps).

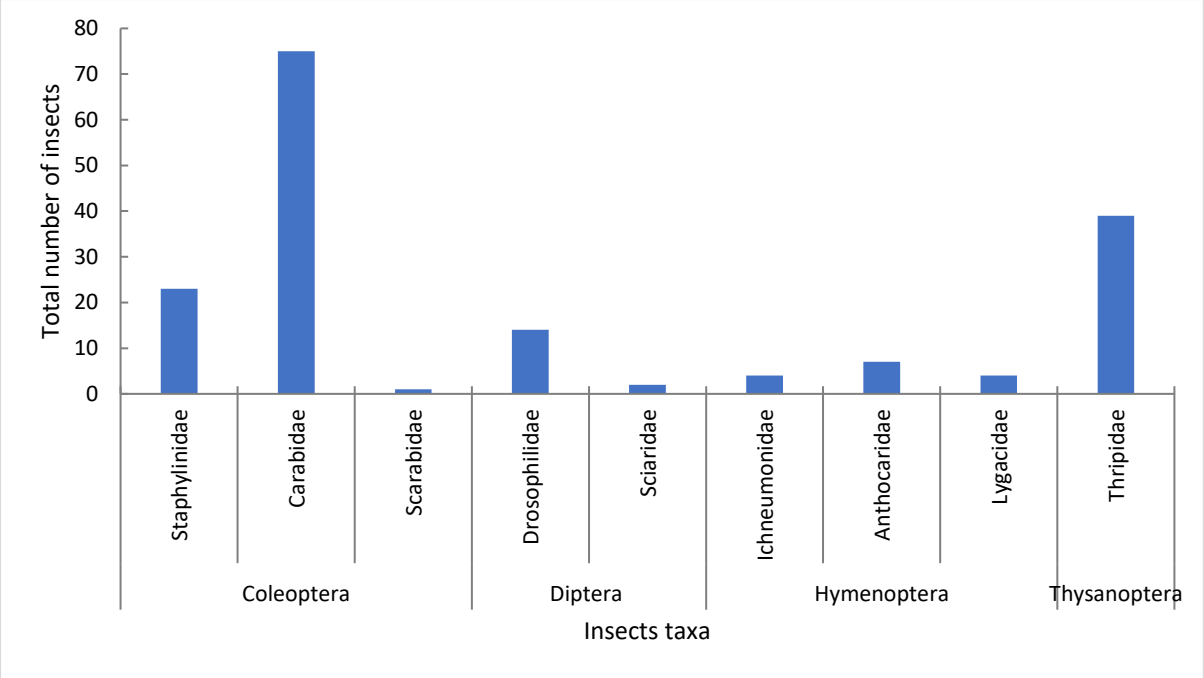


Figure 4:3: Number of insects identified from *Protea longifolia* flowers post ethyl formate fumigation. The treatments used were 1) 10.00 g/m^3 for 2 hours, 2) 18.53 g/m^3 for 1.75 hours, 3) 20.00 g/m^3 for 1 hour and 4) 20.00 g/m^3 for 2 hours.

4.4 Discussion

The presence of arthropods on cut flowers requires disinfestation before export, especially to discerning international markets. This study demonstrates that EF fumigation is effective in controlling a wide range of Proteaceae pests. In this study, thrips (Thripidae) were found to be the most susceptible to EF fumigation, 100% mortality was achieved using the lowest concentration of EF (10.00 g/m^3). These results echo the previous studies that effectively controlled western flower thrips with low EF concentration of 10.20 g/m^3 (Simpson *et al.,* 2007), 5.80 g/m^3 (Pupin *et al.*, 2013) and 18.75 g/m^3 (Huysamer 2018; Huysamer *et al.,* 2019) and for onion thrips (*Thrips tabaci)* at EF concentration of 8.00 g/m^3, at a short exposure time of 30 minutes. According to Malan (2012), thrips infest actively growing shoot tips and developing flowers of *S. florida* and the insectdamage is worse in late Autumn. Additionally, thrip damage on *S. florida* is associated with *Alternaria* infestation (Malan, 2012). Effective postharvest control of thrips on *S. florida* will additionally aid in preventing *Alternaria* infection, especially on flowers which were harvested prematurely.

The concentration used in the present study was significantly lower compared to concentrations which were reported in previous studies which specifically focussed on species from the Lygaeidae

family. Grout and Stoltz (2016) successfully controlled grain chinch bug, *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae) using EF concentration of 42.00 g/m^3 for 6h. Similarly, Smit *et al.* (2020) reported that an EF concentration of 50.00 g/m^3 for 1 h was necessary to achieve 100% mortality of grain chinch bug. However, both the studies were carried out in the presence of fruit rather than ornamentals. In the present study, vinegar flies, *Drosophila melanogaster* (Hemiptera: Drosophilidae) were effectively controlled at all the tested EF concentrations, including the lower EF concentration of 10.00 g/m^3 for 2 h. In accordance, complete control using a lower EF concentration of 1.0 g/m^3 and treatment time of 4 h was recently reported on adult stage *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) (Kwon *et al.,* 2021).

In the present study, the order Coleoptera required higher EF concentration to achieve 100% mortality. For species from the Carabidae and Staphylinidae family, mortality was achieved when EF concentration was doubled from 10.00 g/m^3 to 20.00 g/m^3. This rapid action of EF on other Coleoptera species has been demonstrated by Damcevski and Annis (2006) who reported to achieve 99% mortality of *Sitophilus oryzae* (Coleoptera: Curculionidae) at 11.2 mg/L^1, for the exposures without wheat and 81.20 mg/L EF, for the exposures carried out on 1500g wheat at 97% relative humidity. Ethyl formate is known for its rapid absorption which leads to its accelerated decrease during treatment (Simpson *et al.,* 2004). As a result, EF effectiveness decreased when the product being fumigated absorbed the fumigant (van Epenhuijsen *et al.,* 2007). Therefore, although low concentrations may be required to achieve 100% mortality under experimental conditions, larger scale fumigation will necessitate higher EF concentrations.

Contrary to Weller and Graver (1998) who reported that there was no damage observed at fumigations of 20.00 g/m^3 for 3 hours, this study found that prolonged EF fumigations (18.53 g/m^3 for 1.75 h and 20.00 g/m^3 for 2 h) were not suitable for *S. florida*, as it decreased the vase life of the commodities. The phytotoxic damage at longer treatment times (1.75h and 2h) manifested as discoloration and browning of the foliage. The phytotoxic damage was more evident on mature florets which had already opened during fumigation. The discolouration of foliage can likely be attributed to chlorophyll destruction. Kyung *et al.* (2019) observed that EF fumigated foliage nursery plant species exhibited a decrease in chlorophyll content, which resulted in browning within a week of fumigation.

Ethyl formate's high phytotoxicity has been attributed to the high absorption capacity of plant material, as observed at higher concentrations and longer exposure times (Kyung *et al.,* 2019; Cho *et al.,* 2020). Decreasing the concentration and treatment time reduce lethality of the fumigant.

Therefore, Kyung *et al.* (2019) recommended the combination of EF with other gases (e.g., O_2, CO_2, PH3) to reduce treatment time and dosage rate.

Dipping florets and foliage in a solution containing 4 ml/L Chrysal Viva (with 6-benzyladenine, a synthetic cytokinin, as active ingredient) and pulsing did not prevent phytotoxic damage induced by EF fumigation on *S. florida.* Contrary to the anticipated results, at all EF concentrations investigated in this study, phytotoxic damage observed on Chrysal Viva dipped stems was not significantly different from stems which were only fumigated without any pretreatment. Positive results have previously been reported regarding the impact of benzyladenine in preventing phytotoxic damage of cut flowers under stress. Han (2001) reported that pre-treating Asiatic and Oriental lilies with benzyladenine prior to cold storage at 3.3°C prevented leaf yellowing and browning. Similarly, Fukui *et al.* (2005) reported that vase life of cut *Anthurium* flowers was extended up to 22 days by spraying stems with 200 µg/mL benzyladenine. Applying benzyladenine at an adequate concentration is important to obtain maximum increase in vase life of treated cut flowers (Fukui *et al.,* 2005; Shimizu-Yumoto and Ichimura, 2013). Additionally, the type of cytokinin and method of application has an impact on the effectiveness of the product. According to Shimizu-Yumoto and Ichimura (2013), pulsing cut roses with benzyladenine for 90 minutes is ineffective in hindering petal senescence; however, directly spraying the flower buds extended vase life, whilst directly spraying/dipping were shown to be ineffective for dahlia florets.

The experimental results indicate that Prof 3 (sugar-based preservative) pulsing (without dipping) enhanced the quality of *S. florida* and extended the vase life after EF fumigation at the low concentration of 10.00 g/m^3. However, the pulse did not cause any significant difference when EF concentrations were increased to 18.53 and 20.00 g/m^3. Similar results were observed by Huysamer (2018), who pulsed Proteaceae cut flowers with a 6% (w/v) sucrose solution and 5% (w/v) glucose solution prior to EF fumigation, and still reported high phytotoxic damage across all treated cut Proteaceae.

4.5 Conclusion

The results from this study echoes previous studies on cut flowers, which reported that EF fumigation is highly effective in controlling insects, but can be phytotoxic on plant material, especially at longer treatment times. However, decreasing the treatment time to 1 hour, resulted in a significant decrease in the observed phytotoxic damage. *Serruria florida* remains the most exported Proteaceae cut flower

from South Africa, and for the country to continue exporting high quantities of cut flowers current studies on alternative treatment methods are a necessity. Taking in consideration that, firstly, EF is a naturally occurring plant volatile which breaks down leaving untraceable residues, and secondly, it is enlisted as a 'generally recognised as safe' (GRAS) compound with Threshold Limit Value (TLV) of 100 ppm (Agarwal *et al.,* 2015), the fumigant is worth investigating further. Future studies should investigate the potential of using lower EF concentrations in combination with extended treatment time. Additionally, applying EF in combination with carrier gases such as nitrogen or carbon dioxide will allow vapour to be dispensed quickly and therefore shorten treatment time (Brown *et al.,* 2018), thereby decreasing the potential phytotoxicity of EF.

4.6 References

Agarwal, M., Ren, Y., Newman, J. & Learmonth, S. (2015). Ethyl formate: a potential disinfestation treatment for eucalyptus weevil (*Gonipterus platensis*) (Coleoptera: Curculionidae) in apples. *Journal of Economic Entomology*, 108*,* 2566-2571.

Brown, S.D., Page-Weir, N.E., Hawthorne, A.J., Wilkinson, R.T., Jamieson, L.E., Hartnett, D.E. & Chhagan, A. (2018). Mortality of kiwifruit pests (Coccoidea) exposed to ethyl formate delivered using either carbon dioxide or nitrogen carrier gases. *New Zealand Plant Protection*, 71, 102-111.

Cape Flora SA. (2019). www.capeflorasa.co.za/statistics/ Cape Flora SA statistics (Accessed September 2019).

Cho, S.W., Kim, H.K., Kim, B.S., Yang, J.O. & Kim, G.H. (2020). Combinatory effect of ethyl formate and phosphine fumigation on *Pseudococcus longispinus* and *P. orchidicola* (Hemiptera: Pseudococcidae) mortality and phytotoxicity to 13 foliage nursery plants. *Journal of Asia-Pacific Entomology*, 23, 152-158.

Criley, R.A. (2001). Proteaceae: Beyond the big three. *Acta Horticulturae*, 545, 79–85.

Damcevski, K.A. & Annis, P.C. (2006). Influence of grain and relative humidity on the mortality of *Sitophilus oryzae* (L.) adults exposed to ethyl formate vapour. *Journal of Stored Products Research,* 42, 61-74.

de Villiers, M.J. (2004). *Molecular systematics of the Western Cape genus Serruria Salisb. (Proteaceae L.) based on DNA sequence data* (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Fukui, R., Kikuchi, S., Ichida, Y. & Honjo, H. (2005). Vase life of imported Anthurium flowers evaluated in Japan in relation to the effects of post importation benzyladenine treatment. *HortScience*, *40*, 1439-1443.

Grout, T.G. & Stoltz, K.C. (2016). Eliminating *Macchiademus diplopterus* (Hemiptera: Lygaeidae) and *Siculobata sicula* (Acari: Oribatulidae) from export fruit using ethyl formate. *Journal of Economic Entomology*, 109, 2329-2333.

Han, S.S. (2001). Benzyladenine and gibberellins improve postharvest quality of cut Asiatic and Oriental lilies. *HortScience,* 36, 741-745.

Hansen, J.D. & Hara, A.H. (1994). A review of postharvest disinfestation of cut flowers and foliage with special reference to tropicals. *Postharvest Biology and Technology,* 4, 193-212.

Haritos, V.S. & Dojchinov, G. (2003). Cytochrome c oxidase inhibition in the rice weevil *Sitophilus oryzae* (L.) by formate, the toxic metabolite of volatile alkyl formates. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 136, 135-143.

Huysamer, A.J. (2018). An assessment of alternative postharvest technologies for the disinfestation of fresh Cape Flora cut flowers for export from South Africa (MSc Thesis, Stellenbosch: Stellenbosch University).

Huysamer, A., Hoffman, E.W. & Johnson, S. (2019). Postharvest insect pest control for western flower thrips, *Frankliniella occidentalis*, in exported cut Proteaceae flowers. *Acta Horticulturae,* 1263, 507 - 513.

Jamieson, L.E., Griffin, M.J., Page-Weir, N.E.M., Chhagan, A., Redpath, S.P. & Connolly, P.G. (2014). Developing ethyl formate treatment for disinfesting pipfruit. *New Zealand Plant Protection,* 67, 96-102.

Kwon, T.H., Park, C.G., Lee, B.H., Zarders, D.R., Roh, G.H., Kendra, P.E. & Cha, D.H. (2021). Ethyl formate fumigation and ethyl formate plus cold treatment combination as potential phytosanitary quarantine treatments of *Drosophila suzukii* in blueberries. *Journal of Asia-Pacific Entomology,* 24, 129-135.

Kyung, Y., Kim, H.K., Cho, S.W., Kim, B.S., Yang, J.O., Koo, H.N. & Kim, G.H. (2019). Comparison of the efficacy and phytotoxicity of phosphine and ethyl formate for controlling *Pseudococcus longispinus* (Hemiptera: Pseudococcidae) and *Pseudococcus orchidicola* on imported foliage nursery plants. *Journal of Economic Entomology*, 112, 2149-2156.

Matsikidze, S.N. (2018). Feasibility of closed ventilation and automatic ventilation for sea freight of Proteaceae cut flower stems. Master's thesis. Stellenbosch: Stellenbosch University [Online]. Available: https://scholar.ac.za [2019, May 30]

Paull, R.E. & Chantrachit, T. (2001). Benzyladenine and the vase life of tropical ornamentals. *Postharvest Biology and Technology*, 21, 303-310.

Pupin, F., Bikoba, V., Biasi, W.B., Pedroso, G.M., Ouyang, Y., Grafton-Cardwell, E.E. & Mitcham, E.J. (2013). Postharvest control of western flower thrips (Thysanoptera: Thripidae) and California red scale (Hemiptera: Diaspididae) with ethyl formate and its impact on citrus fruit quality. *Journal of Economic Entomology*, 106, 2341-2348.

Rigby, E.M. (2018). Disinfestation of floriculture products with ethyl formate fumigant: Vapormate®. *Acta Horticulturae,* 1205*,* 351-358.

Rourke, J.P. (1990). Four new species of *Serruria* (Proteaceae) from the south-western Cape. *South African Journal of Botany*, 56, 497-505.

Ryan, R.F., Krishna, H., Bishop, S.R., Brash, D. & Mitcham, E. (2005). Development of vapormate™ for postharvest quality. *Acta Horticulturae,* 687, 413-416.

Shimizu-Yumoto, H. & Ichimura, K. (2013). Postharvest characteristics of cut dahlia flowers with a focus on ethylene and effectiveness of 6-benzylaminopurine treatments in extending vase life. *Postharvest Biology and Technology*, 86, 479-486.

Simpson, T., Bikoba, V. & Mitcham, E.J. (2004). Effects of ethyl formate on fruit quality and target pest mortality for harvested strawberries. *Postharvest Biology and Technology*, *34*, 313-319.

Simpson, T., Bikoba, V., Tipping, C. & Mitcham, E.J. (2007). Ethyl formate as a postharvest fumigant for selected pests of table grapes. *Journal of Economic Entomology*, 100, 1084 -1090.

Smit, R., Jooste, M.M., Addison, M.F. & Johnson, S.A. (2020). Ethyl formate fumigation: Its effect on stone and pome fruit quality, and grain chinch bug (*Macchiademus diplopterus*) mortality. *Scientia Horticulturae*, 261, 1-9.

Song, C. & Scharf, M.E. (2008). Formic acid: A neurologically active, hydrolyzed metabolite of insecticidal formate esters. *Pesticide Biochemistry and Physiology*, 92, 77-82.

Song, C. & Scharf, M.E. (2009). Mitochondrial impacts of insecticidal formate esters in insecticide-resistant and insecticide-susceptible *Drosophila melanogaster*. *Pest Management Science: formerly Pesticide Science*, 65, 697-703.

Stephens, I.A., Jacobs, G. & Holcroft, D.M. (2001). Glucose prevents leaf blackening in 'Sylvia' proteas. *Postharvest Biology and Technology*, 23, 237-240.

van Doorn, W.G. (2001). Leaf blackening in Protea flowers: recent developments. *Acta Horticulturae,* 545, 197-204.

van Epenhuijsen, C.W., Hedderley, D.I., Somerfield, K.G. & Brash, D.W. (2007). Efficacy of ethyl formate and ethyl acetate for the control of onion thrips (Thrips tabaci). *New Zealand Journal of Crop and Horticultural Science*, 35, 267-274.

Vardien, W., Jacobs, G. & Hoffman, E.W. (2018). The efficacy of alternate sugars, trehalose and lactulose, as a postharvest treatment in *Protea* 'Sylvia', to alleviate leaf blackening. *Acta Horticulturae*, 1201, 463-470.

Weller, G.L. & Graver, J.V.S. (1998). Cut flower disinfestation: assessment of replacement fumigants for methyl bromide. *Postharvest Biology and Technology*, 14, 325-333.

Williams, P. & Muhunthan, M. (1998). Fumigants for postharvest control of insect pests of cut flowers. *Acta Horticulturae* 464, 291-296.

5. General discussion

Export of Proteaceae cut flowers from South Africa is significantly affected by infestations of arthropods. Being indigenous to South Africa, the Proteaceae is associated with a wide range of insect *pests*, which infest different parts of the shrub, causing a major threat to the South African Proteaceae export industry (Malan, 2012). These pests have the potential to become serious invaders in other regions of the world where Proteaceae or related families are cultivated, especially in areas with climatic similarity. Not only is the cost of rejection and destroying a consignment astronomical, but it can be a major threat to an industry, as continued access to existing and new markets can be seriously jeopardised. The absence of new chemicals and the cost of registering new effective chemicals, as well as the increasing demand for environmentally friendly alternatives, has led to the demand for minimal use of traditional pesticides, therefore increasing the need to investigate and introduce precautionary postharvest phytosanitary mitigation technologies, in order to provide quarantine security for ongoing international export, without decreasing productivity and marketability.

Controlled Atmosphere Temperature Treatment System (CATTS) technology and ethyl formate (EF) fumigation are such alternative phytosanitary control techniques which were investigated in the current study. CATTS technology combines the effects of a short exposure to high temperature and atmospheric stress, in the form of a low oxygen (1%)/ high carbon dioxide (15%) environment to control phytosanitary pests (Neven and Mitcham, 1996). EF hydrolyses into an aliphatic alcohol component (ethanol) and active metabolite formic acid in an organism because of carboxylic acid esterases (Haritos and Dojchinov, 2003). The presence of formic acid inhibits cytochrome *c* oxidase, thereby impairing oxidative phosphorylation leading to depletion of cellular energy stores (Haritos and Dojchinov, 2003) and consequently causing insect mortality.

In Chapter 2, the research focused on assessing the efficacy of CATTS at a target temperature of 40°C. The targeted temperature was reached by using a 35°C/hr ramp rate from 23°C to 40°C. The potential of pulsing with 10 ml/L Prof 3 (Chrysal Professional 3 vase and foam solution) and hydrating with 5 ml/L Prof 2 (Chrysal Professional 2 transport and display solution) in preventing CATTS-induced leaf blackening and other phytotoxic damage was investigated. The feasibility of CATTS treatments was studied on export quality *Protea magnifica* 'Barbi', *Leucospermum lineare x cordifolium* 'Succession', *Leucospermum patersonii x cordifolium* 'High Gold' and *Leucadendron salignum* 'Goldstrike' cut flower stems. The treated cut flowers were subjected to immediate vase life evaluations and freight

simulation studies. Thrips infested flowers of *Leucospermum* cultivars 'High Gold' (*Leucospermum patersonii x cordifolium*) and 'Jelena' (*Leucospermum cuneiforme x cordifolium*) flowers were CATTS treated to evaluate insect mortality posttreatment. Western flower thrips (WFT), *Frankliniella occidentalis* was the targeted pest. The achieved mortality percentage was relatively low, as counting the number of live insects proved that more than 50% of the encountered insects were alive. These results were contrary to Huysamer (2018) who reported that 100% mortality was achieved within 24 hours of subjecting the WFT and protea itch mite, *Proctolaelaps vandenbergii,* using similar treatment conditions.

CATTS treatments caused severe damage on *Protea* 'Barbi'. Phytotoxic damage on this cultivar manifested as premature leaf blackening, inflorescence wilting and intense discoloration. Results from when *Protea* 'Barbi' was evaluated immediately post CATTS treatment and after air freight simulation indicate that pulsing inhibited the resultant phytotoxic damage. Similarly, hydrating was effective in preventing phytotoxic damage during immediate evaluation however, had no significant impact when the stems were subjected to freight simulation. Additionally, hydrating 'Barbi' during CATTS treatment promoted high humidity and condensation of water inside the inflorescence, which resulted in inflorescence decay during the simulated sea freight storage. Neither pulsing nor hydrating the stems during CATTS treatment inhibited the resultant phytotoxic damage when *Protea* 'Barbi' stems were subjected to sea freight simulation. Similarly, *Leucadendron* 'Goldstrike' did not withstand the combination of CATTS treatment and sea freight simulation. Phytotoxic damage manifested as foliage discolouration, wilting and exhibited a condition which resembled severe chilling injury. However, when *Leucadendron* 'Goldstrike' were pulsed and/or hydrated during CATTS treatment, phytotoxic damage was decreased, although the treated stems had a significantly shorter vase life compared to untreated control stems. *Leucospermum* cultivars 'Succession' and 'High Gold' withstood CATTS treatments and maintained good quality during vase life studies. Pretreatment pulsing and hydrating resulted in premature style reflexion, which made package for transport and long-term cold storage challenging. Therefore, pretreatments are not feasible for these cultivars.

Chapter 3 assessed the potential of thiabendazole (TBZ) in preventing CATTS-induced leaf blackening through evaluating posttreatment quality, storage capacity and shelf life of the treated *Protea* 'Barbi' and *Protea eximia x susannae* 'Sylvia'. Treatments were applied by dipping the cut stems in 2% thiabendazole solution. Post TBZ dipping, the flowers were stored at room temperature to allow the foliage to dry before being subjected to CATTS treatments. Thiabendazole dipping significantly reduced the incidence of leaf blackening of *Protea* 'Barbi', both without and with freight (air and sea)

simulations. These results proved that TBZ dipping is a more viable preharvest technique for *Protea* cultivars, compared to pretreatment pulsing with Prof 3 or hydrating with Prof 2 which was assessed in Chapter 2. On *Protea* 'Sylvia' stems which were subjected to sea freight simulation, TBZ dipped stems were not significantly different from untreated stems. Consequently, both the untreated and TBZ dipped stems exhibited severe yellowing. This yellowing condition was recently attributed to a decrease in chlorophyll which happens during *Protea* 'Sylvia' cold storage (Matsikidze *et al.,* 2018).

More research is required to further develop CATTS technology, and future studies need to combine it with posttreatment cooling down of the treated flowers to eliminate inflorescence decay, especially of *Protea* cultivars. Using closed ventilation during storage with modified atmosphere of low O_2 and 15% CO_2. maintains high relative humidity and decrease *vapor pressure differentials c*onsequently, limiting moisture loss. Therefore, its addition to the postharvest protocol will aid in decreasing the factors which contributes towards product deterioration. The use of modified atmosphere will be a necessity for both *Protea* and *Leucadendron* cultivars as they were the most affected by prolonged cold storage. Philosoph-Hadas *et al.* (2010) reported that this technique aided in preserving the quality of *Leucadendron* 'Safari Sunset' during prolonged sea transport.

Lastly, Chapter 4 determined the feasibility of ethyl formate (EF) fumigation on export quality 'Blushing Brides', *Serruria florida* and insect mortality. The dose ranges and exposure times were 10.00 g/m^3 for 2 hours; 18.53 g/m^3 for 1.75 hours; 20.00 g/m^3 for 1 hour and 20.00 g/m^3 for 2 hours. Additionally, the study determined the impact of dipping the stems in solution of 4 ml/L Chrysal Viva solution and/or pulsing with 10 ml/L Prof 3 in preventing phytotoxic damage caused by EF fumigation. The treated insects were from the Coleoptera order, specifically Carabidae family (ground beetles), Staphylinidae family (rove beetles), Scarabidae family (scarab beetles), Thysanoptera order, which was dominated by the western flower thrips, *Frankliniella occidentalis*,Diptera order which consisted of the Drosophilidae family (flies) and Sciaridae (gnats), and the Hymenoptera order which consisted of the Anthocaridae (pirate bugs or flower bugs), Lygaeidae (milkweed bugs or seed bugs) and Ichneumonidae (wasps).

Effective insect control (100% mortality) was achieved during fumigation at EF concentration of 18. 53 g/m^3 for 1.75 h and 20.00 g/m^3 for 1 and 2 h treatment times. These results are consistent with other studies reporting that EF exhibits strong toxicity against insects. Although, EF fumigation is highly effective in causing insect mortality, no further research can be recommended for this technology. The results concur with previous studies which reported high sorption rates of EF and severe

phytotoxicity on fresh commodities (Huysamer, 2018; Rigby, 2018). The current study focused on 'Blushing Brides', based on studies by Weller and Graver (1998) and Williams and Muhunthan (1998) who reported that 'Blushing Brides' withstood EF fumigation. However, the results obtained in our study proved that fumigating 'Blushing Brides' significantly reduced the vase life of the treated stems, especially at longer exposure times. Phytotoxic damage mainly manifested as discoloration and browning of the foliage especially on mature florets which had already opened during fumigation. *Serruria florida* remains the most exported Proteaceae cut flower from South Africa, and for the country to continue exporting high quantities of cut flowers, further studies on alternative treatment methods are a necessity.

In conclusion, CATTS treatments seem to be the more viable option in terms of flower quality maintenance but require further research on more export cultivars and additional insect pests to find feasible solutions to the problem of phytosanitary pests on our valuable and unique cut flower commodities.

5.1 References

Haritos, V.S. & Dojchinov, G. (2003). Cytochrome *c* oxidase inhibition in the rice weevil *Sitophilus oryzae* (L.) by formate, the toxic metabolite of volatile alkyl formates. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 136, 135-143.

Huysamer, A.J. (2018). An assessment of alternative postharvest technologies for the disinfestation of fresh Cape Flora cut flowers for export from South Africa (MSc Thesis, Stellenbosch: Stellenbosch University).

Malan, G. (2012). Protea cultivation: from concept to carton. Stellenbosch: SUN MeDIA.

Matsikidze, S.N., Botes, W.J., Huysamer, M. & Hoffman, E.W. (2018). Feasibility of automatic ventilation technology for sea freight of *Protea* cut flower products. *Acta Horticulturae*, 1201, 455-462.

Neven, L.G. & Mitcham, E.J. (1996). CATTS (Controlled Atmosphere Temperature Treatment System): A novel tool for the development of quarantine treatments. *American Entomologist*, 42, 56-59.

Philosoph-Hadas, S., Perzelan, Y., Rosenberg, I., Droby, S. & Meir, S. (2010). *Leucadendron* 'Safari Sunset': postharvest treatments to improve quality of cut foliage during prolonged sea shipment. *Acta Horticulturae*, 869, 207-218.

Rigby, E.M. (2018). Disinfestation of floriculture products with ethyl formate fumigant: Vapormate®. *Acta Horticulturae,* 1205*,* 351-358.

Weller, G.L. & Graver, J.V.S. (1998). Cut flower disinfestation: assessment of replacement fumigants for methyl bromide. *Postharvest Biology and Technology*, 14, 325-333.

Williams, P. & Muhunthan, M. (1998). Fumigants for postharvest control of insect pests of cut flowers. *Acta Horticulturae*, 464, 291-296.