AN ASSESSMENT OF ALTERNATIVE POSTHARVEST TECHNOLOGIES FOR THE DISINFESTATION OF FRESH CAPE FLORA CUT FLOWERS FOR EXPORT FROM SOUTH AFRICA

By

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

A successful industry has developed around the export of fresh Proteaceae cut flowers from South Africa. Phytosanitary insects are a barrier to export, as South African Proteaceae associates with a considerable entomofauna. The development of alternative postharvest disinfestation technologies could reduce these interceptions and promote market access. Surveys on export material were conducted to determine which pests are most problematic when exporting Proteaceae. A total of 82 interceptions were made, comprising of eight insect orders and 26 insect families. Although many interceptions were as a result of solitary individuals, multiple interceptions consisted of many individuals of western flower thrips (*Frankliniella occidentalis*) and protea itch mite (*Procotolaelaps vandenbergii*). These pests were selected as the key pests on which to focus for disinfestation using alternative postharvest technologies not yet utilised for Proteaceae. Controlled Atmosphere and Temperature Treatment Systems (CATTS) technology was assessed as a potential disinfestation tool for fresh Proteaceae cut flowers. The tested commodities were *Leucospermum* 'Veldfire', *Protea magnifica* 'Barbi', *Leucadendron* 'Safari sunset' and 'Jade pearl', and Geraldton wax 'Ofir' (Myrtaceae). CATTS treatments consisted of temperature ramps of 35°C/hour and 30°C/hour from 23°C to 40°C, with a 15 min soak at 40°C, and 35°C/hour and 30°C/hour from 23°C to 50°C, with a 15 min soak at 50°C, under modified atmospheres of 1% O_2, 15% CO_2 in N_2. Treated stems were subjected to vase life studies after treatment, or following air- and sea-freight storage simulations at 2°C for 3 or 21 days respectively. *Leucospermum* 'Veldfire' did not withstand treatments, as style wilting reduced overall quality. *Protea magnifica* 'Barbi' withstood some treatments, maintaining

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comparable quality to control stems in the vase immediately after treatment. Both *Leucadendron* commodities withstood treatments well, and maintained marketable quality following treatment, air- and sea-freight simulations. Geraldton wax 'Ofir' maintained quality in vase immediately after and following air-freight simulations. CATTS treatments of 35°C/hour and 30°C/hour to 40°C in 1% O_2, 15% CO_2 in N_2 resulted in 100% mortality in western flower thrips and protea itch mites within 24 hours of treatment. Postharvest fumigation treatment with ethyl formate (EF) was also assessed as a potential disinfestation technology. Concentrations ranged from 18.53g/m^3 to 151.47g/m^3 EF, and durations ranging from 30 mins to 3 hours for the same cut flower commodities listed above for CATTS treatments. Further trials on Geraldton wax 'Ofir' consisted of 10g/m^3 and 20g/m^3 for 1 and 2 hours. All treatments resulted in reduction in overall quality of treated fresh goods. EF fumigations of 18.53g/m^3 for 1 and 2 hours achieved 100% mortality within 24 hours of treatment in western flower thrips and protea itch mites, but excessive post fumigation damage renders EF unsuitable. The information generated from this study has highlighted the most problematic phytosanitary pests in export consignments of fresh Proteaceae from South Africa. It has also highlighted a potential postharvest technology for integration into current disinfestation strategies, and refuted another. This information can assist in the development of postharvest disinfestation strategies, ultimately reducing the phytosanitary pressures and promoting the export of fresh Proteaceae cut flowers from South Africa.

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Opsomming

'n Suksesvolle bedryf het ontwikkel rondom die uitvoer van vars Proteaceae snyblomme uit Suid-Afrika. Fitosanitêre insekte is 'n belangrike handelsversperring, aangesien Suid-Afrikaanse Proteaceae met 'n aansienlike en diverse entomofauna geassosieer word. Die ontwikkeling van alternatiewe na-oes-ontsmettingstegnologieë kan fitosanitêre insek onderskeppings verminder en marktoegang bevorder. Fitosanitêre opnames is uitgevoer om vas te stel watter peste die mees problematies is met die uitvoer van Proteaceae. Altesaam 82 onderskeppings is gemaak, bestaande uit agt insek ordes en 26 insek families. Alhoewel baie onderskeppings toe te skryf was aan geïsoleerde individue, het verskeie onderskeppings bestaan uit veelvuldige individue van westerse blomblaaspootjies (Frankliniella occidentalis) en protea-kliermyt (Procotolaelaps vandenbergii). Hierdie peste is gekies as fokus spesies vir ontsmetting deur middel van alternatiewe na-oes tegnologie wat nog nie voorheen in die bedryf gebruik is nie. Beheerde Atmosfeer- en Temperatuurbehandelingstelsels (CATTS) -tegnologie is geassesseer as 'n potensiële ontsmettingsmetode vir vars Proteaceae snyblomme. Die produkte wat geëvalueer was sluit in Leucospermum 'Veldfire', Protea magnifica 'Barbi', Leucadendron 'Safari Sunset' en 'Jade pearl', en Geraldton wasblom 'Ofir'. CATTS behandelings het bestaan uit temperatuur-verhogingsskale van 35°C/uur en 30°C/uur vanaf 23°C tot 40°C, met 'n 15 min wekingsperiode by 40°C en 35°C/uur en 30°C/uur. vanaf 23°C tot 50°C, met 'n 15 min weekingsperiode by 50°C, onderhewig aan 'n gemodifiseerde atmosfeer van 1% O2, 15% CO2 in N2. Behandelde stele is onderworpe aan vaaslewe-studies direk behandeling, of na lug- en seevragopbergingsimulasies by 2°C vir 3 of 21 dae onderskeidelik.

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Leucospermum 'Veldfire' het nie die behandelings goed hanteer nie, aangesien ernstige stylverwelking drastiese algehele gehalte vermindering tot gevolg gehad het. Protea magnifica 'Barbi' het sekere behandelings weerstaan, met die handhawing van vergelykbare gehalte gedurende vaaslewe in vergelyking met die kontrole. Beide Leucadendron-produkte het die behandelings goed weerstaan, met die behoud van bemarkbare gehalte direk na behandeling, asook gevolg deur lug- en see-vrag simulasies. Geraldton wasblom 'Ofir' het gehalte gehandhaaf in die vaas direk na behandeling asook na lugvrag-simulasies. CATTS behandelings van 35°C/uur en 30°C/uur tot 40°C in 1% O_2, 15% CO_2 in N_2 het daartoe gelei tot ‘n 100% mortaliteit in beide westerse blomdruppels en protea-kliermyt binne 24 uur vanaf behandeling. Etielformaat (EF)-beroking is geassesseer as 'n potensiële ontsmettings-tegnologie. Konsentrasies het gewissel van 18.53g/m3 tot 151.47g/m3 EF, en het geduur van 30 minute tot 3 uur vir dieselfde produkte wat in Hoofstuk 3 getoets is. Verdere toetse op Geraldton wasblom 'Ofir' het bestaan uit 10g/m3 en 20g/m3 vir 1 en 2 ure. Alle behandelings het gelei tot 'n afname in die algehele gehalte van die behandelde vars produkte. EF-berokings van 18.53g/m3 vir 1 en 2 ure het 100% mortaliteit binne 24 uur van toediening in beide Westerse blomdruppels en protea-kliermiddye veroorsaak, maar weens die buitensporige skade is bevind dat EF nie geskik is nie. Die inligting bekom uit hierdie studie, het die mees problematiese fitosanitêre plae in uitvoerbesendings van vars Proteaceae uit Suid-Afrika uitgelig. Dit het die gebruik van ŉ potensiële na-oes tegnologie vir integrasie in huidige ontsmettings strategieë beklemtoon, terwyl 'n ander afgekeur is. Resultate van hierdie studie kan help met die ontwikkeling van na-oes-ontwrigtingstrategieë, wat uiteindelik die fitosanitêre druk verminder en die uitvoer van vars Proteaceae snyblomme uit Suid-Afrika bevorder.

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Chapter 1: General introduction and literature review

1.1 Floral diversity of the Cape

1.1.1 Cape Floristic Region

South Africa's Cape Floristic Region (CFR) is the smallest and most diverse of the six recognized floral kingdoms of the world, and the only one to be fully contained within the borders of a single country. Its royalty-status is justified, as 19.5% of the plant genera and 68.2% of the estimated 9 000 vascular plant species are endemic to the region (Giliomee, 2003). Confined to the southwestern tip of the African continent and spanning an area of only 87 892 km^2, the CFR boasts extraordinary floral diversity comparable to most tropical regions (Cowling et al., 2003; Rebelo, 2011), and endemism comparable to tropical islands (Linder, 2003). Two of the world's 25 biodiversity hotspots are contained in the CFR (Myers et al., 2000). The CFR also houses 1 406 Red Data Book species, representing over 60% of the Red Data Book species of southern Africa, making it the region of the world with the highest concentration of rare plant species (Rouget et al., 2003). The CFR is listed as a Centre of Plant Diversity, an Endemic Bird Area, as well as a centre of diversity and endemism for mammals, other vertebrates and invertebrates (Cowling et al., 2003). The CFR is home to a remarkable diversity of both fauna and flora.

Most of these accolades are accredited to the presence of fynbos, a vegetation type subset of the Fynbos Biome (McDonald & Cowling, 1995). The Fynbos Biome is a constituent of the global Mediterranean Biome and, like other constituents, is fire-

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prone, characterised by having a Mediterranean-type climate and is dominated by evergreen sclerophyllous shrublands (Bond et al., 1988; Rebelo et al., 2006). An estimated 7 500 of the 9 000 vascular plants of the CFR associate explicitly with fynbos communities (Mucina & Rutherford, 2006). The dominant growth forms and defining floral families include Ericaceae, Restionaceae and Proteaceae (Bond et al., 1988; Theron, 2011).

1.1.2 Proteaceae taxonomy

In Greek mythology, the aquatic deity Proteus had the power of physical transmutability, allowing him to take on innumerable shapes and forms. It is from these powers that the adjective "protean" is derived, which means "great versatility and diversity". In 1735, Carl Linnaeus deemed it fitting to name the floral genus *Protea* such, due to the astounding diversity and multiple shapes and colours that the flora exhibited (Blomerus et al., 2010). In 1789, Antoine Laurent de Jussieu went on to name the floral family Proteaceae, recognizing the same protean traits within the other constituent genera (Leonhardt & Criley, 1999). South African Proteaceae consists of approximately 330 species belonging to 14 genera (Malan, 2012).

1.2 Development of the Proteaceae cut flower industry

1.2.1 History abroad

Proteaceae has long been studied and admired by botanists. The first *Protea* to receive the attention of the scientific world was the oleander leaf protea (*Protea neriifolia*), described and drawn by Carolus Clusius and officially published in 1605 (Leonhardt & Criley, 1999). Many honourable botanists and scientists found great

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pleasure in the description, collection and cultivation of Proteaceae. The first true cultivation of Proteaceae did not occur within South Africa, but was instead achieved when William Aiton succeeded in bringing the sugarbush (*Protea repens*) to flower in the Royal Gardens at Kew in 1789 (https://www.proteaatlas.org.za/comsugar.htm). Not long after, *Protea cynaroides* flowered in the private gardens of the Earl of Coventry in 1803 (Janick, 2007). George Hibbert, a wealthy merchant and amateur botanist, owned the largest collection of Proteaceae of the time, boasting a total of 35 flowering species in London in 1805. Joseph Knight, Hibbert's apprentice, was also a notable and avid botanist. He eventually managed to master the cultivation of Proteaceae under artificial conditions and published the first definitive guide to the growing and care of Cape flora, "On the Cultivation of the Plants Belonging to the Natural Order Proteeae" in 1809 (Ziskovsky, 2015). Upon Hibbert's retirement, he bequeathed his entire collection to his protégé Knight who used this gift to establish the Royal Exotic nursery. This would be the first recorded commercial cultivation and selling of Proteaceae plants (Ziskovsky, 2015).

The exotic Cape flora captivated the minds of botanists and collectors for centuries abroad, and many European countries participated in the cultivation and collection of the flora. Ultimately, this was not to last as the industrial revolution in Europe and the British Isles in the early 1800's brought with it alterations to greenhouses which, utilising humidity-based heating systems, made cultivation of Proteaceae within them nearly impossible (Janick, 2007). The industrial revolution would indirectly end the reign of Proteaceae in Europe for the next century.

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1.2.2 Humble origins in the Cape

Even with the booming success and ultimate collapse of Proteaceae overseas, South Africa and its inhabitants did not express the same enthusiasm for the local flora until much later. William Burchell, renowned British naturalist and flora enthusiast, travelled through the Cape colonies to collect and classify various fauna and flora while simultaneously describing his adventures in a two part series titles "Travels in the interior of southern Africa (Burchell, 1822). He described the mountainsides of the Cape Peninsula as "a botanic garden, neglected and left to grow to a state of nature", and was captivated that "Many beautiful flowers, well known in the choicer collections in England, grow wild on this mountain". It was much to his disapproval that, despite being surrounded by flora of exceptional beauty and diversity, the colonial settlers opted to rather create nurseries and gardens consisting almost entirely of European flowers (van Sittert, 2007) and that the flora of the Cape was viewed with disdain. He grieved that, "[T]hey viewed all the elegant productions of their hills as mere weeds". It was because of this that the South African Proteaceae industry did not develop until much later in the 20th century.

The industry itself began from the most humble of roots within the South African context. Initially, Proteaceae was sold on street corners and at local markets by the disadvantaged communities who would harvest the blooms from the wild (Janick, 2007). This practice is still seen today within the disadvantaged communities of the Cape (Coetzee & Littlejohn, 1994). European religious bodies established mission stations within South Africa to support and empower disadvantaged communities and international slaves. The inhabitants of two such mission stations built in the Western Cape, Elim and Genadendal, were the first South African exporters of dried

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flora to Europe in 1886. Commercial cultivation of indigenous fynbos for floricultural use was practically non-existent before the 1900's (Janick, 2007).

1.2.3 Development of the local industry

The first recorded commercial plantation was of *P. cynaroides*, planted by A.C. Buller on his farm outside of Stellenbosch, South Africa, in 1910. The establishment of Kirstenbosch National Botanical Gardens in 1913 had initial plantings of mostly indigenous flora (Brits et al., 1983) and shortly after its establishment also began selling Proteaceous achenes to the general public (Janick, 2007). Kate Standford also cultivated on a broad-scale in 1920, and became the first trader to sell Proteaceous achenes from a catalogue in 1933 (Janick, 2007). Ruth Middelmann exported Proteaceous achenes to various countries which had, at the time, already begun developing their own wildflower industries which also included the production of South African Proteaceae. The target countries were Australia, New Zealand and the United States of America, which are currently major international production areas (Janick, 2007). Frank Batchelor, considered to be one of the pioneers of the Proteaceae floricultural industry in South Africa, collected wild-growing variants and hybrids of Proteaceae between 1940 and 1970, and through much trial and error gained insights into the ecological and horticultural requirements of effective Proteaceae cultivation (Ziskovsky, 2015). These selection, hybridization, and vegetative propagative techniques were the foundations for the industry developing methods for producing commodities of reliable and superior quality (Leonhardt & Criley, 1999).

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The first commercial harvest of *P. cynaroides* was on Frank Batchelor's farm (later to be named Protea Heights) on the outskirts of Stellenbosch, in 1948. On the eve of her coronation in 1953, Queen Elizabeth received a floral basket containing *P. cynaroides* as a gift from the people of South Africa. This is the first known record of the export of fresh *Protea* cut flowers from South Africa (Janick, 2007). Later that decade, in 1959, Marie Vogts published "Proteas, Know Them and Grow Them", a critically important document which not only acted as a definitive guide to the cultivation of the plants, but encouraged cultivation instead of wild-harvesting, helping reduce the already high stress load placed on wild populations (Parvin et al., 2003).

The commercialization of the dried flower industry began in the 1950's, with the Middelmann family shipping vast quantities to European markets (Janick, 2007). The fresh cut flower industry took off a decade later, as the growing wealth of European nations coincided with drastic drops in airfreight costs (Littlejohn, 2001). The vast majority of exported products were wild-harvested, as true cultivation was limited to a few pioneer cultivators. These wild sourced blooms were mostly inconsistent with regard to quality and flowering times, and were generally also of inferior quality (Gerber & Hoffman, 2012). In order to supply European markets with a steady stream of Cape flora throughout the year, many species and variants were incorporated. Through collaboration between harvesters and exporters, many of the smaller and inconsistent commodities were removed from consignments, especially once the European market began expecting blooms of higher and more consistent quality (Coetzee & Littlejohn, 1994; Littlejohn, 2001).

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In 1965, the South African Wildflower Growers Association came into being. A decade later, in 1976, the name was changed to the South African Protea Producers and Exporters Association (SAPPEX), emphasising the dire need for cultivation instead of wild harvesting (Parvin et al., 2003). By 1981, various countries were independently producing Proteaceae for their floricultural industries. A total of 116 international industry participants congregated in Australia to discuss the possibility of forming an international organization for collaboration and sharing of research and information. At the conclusion of the meeting, the International Protea Association (IPA) came into being (Parvin et al., 2003).

Members of the IPA proposed the creation of a scientific group under the International Society of Horticultural Sciences (ISHS), through which specific issues within the industry could be addressed directly, and the findings be made available to researchers and growers alike. Thus, the International Protea Working Group (IPWG) came into being, and joined the IPA in 1985 (Parvin et al., 2003).

In 2014, the merging of SAPPEX and Protea Producers of South Africa (PPSA) created Cape Flora SA, an umbrella organization representing various stakeholders within the industry. The industry body provides platforms for communication across and between stakeholders, promotes market access for Cape flora abroad, supports research on products and transport chains to ensure global competitiveness, but mostly unites stakeholders under a common name (Gollnow & Gerber, 2015).

These entities and organizations have helped steer the Proteaceae cut flower industry, both locally and internationally, into becoming a recognized agricultural crop across the world.

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1.3 The industry today

1.3.1 South African industry today

Proteaceae production occurs worldwide in countries exhibiting Mediterranean or Mediterranean-like climates. As of 2015, there are 15 countries or regions which produce Proteaceae on levels deemed significant by the IPA, and these are considered "member countries" (Gollnow & Gerber, 2015). These recognized member countries include Australia, the Azores, California, the Canary Islands, Chile, Hawaii, Israel, New Zealand, Portugal, South Africa and Zimbabwe. Production regions are limited by a variety of production factors, including but not limited to well-draining and slightly acidic soils, warm and dry summers, and frost-free winters (Gollnow & Gerber, 2015).

Proportionately, South Africa is still the leading producer of South African Proteaceae, boasting 1041 hectares of production units (www.capeflorasa.co.za – Statistics). This constitutes 666 ha *Protea*, 181 ha *Leucospermum*, 132 ha *Leucadendron*, and 62 ha Cape flora greenery. As is commonly seen within the industry internationally, the majority of production is owned and cultivated by a minority of producers, highlighting the shift towards large-scale, professional production replacing opportunistic crops (Gollnow & Gerber, 2015). Export commodities consist almost entirely of cultivated crops, as wild harvested products tend to be of inferior quality. That being said, wild harvesting still occurs and is a critical component of both the dried flower export industry, as well as supplying filler material for the increasingly popular mixed Cape Flora bouquets (Gerber & Hoffman, 2012).

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According to Cape Flora SA statistics (www.capeflorasa.co.za), an estimated 19 105 727 loose stems of South African Proteaceae (excluding greenery and bouquets) were exported during the 2016/2017 season. This was made up of 2 948 242 *Protea* stems (15.43%), 6 998 834 *Leucospermum* stems (36.63%), and 9 158 651 *Leucadendron* stems (47.94%).

The most exported species, selections and cultivars of the 2016/2017 season for *Protea* were 'Pink Ice' (313 151 stems), *P. magnifica* (300 929 stems), *P. cynaroides* (283 361 stems), 'Sylvia' (223 115 stems) and 'Carnival' (165 056 stems). The top exported *Leucospermum* commodities were 'Succession' (3 375 414 stems), 'Soleil' (1 127 555 stems), 'Jelena' (1 045 541 stems), 'Tango' (955 360 stems), and 'Gold dust' (559 267 stems). The most popular *Leucadendron* products were 'Safari sunset' (913 421 stems), 'Jade pearl' (741 202 stems), 'Rosette' (699 728 stems), 'Plumosum female' (486 716 stems) and 'Discolour' (358 241 stems). Finally, the most exported foliage stems in descending order was 'Geraldton wax', *Brunia laevis, Eucalyptus* spp., *B. albiflora*, and *Berzelia galpinii*. Despite not belonging to Proteaceae or being indigenous to South Africa, *Eucalyptus* spp products are sold under the category of Cape floral greenery.

The majority of cultivated crops are destined for export, with the domestic market being significantly smaller despite a growing trend in local sales (Gerber & Hoffman, 2012). South Africa's main export regions include the European Union and Russia (41%), the Middle East (28%), the United Kingdom and its particular increase in demand for mixed bouquets (25%), the Far East (4%), Canada and the United States of America (2%), Africa (<1%), and the Indian Ocean Islands (<1%).

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This considerable disparity in international demand is due to various factors. Firstly, South Africa's position globally and relative distance to export markets brings with it the complications of long distance freighting. Airfreight is far superior with regard to duration, as products typically reach markets within 24 to 48 hours. Unfortunately, the environmental conditions are poorly regulated in comparison to sea freight, and flower quality may be compromised as a result. Transport by ship takes longer, anywhere between 1 and 4 weeks, but the environmental conditions can be strictly regulated and enforced, ensuring product quality upon arrival in target countries, provided the products are capable of withstanding these lengthy voyages (Philosoph-Hadas et al., 2007). There is a continuous pressure from internal and external entities to shift freighting to sea instead of air transport, as not only are freighting costs reduced, but the carbon footprint of sea freighting is considered to be far less. In order to fully utilise sea transport, floral commodities need to withstand extended freighting times and simultaneously exhibit adequate vase life once received. This is achieved through appropriate circulation within containers, perforated boxes, carbohydrate pulses prior to or during transport, and the addition of plant growth regulating products (Philosoph-Hadas et al., 2010).

The second factor, and focus of this study, is the presence of live insects within export consignments of Proteaceae from South Africa.

1.3.2 Insects associating with Proteaceae

Naturally-occurring Proteaceae is associated with a plethora of insects. Many have proven to be serious pests of cultivated Proteaceae (Myburgh and Rust, 1975; Wright, 1993; Wright and Saunderson, 1995; Leandro et al., 2003; Wright, 2003).

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South African Proteaceae is infested by a considerable entomofauna with indigenous insects attacking almost every functional part of the plant, ranging from roots, stems, buds, foliage and inflorescences (Wright, 2003). This is due to production units occurring within the natural distribution range of both the plants and insects. Bud- and flower borers include the larval stages of a variety of both Lepidopteran and Coleopteran species. The larvae of insects such as the American bollworm (*Helicoverpa armigera*), Protea scarlet butterfly (*Capys alphaeus*), Protea bud weevil (*Euderes spp.*) and Protea black moth (*Argyroploce spp.*) bore into developing and fully-developed receptacles of numerous cultivated Proteaceae, drastically reducing yields (Malan, 2012). Despite the sclerophyllous nature of Proteaceae foliage, various caterpillars and weevils feed or oviposit on- or within them. The pine emperor (*Imbrasia cytherea*) can defoliate entire portions of the host plant, whereas the leaf roller (*Tanyzancla haematella*) and green fruit nibbler (*Prasoidea sericea*) can cause severe damage in a highly localized region of the host plant (Malan, 2012). While many of these insects cause direct damage to the plant, reducing plant vigour, bloom quality and even sometimes resulting in death, many are considered non-destructive. Irrespective, the presence of insects has related phytosanitary concerns for international trade (Wright, 2003).

1.3.3 Phytosanitary pests associated with Proteaceae and current control measures

The transport, trade and export of plant products has acted as a vector for the movement of exotic organisms since as far back as the 1500's (Hulme, 2009). It is believed that biological invasions are the second largest reason for loss of biodiversity (Keane & Crawley, 2002). With the increase in international trade of

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products and goods, there will most likely be an increase in the number of exotic organisms introduced throughout the world, intentional or not (Keane & Crawley, 2002).

From an agricultural perspective, the phytosanitary risk is that these introduced organisms could become pests within production units in the country of introduction. The introduction of novel pests could result in major financial losses for the foreign country, as the pests may decimate production units in absence of their natural enemies (Wolfe, 2002). Further financial losses are incurred when control measures for the novel pest have to be created and implemented (Born et al., 2005), and even a loss of potential markets for the affected country (Baker & Cowley, 1989). Currently, strict phytosanitary regulations are enforced globally, to reduce and limit the introduction of exotic organisms. Due to Proteaceae's extensive interactions with insects, the cut flower industry faces serious phytosanitary barriers when exporting, particularly to stricter countries. Phytosanitary pests of Proteaceae are controlled throughout the production chain and up to the point of export.

Preharvest control of insects within production units can drastically decrease the overall insect abundance and diversity at point of export (Hansen et al., 1992). Though limited, pesticides and spray regimes are the first and most commonly used preharvest disinfestation technique (Wright, 2003). Due to the Proteaceae industry being niche and far smaller than other agricultural crop industries within the Cape, registered pesticides for the industry are minimal and dwindling (Janick, 2007). Plantation sanitation, through removal of infested blooms, foliage and stems, is critically important but fairly underutilised, particularly when controlling Lepidopteran and Coleopteran borers (Janick, 2007; Leonhardt & Criley, 1999). The

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implementation of an integrated pest management (IPM) system, encouraging natural predators and holistic approaches to pest control, may not be a stand-alone technique, but reduces pesticide loads and promotes healthy ecosystem functioning while simultaneously maintaining pests at controllable levels (Wright, 1993; Hansen & Hara, 1994; Leandro et al., 2003; Janick, 2007).

Although the preharvest techniques may reduce overall insect presence within export consignments, they do not guarantee an insect-free product at the point of export (Hansen et al., 1992). Therefore, postharvest disinfestation techniques have been the most effective manner by which to guarantee insect-free products (Hansen et al., 1992). Current postharvest treatments in the South African Proteaceae industry consist almost entirely of fumigation regimes. Methyl bromide was used extensively within the industry due to its short treatment durations and its effectiveness against a wide range of pests, but due to its ozone depleting factors and it being phased out, its use is greatly restricted to pre-shipment fumigation which too will eventually be banned (Weller & van Graver, 1998; Williams, 1998). Other fumigants utilised for postharvest control include various pyrethoids, dichlorvos and phytoxin accelerants (J. Walsh, pers. comm.).

There exists an arsenal of postharvest techniques for the disinfestation of cut flower. Currently available physical control measures include cold- and heat treatments, hand removal, hyper- and hypobaric pressures, radio frequency and far ultra-violet radiation, to name a few (Hansen & Hara, 1994; Usall et al., 2016). There is also an ever-expanding range of chemical treatments available for use as fumigants, varying drastically between industries and even commodities within them (Follet & Neven, 2006). Many of these treatments are considered to be effective and simultaneously

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friendlier towards the environment, yet none have been regarded for use in the South African Proteaceae cut flower industry. Due to the industry's extensive limitations as a result of phytosanitary insects, alternative postharvest treatments should always be considered.

1.3.4 Controlled Atmosphere Temperature Treatment Systems (CATTS) technology

A fairly recent development in postharvest quarantine technologies is the combination of two physical treatments, heat and atmospheric stress, and was termed a novel tool for postharvest treatment development in 1996 (Verschoor et al., 2015). The postharvest technology known as CATTS (Controlled Atmosphere and Temperature Treatment Systems technology) utilises short-term heat treatments in atmospheres consisting of low oxygen and high carbon dioxide. The combination of these stresses reduces treatment time significantly, thereby maintaining fresh produce quality after treatment while simultaneously controlling a variety of postharvest pests (Neven & Johnson, 2018).

CATTS technology has been proven effective in maintaining postharvest quality in peaches and nectarines (Neven et al. 2006), sweet cherries (Shellie et al., 2001), apples and pears (Neven et al., 2001), as well as other stone and pome fruits. CATTS technology has also been shown to effectively control both tarsonemid mites and root knot nematodes in strawberry root stock and runners (van Kruistum et al., 2014). Although the research on CATTS for use as a tool for the disinfestation of cut flowers is limited, Slootweg (2007) found that both roses and *Freesia* were capable of withstanding certain CATTS treatments.

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CATTS has also proven to be an effective postharvest technology for the control of codling moth (*Cydia pomonella*), oriental fruit moth (*Grapholita molesta*), western cherry fruit fly (*Rhagoletis indifferens*), plum curculio (*Conotrachelus nenuphar*) and apple maggot (*Rhagoletis pomonella*). Extreme temperature treatments and controlled atmospheres have been thoroughly researched for the use of cut flower disinfestation (Jones & Faragher, 1991; Shelton et al., 1996; Lurie, 1998; Hara et al., 2003; Philosoph-Hadas et al., 2010), but CATTS has not yet been considered for use on Proteaceae cut flowers.

The exact mechanisms of CATTS treatments on insect physiology are not well-understood, but various suggestions have been made. High CO_2 levels reduces the formation of reduced nicotinamide adenine dinucleotide phosphate (NADPH), hinders adenosine triphosphate (ATP) affiliated reactions and reduces production of acetylcholine from choline (Neven & Mitcham, 1996; Zhou et al., 2000). The accumulation of CO_2 in the insect lowers the haemolymph pH due to the formation of carbonic acid (Neven & Mitcham, 1996). High temperatures also result in a decrease of pH. The lowered pH may greatly reduce insect physiological capacity through the inhibition of membrane function and cellular metabolism (Zhou et al., 2000). The opening and closing of spiracles is largely regulated by internal CO_2 levels and O_2 requirements. Higher temperatures increase the metabolism of insects, and the demand for O_2 keeps the spiracles open. Low levels of O_2 and higher levels of metabolism can induce anaerobic metabolism (Zhou et al., 2000), which may greatly hinder efficient functioning of neurons throughout the insect. The combination of high temperatures, high levels of CO_2 and low levels of O_2 synergistically result in the breakdown of metabolic functioning in insects, ultimately resulting in mortality.

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CATTS technology could potentially be used as an effective postharvest technique for control of phytosanitary insects on export Proteaceae from South Africa.

1.3.5 Ethyl formate fumigation

Ethyl formate (EF) has historically been used as a fumigant in the dried fruit industry (Cotton & Roark, 1928). The chemical is a naturally occurring plant volatile, which easily degrades into ethanol and formic acid when exposed to water (Grifin et al., 2013). EF is considered to be a “GRAS” (generally regarded as safe) compound, and is recognised as being so due to prolonged use in the food industry (Mitcham, 2001). EF is commercially available and registered for use in certain countries in the form of VAPORMATE™, a non-flammable mixture of EF (16.7% by weight) and liquid CO_2 (83.3% by weight), thereby including atmospheric stress (Grifin et al., 2013).

Both EF and VAPORMATE™ have shown to be effective fumigants against a variety of pests and phytosanitary insects. Simpson et al. (2007) found that all stages of the western flower thrips (WFT) (*Frankliniella occidentalis*) and the grape mealy bug (*Pseudococcus maritimus*) were susceptible to EF fumigation, as well as with EF in 10% CO_2. Negligible phytotoxicity was found in treated grapes. Similarly, Pupin et al., (2013) found EF to be effective against both WFT and California red scale (*Aonidiella aurantii*), while maintaining post-treatment quality in sweet oranges. Both EF and VAPORMATE™ proved effective in controlling onion thrips (*Thrips tabaci*), and no phytotoxicity was noted on treated onions (van Epenhuijsen et al., 2007).

EF and VAPORMATE™ have also been tested against a range of floricultural products, including both South African and Australian Proteaceae, in order to

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determine its efficacy as a postharvest treatment for phytosanitary pests. While comparing an array of potential postharvest fumigants for various cut flowers, Weller & van Graver (1998) noted that, of the fumigants tested, EF caused the most damage, and deemed it "unsuitable". Extreme damage was noted in *Protea* 'Pink Ice', with some damage in Geraldton wax. They did, however, also note that Blushing bride (*Serruria florida*) exhibited no phytotoxic reactions to the fumigant. Similarly, Williams (1998) noted excessive damage in *Protea*, but no damage in *Serruria*. VAPORMATE™ efficacy trials were conducted on a variety of South African and Australian Proteaceae commodities by Rigby, Gallagher, & Collins (n.d.). Once again, damage was noted across the treated commodities, particularly foliage damage in *P. cynaroides*, *Leucospermum spp*, and Geraldton wax 'Mullering Brooke White' and 'Chantilly Lace'.

While studies suggest that EF and VAPORMATE™ are not suitable for the fumigation of South African Proteaceae, certain exceptions to the case, such as seen in *Serruria*, may unknowingly exist. Further research and inclusion of more commodities is required before this fumigant can be considered as ineffective for use in postharvest disinfestation for South African Proteaceae.

1.4 Thesis structure and objectives

The overall aim of this study is to reduce the phytosanitary pressures of exporting South African Proteaceae by assessing the efficacy of two novel postharvest technologies. This study will assist in a better understanding of the phytosanitary pests of exported Proteaceae, determine the success of the new technologies, and potentially allow for market expansion and access.

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The objectives of the study are: 1) through qualitative survey, determine which phytosanitary insects are most frequently encountered and problematic at export; 2) to assess the potential of CATTS treatments as a postharvest technology for the control of phytosanitary insect pests of Proteaceae; and 3) to assess the potential of EF fumigation as an effective postharvest fumigant for the control of phytosanitary pests of Proteaceae. These study objectives are presented in three chapters.

- Chapter 2 reviews the known pests of Proteaceae, and describes the qualitative survey in which live insects were collected from export points, directly prior to export to various international phytosanitary markets
- Chapter 3 evaluates the potential of CATTS technology as an alternative postharvest treatment for the control of phytosanitary insects, with regard to both post-treatment flower quality and insect mortality
- Chapter 4 evaluates the potential of EF fumigation as an alternative postharvest treatment for controlling phytosanitary insects, with regard to both post-fumigation flower quality and insect mortality

Chapter 5 is the concluding chapter of the study, where the results of other chapters will be summarised, and the main aim of the study will be re-evaluated and discussed.

1.5 References cited

Baker, R., & Cowley, J. (1989). Evaluation of the economic impact of newly introduced pests. *New Zealand Journal of Forestry Science*, 19, 330–334.

Blomerus, L., Joshua, S., & Williams, J. (2010). Breeding Proteaceae varieties for changing market trends. *Acta Horticulturae*, 869, 173–181.

Stellenbosch University https://scholar.sun.ac.za

Bond, W.J., Midgley, J., & Vlok, J. (1988). When is an island not an island? Insular effects and their causes in fynbos shrublands. *Oecologia*, 77, 515–521.

Born, W., Rauschmayer, F., & Bräuer, I. (2005). Economic evaluation of biological invasions - a survey. *Ecological Economics*, 55, 321–336.

Brits, G.J., Jacobs, G., & Vogts, M. (1983). Domestication of fynbos Proteaceae as a floricultural crop. *Bothalia*, 14, 641–646.

Burchell, W.J. (1822) Travels in the Interior of Southern Africa, volume 2 (London, Batchwork Press).

Coetzee, J.H., & Littlejohn, G. M. (1994). Ornamental horticulture in rural areas in South Africa. *Horticulture in Human Life, Culture and Environment*, 391, 173–182.

Cotton, R.T., & Roark, R.C. (1928). Fumigation of stored–product insects with certain alkyl and alkylene formates*. Industrial & Engineering Chemistry*, 20, 380–382.

Cowling, R.M., Pressey, R.L., Rouget, M., & Lombard, A.T. (2003). A conservation plan for a global biodiversity hotspot - the Cape Floristic Region, South Africa. *Biological Conservation*, 112, 191–216.

Follett, P. A., & Neven, L. G. (2006). Current trends in quarantine entomology. *Annual Review of Entomology*, 51, 359-385.

Gerber, A.I., & Hoffman, E.W. (2012). 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.

Stellenbosch University https://scholar.sun.ac.za

Gollnow, B., & Gerber, A.I. (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.

Grifin, M.J., Jamieson, L.E., Chhagan, A., Page-Weir, N.E.M., Poulton, J., Davis, V.A., Connolly, P.G. (2013). The potential of ethyl formate + carbon dioxide to control a range of horticultural pests. *New Zealand Plant Protection*, 66, 54–62.

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.

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

Hulme, P.E. (2009). Trade, transport and trouble: Managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, 46, 10–18.

Janick, J. (2007). Proteaceous Ornamentals: Banksia, Leucadendron, Leucospermum and Protea. *Scripta Horticulturae*, 5, 1–161.

Jones, R., & Faragher, J. (1991). Cold storage of selected members of the Proteaceae and Australian native cut flowers. *Hortscience*, 26, 1395–1397.

Keane, R.M., & Crawley, M.J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, 17, 164–170.

Leandro, M.J., Oliveira, M., Melo, C., & Mexia, A. (2003). Survey of the insect population on a Protea plantation in the Southwest of Portugal. *Acta Horticulturae*, 602, 67–74.

Stellenbosch University https://scholar.sun.ac.za

Leonhardt, K.W., & Criley, R.A. (1999). Proteaceae floral crops : Cultivar development and underexploited uses. *Perspectives on New Crops and New Uses*, 410–430.

Linder, H.P. (2003). The radiation of the Cape flora, southern Africa. *Biological Reviews of the Cambridge Philosophical Society*, 78, 597–638.

Littlejohn, G.M. (2001). The challenges of breeding wild flower cultivars for use in commercial floriculture: African Proteaceae. *Acta Horticulturae*, 552, 25–37.

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.

McDonald, D.J., & Cowling, R. (1995*).* Towards a profile of an endemic mountain fynbos flora: implications for conservation. *Biological Conservation*, 72, 1-12.

Mitcham, E. (2001). Quarantine issues in 2000. *Acta Horticulturae*, 553, 451–455.

Mucina, L., & Rutherford, M.C. (2006). The vegetation of South Africa, Lesotho and Swaziland. *South African National Biodiversity Institute*. Pretoria, South Africa.

Myburgh, A.C., & Rust, D. (1975). A survey of pests of the Proteaceae in the western and southern Cape Province. *Journal of the Entomological Society of Southern Africa*, 38, 55–60.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.

Neven, L.G., Drake, S.R., & Shellie, K.C. (2001). Development of a high temperature controlled atmosphere quarantine treatment for pome and stone fruits. *Acta Horticulturae*, 533, 457–460.

Stellenbosch University https://scholar.sun.ac.za

Neven, L.G. and Johnson, S.A. (2018). Combination hot forced air treatments and controlled atmosphere treatments: CATTS — Controlled Atmosphere Temperature Treatment System. *In: Novel postharvest treatments of fresh produce* (Edited by Sunil Pareek). CRC Press, Boca Raton, Florida.

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., Rehfield-Ray, L., & Obenland, D. (2006). Confirmation and efficacy tests against codling moth and oriental fruit moth in apples using combination heat and controlled atmosphere treatments. *Journal of Economic Entomology*, 99, 1620–1627.

Parvin, P.E., Criley, R.A., & Coetzee, J.H. (2003). Proteas – a dynamic industry. *Acta Horticulturae*, 602, 123–126.

Philosoph-Hadas, S., Droby, S., Rosenberger, I., Perzelan, Y., Salim, S., Shtein, I., & Meir, S. (2007). Sea transport of ornamental branches: Problems and solutions. *Acta Horticulturae*, 755, 267–276.

Philosoph-Hadas, S., Perzelan, Y., Rosenberger, I., Droby, S., & Meir, S. (2010). Leucadendron "Safari Sunset": Postharvest treatments to improve quality of cut foliage during prolonged sea shipment. *Acta Horticulturae*, 869, 207–217.

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.

Stellenbosch University https://scholar.sun.ac.za

Rebelo, A. (2011). The preservation of the Cape flora: status, causes of rarity, ideals and priorities. Doctoral Dissertation. University of Cape Town.

Rebelo, A.G., Boucher, C., Helme, N., Mucina, L., Rutherford, M.C. (2006). Fynbos Biome. In: The Vegetation of South Africa, Lesotho, and Swaziland. *South African National Biodiversity Institute*. Pretoria, South Africa.

Rigby, E., Gallagher, E., & Collins, A. (n.d.). Vapormate ® phytotoxicity trials on Australian Wildflowers. *Cedar Hill Flowers & Foliage Pty Ltd*, 1–20.

Rouget, M., Richardson, D.M., Cowling, R.M., Lloyd, J. W., & Lombard, A.T. (2003). Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region, South Africa. *Biological Conservation*, 112, 63–85.

Shellie, K.C., Neven, L.G., & Drake, S.R. (2001). Assessing "Bing" sweet cherry tolerance to a heated controlled atmosphere for insect pest control. *HortTechnology*, 11, 308-311.

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.

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.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.

Theron, N. (2011). Mite communities within Protea infructescences in South Africa. Doctoral Dissertation. Stellenbosch University.

Stellenbosch University https://scholar.sun.ac.za

Usall, J., Ippolito, A., Sisquella, M., & Neri, F. (2016). Physical treatments to control postharvest diseases of fresh fruits and vegetables. *Postharvest Biology and Technology*, 122, 30-40.

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). CATT as a non-chemical pest and nematode control method in strawberry mother planting stock. *Journal of Berry Research*, 4, 29–35.

Van Sittert, L. (2003). Making the Cape Floral Kingdom: the discovery and defence of indigenous flora at the Cape ca. 1890-1939. *Landscape Research*, 28, 113-129.

Verschoor, J., Otma, E., Qiu, Y.T., Van Kruistum, G., & Hoek, J. (2015). Controlled Atmosphere Temperature Treatment: non-chemical (quarantine) pest control in fresh plant products. *Acta Horticulturae*, 1071, 253–258.

Weller, G.L., & van Graver, J. E. (1998). Cut flower disinfestation: Assessment of replacement fumigants for methyl bromide. *Postharvest Biology and Technology*, 14, 325–333.

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

Wolfe, L.M. (2002). Why alien invaders succeed: support for the escape-from-enemy hypothesis. *The American Naturalist*, 160, 705–711.

Wright, M.G. (1993). Integrated pest management - concepts and potential for the control of borers on proteas. *Acta Horticulturae* 387, 153–158.

Stellenbosch University https://scholar.sun.ac.za

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., & Saunderson, M. (1995). Protea plant protection: From the African context to the international arena. *Acta Horticulturae*, 387, 129-139.

www.capeflorasa.co.za/statistics/ Cape Flora SA statistics (Accessed October 2017).

www.proteaatlas.org.za/comsugar.htm Protea Atlas Project (Accessed June 2016)

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.

Ziskovsky, B. (2015). Expanding the seasonal potted plant floral market in North America with containerized Protea cynaroides L. Doctoral Dissertation. University of Minnesota.

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Chapter 2: Invertebrates of phytosanitary importance associated with exported Cape Flora

2.1 Introduction

Insect diversity and endemism within the Cape Floristic Region (CFR) of South Africa is both understudied as well as misunderstood. The sheer magnitude of species, the daunting task of trapping and collecting them, coupled with the taxonomic expertise required to identify all the individuals makes the compiling of a complete invertebrate faunal list a near impossible task (Picker and Samways, 1996; Giliomee, 2003). It has been suggested that, despite having incredibly rich plant diversity, the CFR is notably lacking in insect diversity (Giliomee, 2003). This viewpoint is centred around the nutrient-poor substrates of the Cape region, and the subsequent sclerophyllous and unpalatable nature of the indigenous vegetation. Putative defence mechanisms against insect herbivory are suggested to occur within members of the *Protea* genus (Wright and Giliomee, 1992), where the presence of trichomes on young leaves and condensed tannin levels in older leaves rendered plants unpalatable to insects which had not developed a gut pH capable of processing them. On the other hand, Procheş and Cowling (2006) and Procheş et al. (2009) state that broad-scale insect diversity within the fynbos vegetation is not as low as previously thought, but is comparable to the diversity found within the neighbouring biomes of Grassland, Nama-karoo and Albany thicket.

Despite the uncertainty around overall diversity of insects in the Fynbos biome, it is well-known that naturally-occurring Proteaceae is associated with a considerably rich

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entomofauna. Proteaceae is considered the structurally dominant component within the fynbos (Theron, 2011), as trees are mostly rare or entirely absent across regions (Giliomee, 2003). The architecture of Proteaceae, some growing to be tree-like shrubs, provides a multitude of niches for insects and other invertebrates to exploit. These insects associate with almost every part of the plant in some form or other, from root borers, stem borers and bud borers, to foliage feeders and miners (Wright, 2003). By far the most notable and subsequently well-studied place of interaction is between the inflorescences and the multitude of creatures which interact with and within them.

Of Proteaceae's constituents, the genus *Protea* is most severely infested, followed by other members such as *Leucospermum*, *Leucadendron, Brabejum* and *Faurea* (Gess, 1968; Myburgh and Rust, 1975). The genus *Protea* produces the largest inflorescences within the Cape Flora group, the extreme being *Protea cynaroides* with a diameter of almost 30cm (Coetzee and Littlejohn, 2001; Ziskovsky, 2015). A correlation was noted between infructescence size and arthropod richness and abundance in Proteaceae members, where the larger the infructesences housed the more diverse fauna (Roets et al., 2006). Many genera exhibit large, structurally complex and cup-shaped flowers. This, coupled with the tendency to produce ample nectar and pollen, provides ideal conditions for insects to congregate and feed (Coetzee and Giliomee, 1985). The entomofauna associating with these inflorescences are remarkable, not only from a species-diversity viewpoint, but also due to the immense numbers representing a single species. A large survey undertaken by Gess (1968) reported a total of 200 insect species associating only with the flowering structures of *Protea*. This did not include the vast number of Arachnida species of mites, spiders and pseudoscorpions that are also present

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within the inflorescences. An astounding total of >60 000 individuals of protea itch mite (*Proctolaelaps vandenbergii)* were found in only 4 flower heads of wild-growing Laurel protea (*Protea laurifolia*) by Myburgh et al., (1973).

In comparison to *Protea,* the other genera have far fewer associated insects which tend to also inhabit the other components of the plant, such as the foliage and stems. This is most likely due to their more exposed and open flower structures, thus exhibiting a lack of prominent involucral bracts, or a deeper cup-shape structure as seen in *Protea* (Gess, 1968). Despite major differences in floral morphology, the genera *Leucospermum*, *Leucadendron, Brabejum* and *Faurea* still associate with a considerable array of insects.

Many species of Proteaceae are utilised in a prominent indigenous cut flower industry in South Africa (Coetzee et al., 1997). The sometimes substantial and often showy inflorescences, incredible diversity, long vase life and exotic appeal of these flowers have contributed to a leading global ornamental industry (Reinten et al., 2011). As many of the constituents are endemic, the industry consists of both a commercial and wild harvested sector. Cultivated products from commercial orchards have mostly replaced wild harvested products, as product consistency and the need for chemical pest control makes natural stands less feasible (Coetzee and Littlejohn, 1994). However, natural stands still provide important filler material for mixed bouquets which are ever growing in popularity (Gollnow and Gerber, 2015). Commercial plantations consist almost entirely of clonal cultivation, allowing for uniformity in product quality (Gerber, 2008). These monocultural systems create conditions for more effective control of insect pests, a practice almost impossible in wild harvested stands, and lead to an overall decrease in insect pest diversity and

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abundance. However, these systems also increase the risk of an outbreak of one or more pests as seen in other monocultural systems (Rust and Myburgh, 1975; Wright, 1993). The chemical and physical control of insect pests in plantations reduces the risk of insects being present at export, but cannot ensure insect-free products, especially if the plantations are adjacent to natural veld.

From the industry's perspective, any and all insects are of highest concern. Firstly, insect pests reduce overall productivity and product quality of various Proteaceae stems. Furthermore, these insects also pose a significant barrier when exporting the cut flowers, as many are cryptic and undescribed and can easily be missed when picking and packing the flowers. One of the most seriously limiting factors to the expansion and success of the South African Proteaceae industry is the presence of a variety of indigenous insects and invertebrates pests, both pre- and post-harvest (Rust and Myburgh, 1975; Wright and Saunderson, 1995; Coetzee et al., 1997). The transport of these insects and invertebrates within export consignments pose a serious phytosanitary threat, as their accidental introduction into novel distribution ranges could result in dire agricultural, economic and ecological consequences (FAO, 2011). This is further compounded when importing countries are producers of Proteaceae products or closely related agricultural goods, as native pests may become established in non-native production units and cause massive agricultural concerns abroad (Wright and Saunderson, 1995).

During inspection of export consignments, depending on the encountered insect, abundance of said insect and the country in question, a variety of outcomes are possible. If the insects are few and large, as seen with Coleopterans, they are removed by hand or isolated by knocking of the flowers, where after the particular

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box with flower stems is still permitted to be exported. If there are many insects, a secondary fumigation may be mandated. If a box contains “too many” individuals, or individuals of a high risk species, the flowers may be rejected entirely. Should insects be found upon arrival in special market countries (those which exhibit a zero-tolerance for insects in consignments), another fumigation is mandated of which the costs are shared with the exporter, or the consignment is rejected entirely. In very rare cases, the presence of certain invertebrates may result in the burning of entire consignments, such as on the detection of snails from the genus *Helix* (J. Walsh, pers. comm.) Of all these possible outcomes, the first is the only one which does not negatively influence impact on the export of South African Proteaceae. The extra required fumigations and risk of possible rejections induce significant economic and reputational costs for the industry. Additional fumigations either in South Africa or overseas also reduce the product-life of the fumigated commodities, which too negatively impacts the industry (J. Walsh, pers. comm.).

To date, there has not been a thorough survey of phytosanitary insects as found in export consignments of Cape flora directly prior to export. This study is a qualitative review of insects and invertebrates of potential phytosanitary importance found on a variety of Cape flora products prior to export to various phytosanitary markets. It aims to highlight the most serious pests, due to repetitive interception or excessive abundance, and identify the products rendered most susceptible to rejections due to insect infestation.

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2.2 Materials and Methods

Insects were collected from floral commodities presented for export over a period of two years. During the 2016 season (September-November), collections were carried out during official DAFF (Department of Agriculture, Forestry and Fisheries) inspections of Cape flora presented for export at Cape Town International Airport. Due to time constraints and risk of demurrage, collections in 2017 (March-April and August-October) were not done during official inspections, but directly before or after inspections had already taken place. Floral commodities included in the survey were determined by the time of year inspections took place, and included species belonging to *Protea*, *Leucospermum* and *Leucadendron*, floral greenery such as *Chamelaucium*, *Eucalyptus, Brunia* and *Berzelia* products, and mixed bouquets consisting of mixtures of many. Over the two seasons a total of 13 inspections were done.

Export products were removed from cold storage (2°C) and their boxes packed next to the inspection table. Boxes were examined individually, where the top half of the floral commodities were gently tapped against one another above a bottom-lit inspection table. This was repeated until the top half of the stems in each box was inspected independently. Any visible insects which were clearly moving were collected using entomological forceps and placed in specimen jars, on which the date, product and importing country were recorded. Once all the clearly visible insects were removed, a magnifying glass and paintbrush were used to collect the smaller specimens. After the table had been thoroughly looked over, the remaining flowers in the box were lifted to reveal any insects hiding within or under them, after which the box was repacked, the table was brushed off and the next box was

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inspected. Due to the limited time and often voluminous commodity load, insects were not counted, but their presence or absence in commodities was recorded.

The collected insects were mostly identified to family level using taxonomic keys (Picker et al., 2012) or to species level when frequent interceptions were encountered. Any larval stages, particularly Lepidopteran larvae, were kept in ventilated containers with Proteaceae inflorescences to assist with rearing and later identification.

2.3 Results and Discussion

A total of 82 interceptions (the detection of a pest during the inspection of a consignment) were listed over the two-year collection period during 13 total inspections, along with their associated floral commodities prior to export from Cape Town International Airport to various international markets (Table 2.1). These interceptions consisted of 8 orders and 26 families. The various veld-harvested Cape flora foliage- and filler material had the highest overall insect diversity, followed by *Leucospermum*, *Protea* and *Leucadendron* commodities.

The most commonly encountered insects within and across commodities were those from the family Thripidae, particularly the western flower thrips (*Frankliniella occidentalis*). These were not only the most regularly found insect, but also the most abundant, where a single interception could account for an abundance in excess of 100 individuals. All *Leucospermum* commodities found to contain thrips were infested with *F. occidentalis* individuals. The second most encountered insects were a variety of weevils. These, as with the other Coleopterans, were mostly solitary individuals, with the exception of the banded fruit weevil (*Phlyctinus callosus*) and

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Fuller rose weevil (*Naupactus godmanni*), of which the former was once found to be approximately 21 individuals within a single box of *Leucospermum* 'Tango'.

Insects found within export consignments are a major hindrance to the South African Proteaceae cut flower industry, particularly when exporting to stricter, zero-tolerance countries such as the USA and Japan (G. Malan, pers. comm.). Each of the above interceptions would result in consignment rejection to these stricter phytosanitary markets.

Table 2.1: Frequency of insect taxa intercepted on export commodities of Proteaceae from Cape Town International Airport (South Africa) between March and November 2016-2017**.**

Taxon	*Protea*	*Leucospermum*	*Leucadendron*	Foliage-and filler material	Bouquets	*Banksia*	TOTAL
Dermaptera							
Forficulidae		-	-	1	-	-	1
Blattodea							
Blattellidae	-	-	1	-	-	-	1
Hemiptera							
Aphididiae	-	-	-	2	-	-	2
Cicadellidae	-	-	-	3	-	-	3
Coccidae	1	-	-	-	-	-	1
Lygaeidae	-	-	-	3	-	-	3
Pentatomidae	-	-	-	2	-	-	2
Pseudococcidae	2	4	-	-	-	-	6
Pyrrochoridae	-	-	1	1	-	-	2
Thysanoptera							
Thripidae	3	9	-	7	1	-	20
Coleoptera							
Carabidae	-	-	1	2	-	1	4
Chrysomelidae	-	1	-	-	-	-	1
Coccinelidae	-	1	1	-	-	-	2
Curculionidae	-	3	1	6	-	-	10
Histeriidae	-	1	-	-	1	-	2
Scarabidae	-	1	-	-	1	-	2
Hymenoptera							
Braconidae	-	-	1	-	-	-	1
Evaniidae	-	-	-	1	-	-	1
Formicidae	-	1	-	4	-	-	5
Lepidoptera							
Noctuidae	-	1	-	-	-	-	1
Oecophoridae	-	1	-	-	-	-	1
Larvae	-	-	-	4	-	-	4
Diptera							
Cecidomyiidae	1	-	-	-	1	-	2
Calliphoridae	-	1	-	-	-	-	1
Culicidae	-	-	-	2	-	-	2
Tipulidae	-	-	-	1	-	-	1
Bombyliidae	-	-	-	1	-	-	1
TOTAL	7	24	6	40	4	1	**82**

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Many of the interceptions were solitary individuals from a variety of insect families. These insects, such as the earwig (Forficulidae) and cockroach (Blatellidae) belong to the "tourist" (Wright and Giliomee, 1990) or "hitchhiker" guild of insects, which do not explicitly associate with Proteaceae but end up in the boxes during the packing or transport stages. Although mostly consisting of generalist, low-risk species, they can sometimes result in rejection prior to export.

The Hemipterans were most diverse and abundant on Cape flora greens. The large variety in products, and the less intensive manner in which the greenery and bouquet fillers are harvested can result in higher instances of insect presence. Mealybugs (Pseudoccocidae) and scale insects (Coccidae) found within *Protea* commodities are of far higher concern. These insects occur between the bracts of *P. cynaroides*, and can result in serious sooty mould infections which lower overall aesthetic quality of the flowers (Myburgh and Rust, 1975). Scale insects and mealybugs are considered to be major Proteaceae pests internationally (Wright, 2003).

The regular presence and often astounding abundance of thrips is of major concern. The thrips, by far mostly *F. occidentalis*, were never only a few individuals. Once native to western North America, western flower thrips has reached a near-cosmopolitan distribution through horticultural trade routes and is a pest of many international crops (Kirk and Terry, 2003). The insect causes direct damage through feeding and oviposition and also acts as a vector for viral plant diseases (Pupin et al., 2013). The western flower thrips is particularly difficult to control due to thigmokinetic behaviour and fast development of pesticide resistance (Jensen, 2000). Their cryptic life-cycle and presence deep within the flowers, small size and growing international concern makes these the most threatening of encountered

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insects (Jensen, 2000; Maynard et al., 2004). Thrips are considered one of the most important phytosanitary barriers to the export of South African Proteaceae (J. Walsh; G. Malan, pers. comm.).

The Coleopterans were the second most encountered order, however interceptions were mostly due to the presence of solitary individuals from a variety of families. The fairly large beetles were easily observed and were often few enough to remove by hand. The most commonly intercepted family was the weevils (Curculionidae), mostly consisting of the banded fruit weevil (*P. callosus*) and occasionally the Fuller rose weevil (*N. godmanni*). The banded fruit weevil is indigenous to the south-western Cape of South Africa, where it is a key pest on apples, pears, nectarines, plums, peaches and grapes (Prinsloo and Uys, 2015). The Fuller's rose weevil is a cosmopolitan beetle believed to have originated from South America (Normark, 1996). Despite the rather unpalatable nature of the sclerophyllous leaves, various weevils are recorded as foliage feeders on Proteaceae plants (Gess, 1968).

The Hymenopterans were represented by only three families. Two were solitary individuals of the Evaniid and Braconid families, wasps which fulfill very important roles as parisitoids in natural systems. These insects may be beneficial in plantations for controlling insect populations (Leandro et al., 2003), but are problematic when encountered in consignments. Ants (Formicidae) were mostly found in Cape flora greenery consignments, and are considered to be nectar thieves (Coetzee and Giliomee, 1985). They do not directly reduce flower quality, but the symbiotic relationship between scale insects, mealybugs and ants should be noted (Myburgh and Rust, 1975).

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Lepidopterans are a very notable pest of Proteaceae. They negatively influence production, and their often cryptic nature poses a serious problem in export consignments (Myburgh and Rust, 1975). As many as 21 species of borers infest Proteaceae within the Cape region (Wright, 2003). *Protea* is particularly susceptible, where infestation levels can be as high as 35%, resulting in crop losses in plantations anywhere from 1% to 35% (Wright, 2003). Both in natural and cultivated conditions, a 50% reduction in flower production can occur as a result of various stem and shoot borers (Rust and Myburgh, 1975). No adult Lepidopterans were encountered during inspections, as all individuals were still in their larval stages. This compounds the problem, as Lepidopteran identification is easiest once the insect has reached adult stage. A total of six larvae were intercepted, of which only two were successfully reared to adult stage. The speckled stem borer (*Orophia spp*), is a major Oecophorid pest of *Protea* species and a serious threat to production units. The American bollworm (*Helicoverpa armigera*), is an exotic Noctuid of similar pest and threat status, which has not only attacked Proteaceae within South Africa but has been found to attack Proteaceae in Portugal as well (Leandro et al., 2003).

The Dipterans were represented by five families, of which only one, Cecidomyidae, is considered a pest of Proteaceae. The tip fly (*Reseliella proteae*) infests bracts of Proteaceae flowers by laying eggs in between them, causing a browning of bracts and an overall decrease in flower quality (Coetzee and Giliomee, 1987).

Not incorporated into this survey but most definitely an important factor is the presence of invertebrates other than insects within export consignments. The protea itch mite (*P. vandenbergii*) was intercepted numerous times and often with high abundance. The mites also exhibit thigmokinetic behaviour, where they retreat into

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the flower head as temperatures are lowered during packing, shipment and post-harvest treatments (Coetzee et al., 1985). The high population turnover rates of the mites may lead to shipping births, where seemingly uninfested flowers are packed only to arrive overseas filled with mites awaiting warmer conditions (G. Malan, pers comm.). Once the flowers are subjected to room temperature again, the mites swarm out (Coetzee et al., 1985), where they are able to persist and multiply during the vase life stage of the cut flowers (Rust and Myburgh, 1975). Although the mites are unlikely to ever leave the flower head during shipment or once in vase (Ryke, 1964) they can cause severe skin irritation in humans (Coetzee et al., 1985). Although not found in the present study, the witches' broom mite (*Aceria proteae*) is believed to be the vector of the agent which causes witches' broom outbreaks in plantations, and so mites should not be dismissed as merely hitchhikers (Coetzee et al., 1985). Often found, particularly among Cape flora greenery, were many species of spiders. These could also easily result in mandatory fumigations and rejections, and should be considered in future surveys.

The faunal list compiled in this study allows one to compare phytosanitary insects in export consignments with the known pests of Proteaceae and other crops abroad. A comprehensive survey by Wright (2003) highlighted major pests of each notable Proteaceae producing country or region. In Hawaii, the Fuller rose weevil (*N. godmanni*), western flower thrips (*F. occidentalis*), various aphids, mealybugs and scale insects have become established in Proteaceae plantations. Californian grown Proteaceae has also suffered damage from Fuller rose weevil (*N. godmanni*) and western flower thrips (*F. occidentalis*). Australian-grown Proteaceae has not experienced serious infestations of any particular pest as of yet, and local pests of *Banksia* have not yet shifted their host range to include South African crops. New

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Zealand appears to be comparatively free of pests on South African Proteaceae, and research is more focused on crop diseases. Portugal has suffered crop damage to a great extent as a result of Lepidopteran borer infestations. The American bollworm (*H. armigera*) was a severe pest on *Leucadendron* crops, but was eventually replaced by the carnation worm (*Cacoecimorpha pronubana*) which attacks both *Leucospermum* and *Leucadendron* crops (Wright, 2003).

2.4 Conclusion

This qualitative survey of insect collections at export points for Proteaceae products shows how diverse the plant-insect interactions are between the flowers and their insect counterparts. However, the faunal list is by no means complete. Commodities were limited not only by the time of year inspections took place, but also by the exporter themselves, as different exporters place emphasis on different commodities, depending on the country of import and overall desirability. Incorporating a larger variety of export commodities will undoubtedly increase the faunal diversity drastically.

That being said, a clear trend is noted in the presence and abundances of insects. Many of the interceptions were the presence of single individuals representing entire families, and even entire orders. These once-off interceptions consisting of “tourists” and “hitchhikers” boosts overall diversity of taxa found within consignments.

What should be of most concern is the presence of some very notable pests of Proteaceae and other crops within export consignments. Very few international pests have become established within Proteaceae plantations in South Africa, and it is suggested that the generally low quality, sclerophyllous nature of the plants is the

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reason for this. It is further suggested that Proteaceae grown in countries other than South Africa would also experience low levels of infestation from their local pests for the same reasons. Therefore, any internationally important pests of Proteaceae would most likely originate from South Africa (Wright and Saunderson, 1995), and importing countries which grow South African Proteaceae and related plants would have to rely on strict phytosanitary measures to ensure they remain pest-free. Furthermore, the introduction of South African pests in other countries does not only threaten Proteaceae production abroad, but also potentially other cut flowers products, along with far larger industries such as that of fruit and vegetable crops.

Possibly the most important conclusion from this survey is the dire need for effective postharvest treatments for the South African Proteaceae industry. Pesticides registered for the Proteaceae industry are minimal and the list of target insects is substantial. Effective postharvest techniques could drastically decrease overall consignment rejection, allow for industry expansion and promote the reputation of the South African Proteaceae industry.

2.5 References cited

Coetzee, J.H., and Giliomee, J.H. (1985). Insects in association with the inflorescence of Protea repens (L.) (Proteaceae) and their role in pollination. Journal of the Entomological Society of southern Africa, 48, 303–314.

Coetzee, J.H., and Gilomee, J.H. (1987). Borers and other inhabitants of the inflorescences and infrutescences of *Protea repens* in the western Cape. *Phytophylactica* 19, 1–6.

Stellenbosch University https://scholar.sun.ac.za

Coetzee, J.H., and Littlejohn, G.M. (1994). Ornamental horticulture in rural areas in South Africa. *Horticulture in Human life, Culture and Environment*. 391, 173–182.

Coetzee, J.H., and Littlejohn, G.M. (2001). *Protea*: A floricultural crop from the Cape Floristic Kingdom. *Horticultural Reviews*. 26:1–48.

Coetzee, J.H., Rust, D.J, and Latsky, L. (1985). Mites (Acari) on Proteas. In *1st International Protea Research Symposium*, pp. 247–252.

Coetzee, J.H., Wright, M.G., and Gilomee, J.H. (1997). Anti-herbivore mechanisms of economically important Proteaceae species in the African Cape Fynbos. *Journal of Applied Entomology*. 121, 367–372.

FAO. (2011). ISPM 5: Glossary of Phytosanitary Terms.

Gerber, A.I. (2008). Contributions of research to the evolution of the *Protea* industry. *Acta Horticulturae*. 805, 15–20.

Gess, F.W. (1968). Insects found on Proteas. *Veld Flora,* 54, 29.

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

Gollnow, B., and Gerber, A.I. (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*. 73–84.

Stellenbosch University https://scholar.sun.ac.za

Jensen, S. (2000). Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integrated Pest Management Reviews*. 5, 131–146.

Kirk, W.D.J., and Terry, L.I. (2003). The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology*. 5, 301–310.

Leandro, M.J., Oliveira, M., Melo, C., and Mexia, A. (2003). Survey of the insect population on a *Protea* plantation in the Southwest of Portugal. *Acta Horticulturae*. 602, 67–74.

Maynard, G. V., Hamilton, J.G., and Grimshaw, J.F. (2004). Quarantine - Phytosanitary, sanitary and incursion management: An Australian entomological perspective. *Austral Entomology*. 43, 318–328.

Myburgh, A.C., and Rust, D.J. (1975). A survey of pests of the Proteaceae in the western and southern Cape Province. *Journal of the Entomological Society of southern Africa*. 38, 55–60.

Myburgh, A.C., Rust, D.J. and Starke, L.C. (1973). Pests of protea cut-flowers. *Journal of the Entomological Society of southern Africa.* 36, 251–255.

Normark, B.B. (1996). Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): evidence from mitochondrial DNA sequences. *Evolution*. 50, 734–745.

Picker, M. (2012). Field guide to insects of South Africa. *Penguin Random House,* South Africa.

Stellenbosch University https://scholar.sun.ac.za

Picker, M., & Samways, M. (1996). Faunal diversity and endemicity of the Cape Peninsula, South Africa - a first assessment. *Biodiversity & Conservation.* 5, 591–606.

Prinsloo, G. L., & Uys, V. M. (Eds.). (2015). Insects of cultivated plants and natural pastures in Southern Africa. *Entomological Society of Southern Africa*. Pretoria: Southern Africa

Procheş, Ş., and Cowling, R.M. (2006). Insect diversity in Cape fynbos and neighbouring South African vegetation. *Global ecology and biogeography*. 15, 445–451.

Procheş, Ş., Forest, F., Veldtman, R., Chown, S.L., Cowling, R.M., Johnson, S.D., Richardson, D.M., and Savolainen, V. (2009). Dissecting the plant-insect diversity relationship in the Cape. *Molecular Phylogenetics and Evolution*, *51*(1), 94-99.

Pupin, F., Bikoba, V., Biasi, W.B., Pedroso, G.M., Ouyang, Y., Grafton-Cardwell, E.E., and 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*(6), 2341-2348.

Reinten, E.Y., Coetzee, J.H., and Van Wyk, B.E. (2011). The potential of South African indigenous plants for the international cut flower trade. *South African Journal of Botany*, *77*(4), 934-946.

Stellenbosch University https://scholar.sun.ac.za

Roets, F., Dreyer, L.L., Geertsema, H., and Crous, P.W. (2006). Arthropod communities in Proteaceae infructescences: seasonal variation and the influence of infructescence phenology. *African Entomology*, *14*(2), 257-265.

Rust, D.J., and Myburgh, A.C. (1975). Pests as a factor limiting the development of the *Protea* industry. *Veld Flora.* 61, 16–19.

Ryke, P.A.J. (1964). Acarina associated with *Protea* flowers in the Cape Province. *Journal of the Entomological Society of Southern Africa,* 26, 337-354.

Theron, N. (2011). Mite communities within *Protea* infructescences in South Africa (Masters dissertation, Stellenbosch: University of Stellenbosch).

Wright, M.G. (1993). Integrated pest management - concepts and potential for the control of borers on proteas. In *III International Protea Research Symposium 387.* 153-158.

Wright, M.G. (2003). Insect pests of proteaceae: Assessment of predictions of new pests internationally, and management implications. In *VI International Protea Research Symposium 602*, 167-171.

Wright, M.G., and Gilomee, J.H. (1990). Guild composition and seasonal distribution of insects on *Protea magnifica* and *P. laurifolia* (Proteaceae). *African Zoology*, *25*, 245-249

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

Stellenbosch University https://scholar.sun.ac.za

Wright, M.G., and Saunderson, M. (1995). Protea plant protection: From the African context to the international arena. In *III International Protea Research Symposium, 387,* 129-140.

Ziskovsky, B. (2015). Expanding the seasonal potted plant floral market in North America with containerized *Protea cynaroides* L. (Masters dissertation, Stellenbosch: University of Minnesota).

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Chapter 3: Controlled Atmosphere and Temperature Treatment Systems (CATTS) technology as a potential postharvest disinfestation technique for South African export Proteaceae cut flower stems

3.1 Introduction

The South African Proteaceae export industry is required to deliver insect-free commodities in order to access phytosanitary markets such as the United States of America and Japan. Phytosanitary restrictions limit market access and pose barriers to the industry, as both wild and cultivated Proteaceae associate with a considerable entomofauna, which can be both direct and phytosanitary pests of export products (Wright and Saunderson, 1995; Wright, 2003; Roets et al., 2006; Janick, 2007; Prinsloo & Uys, 2015). Preharvest control measures such as pesticide spray regimes, plantation sanitation, and integrated pest management (IPM) systems may reduce the overall number of insects reaching the point of export, but cannot guarantee insect-free products (Hansen et al., 1992). This is further compounded by the limited number of pesticides that are registered for use on cultivated Proteaceae, as the crop is considered to be minor in comparison with other horticultural industries in South Africa, and also a fairly young industry. Postharvest treatment is the final and most effective method of ensuring that export consignments are free of living insects.

Currently, there exists an arsenal of postharvest treatments worldwide which are utilised for disinfestation of floricultural goods. This includes hand removal,

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irradiation, fumigation, insecticidal dips and sprays, cold storage, hot water baths, vapour heat and controlled atmospheres, with fumigation being the most utilised disinfestation method (Hansen & Hara, 1994). The South African Proteaceae export industry still relies almost entirely on fumigation as a means of disinfestation, utilising pyrethoids, dichlorvos and phytoxin accelerants, the degree to which varies amongst exporters (Malan, 2012). The exact figures of consignment rejection from South Africa are not known, but for a strict export destination such as Japan, an estimated 35%-40% of consignments exported are intercepted upon arriving and fumigated once again (J. Walsh, pers. comm). This is costly as exporters have to share the disinfestation costs, and repeated fumigation decreases overall flower quality of the exported goods, inducing both financial and reputational costs (J. Walsh, pers. comm).

Major phytosanitary invertebrates hindering the export of South African Proteaceae to special markets are various thrips species, in particular, the western flower thrips (*Frankliniella occidentalis*), and the protea itch mite (*Proctolaelaps vandenbergii*). western flower thrips (WFT) has reached near-cosmopolitan status (Kirk & Terry, 2003) as it is a pest of a variety of agricultural crops (Reitz, 2009), a vector of a variety of plant diseases and has the potential to rapidly develop pesticide resistance (Jensen, 2000). The protea itch mite (PIM) can occur in staggeringly high numbers within both wild and cultivated Proteaceae, particularly bearded proteas, and is capable of persisting and thriving in cut flowers long after they have been harvested (Rust & Myburgh, 1975; Coetzee et al., 1985). A single box of exported Proteaceae stems may contain hundreds, if not thousands of these invertebrates, and can easily result in consignment rejection when intercepted at inspections points in South Africa and the importing country (Taylor, 2000).

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As the global awareness increases with regard to environmental issues, consumers have begun demanding of industries to do the same by delivering agricultural commodities free of pesticide residues and which have, throughout the production and transport chains, not resulted in extreme negative impacts on the environments in which they are produced (Blend & van Ravenswaay, 1999; Opara, 2003; Tzortzakis, 2007). From a postharvest disinfestation perspective, this mind set is pushing industries towards alternative technologies, which include a variety of non-chemical methods, or by utilising chemicals considered to be non-detrimental (Follett & Neven, 2006).

A recently developed disinfestation technology is the combination of two physical treatments, namely high temperature and modified/controlled atmospheres. This combination has been termed Controlled Atmosphere and Temperature Treatment Systems (CATTS) technology whereby combining heat and controlled atmospheres of high CO_2 and low O_2 concentrations, treatment duration can be drastically reduced, maintaining produce quality while simultaneously controlling insect pests (Neven and Johnson, 2018).

CATTS has been utilised for disinfesting stone- and pome fruit from a variety of major agricultural pests, including codling moth (*Cydia pomonella*), oriental fruit moth (*Grapholita molesta*), western cherry fruit fly (*Rhagoletis indifferens*), plum curculio (*Conotrachelus nenuphar*) and apple maggot (*Rhagoletis pomonella*) (Shellie et al., 2001; Neven et al., 2006; Johnson & Neven, 2010). Furthermore, CATTS has been used to disinfest strawberry runners and rootstock of root knot nematodes and tarsonemid mites (van Kruistum et al., 2014). Slootweg (2007) showed that *Freesia*

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and roses could withstand CATTS treatments, although this is the only research available for the effect of CATTS technology on cut flowers.

Considering the hardy, sclerophyllous nature of many Proteaceae products, and the high summer temperatures which they tolerate, CATTS technology has the potential to become a postharvest disinfestation method for the cut flowers of Proteaceae. This study aimed to assess Proteaceae cut flower responses to CATTS treatments, evaluating the marketability and vase life limits of products following treatment, as well as their ability to endure air- and sea freighting and long-term cold storage following post-treatment. The efficacy of CATTS treatments against WFT and PIM was also evaluated.

3.2 Materials and Methods

3.2.1 Flowers and pre-treatment handling

Freshly harvested flowers were procured, in season, from various commercial plantations within the Western Cape, South Africa. Floral commodities were selected according to availability, relative demand from export markets, as well as to include diverse product types and growth forms. A total of four Proteaceae commodities and one Australian wildflower species were tested: 1) *Protea magnifica* 'Barbi'; 2) *Leucospermum* 'Veldfire' (*L. conocarpodendron* x *L. glabrum*); 3) *Leucadendron* 'Safari sunset' (*L. salignum* x *L. laureolum*); 4) *Leucadendron linifolium* 'Jade pearl'; 5) Geraldton wax (*Chamelaucium uncinatum*) 'Ofir'. Geraldton wax 'Ofir', although belonging to the floral family Myrtaceae, is an important component of the Cape flora greenery and bouquet filler material categories, and is sold as such.

Stems were brought to the laboratory within 24 hours of harvesting, and stored overnight at 4°C. The following day stems were removed from storage and the bottom 1 cm of each stem was trimmed off at a 45° angle. Stems were pulsed using various sugar solutions prior to treatment. *Protea* and *Leucospermum* commodities were pulsed with a 6% ($^{w}/_{v}$) sucrose solution, allowing each stem to absorb ~10mL and ~2mL for the respective genera. *Leucadendron* commodities were pulsed with a 5% ($^{w}/_{v}$) glucose solution, until stems absorbed ~2mL each. The Geraldton wax 'Ofir' was also pulsed using a 5% ($^{w}/_{v}$) glucose solution, until individual stems had absorbed ~2mL each. Once pulsing was completed, individual stem weight was recorded. Prior to treatment, flower quality was evaluated according to a scoring system described below.

3.2.2 Flower quality scoring system

Commodity quality was evaluated using a 2-part, 10-point phytotoxicity scoring system. The scoring criteria used were adjusted from a version used in various other studies (Hansen et al.,1991a; 1991b; Hansen et al., 1992). Each stem received scores ranging from 0 to 9, based on the condition of the foliage (referring to the stems and leaves of the product), and the inflorescence (referring to the flower head and bloom itself) (Table 3.1). The individual foliage and inflorescence scores of each stem were summed to achieve an overall score of 0 to 18, representing an overall quality score for each stem. This scale of 0 to 18 was used to determine critical quality thresholds, where 0 = no damage, 4 = limit of marketability (i.e. lowest acceptable quality for commercial sale of product), 8 = limit of vase life (i.e. product deteriorates in vase to the point of being aesthetically unpleasant) and 18 = complete discoloration or destruction of both the foliage and inflorescence. For Geraldton wax

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and coneless *Leucadendron* commodities, the foliage score of 0 to 9 represented overall flower quality, and thresholds were adjusted (0 = no damage, 2 = limit of marketability, 4 = limit of vase life, and 9 = complete discoloration or destruction of the foliage).

Table 3.1: Description of scoring criteria for phytotoxicity ratings and overall flower score in Proteaceae commodities. Overall flower score equals the sum of individual inflorescence- and foliage scores (except Geraldton wax and coneless *Leucadendron*, overall flower score equals foliage score).

Rating	**Inflorescence score**			**Foliage score/ Geraldton wax score/ Coneless *Leucadendron* 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

This scoring system was also used to evaluate flower quality in vase life assessments conducted immediately after CATTS treatments, as well as assessments of flowers subjected to simulated air- and sea freight conditions after treatment.

3.2.3 Treatments and vase life studies

CATTS treatments were performed using a laboratory-scale CATTS unit (Techni-Systems, Chelan, WA, USA) housed at the Department of Conservation Ecology and Entomology, Stellenbosch University. Each treatment per commodity consisted of 10 stem replicates, with 10 control stems per trial. Control stems were subjected to the same pre-treatment handling procedures (pulsing and scoring) and vase solutions following treatment as the treated stems. The four CATTS treatments applied under a controlled atmosphere of 1% O_2, 15% CO_2 in N_2 were:

1) 35°C/hour temperature ramp rate from 23°C to 40°C, with a 15 min soaking period at 40°C
2) 30°C/hour temperature ramp rate from 23°C to 40°C, with a 15 min soaking period at 40°C
3) 35°C/hour temperature ramp rate from 23°C to 50°C, with a 15 min soaking period at 50°C
4) 30°C/hour temperature ramp rate from 23°C to 50°C, with a 15 min soaking period at 50°C

Directly after treatment, individual stems were weighed to calculate mass loss during treatment, and flower quality was either assessed immediately, or following cold-storage as relevant during air- and sea-freight simulation. Flowers assessed

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immediately were placed into vases containing a 2% ($^{w}/_{v}$) sucrose solution before being subjected to vase life studies. Flowers used in freight simulation trials were pulsed with the same pulsing regimes used pre-treatment and stored dry at 2°C in standard S14 boxes for 3 days (air-freight) or 21 days (sea-freight) utilising normal atmospheres. After freight-simulations were completed flowers were removed from boxes and subjected to vase life studies. Table 3.2 summarises the various CATTS treatments and post-treatment handling protocols used per commodity.

Table 3.2: CATTS treatments and Cape Flora cultivar combinations for vase life studies directly after treatment, after treatment plus air-freight simulation and after treatment plus sea-freight simulation.

CATTS treatment	Proteaceae Cultivars		
	Vase life assessed immediately after treatment	Vase life assessed after treatment plus simulated air-freight	Vase life assessed after treatment plus simulated sea-freight
1	'Veldfire' 'Barbi' 'Safari sunset' 'Ofir'	'Safari sunset' 'Jade pearl' 'Ofir'	'Safari sunset' 'Jade pearl'
2	'Veldfire' 'Barbi' 'Safari sunset' 'Ofir'	'Safari sunset' 'Jade pearl' 'Ofir'	'Safari sunset' 'Jade pearl'
3	'Veldfire' 'Barbi' 'Safari sunset'	-[z]	-
4	'Veldfire' 'Safari sunset'	-	-

[z]Air- and sea-freight simulations were not carried out on cultivars that were subjected to Treatments 3 and 4, due to the poor post treatment flower quality resulting from those treatments

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The vase life studies were conducted for 10 days for *Protea magnifica* ('Barbi') and *Leucospermum* ('Veldfire') commodities, and 14 days for *Leucadendron* ('Safari sunset' and 'Jade pearl') and Geraldton wax ('Ofir') commodities. The flowers were kept under a natural photoperiod of 12:12 hours, with a room temperature of 20°C ±2°C. All treatments received a 2% ($^{w}/_{v}$) sucrose vase solution which was replaced on every evaluation day. Flower quality evaluations were performed every second day in *Protea*, *Leucospermum* and in all trials which simulated freight storage (Day 2, 4, 6, 8, and 10) and every second day following day one for *Leucadendron* and Geraldton wax trials (Day 1, 3, 5, 7, 10, and 14). The marketability and vase life critical quality thresholds were estimated by plotting the average overall flower score for each commodity against the time elapsed since the treatments commenced. The critical intercepts on the y-axis were determined and rounded to the nearest full evaluation day. A value of 4 was established as the marketability limit, except for coneless 'Safari sunset' and Geraldton wax 'Ofir to which a value of 2 was allocated, whilst a value of 8 was assigned for vase life limit, except for coneless 'Safari sunset' and Geraldton wax 'Ofir' were the value of 4 was established.

3.2.4 Insect mortality trials

Western flower thrips (WFT), as well as other thrips species coincidentally present at the time, were collected from wild-growing *Trifolioum* and *Tropaeolum* species in Stellenbosch, South Africa throughout the month of October 2017. The protea itch mite (PIM) was collected from wild-growing visibly mite-infested *Protea neriifolia*, growing on the outskirts of Stellenbosch during the month of September 2017. Source plant material was shaken above sheets of white paper to dislodge any insects and mites and any live specimens were collected into specimen jars using a pooter. Specimen jars was sealed with finely-meshed gauze, allowing for heat and atmosphere penetration during treatment application, but preventing the escape of any specimens.

On the same day of collection, thrips and mites were subjected to CATTS treatments 1 and 2. Directly after treatment, the specimen jars were emptied onto a sheet of white paper and the number of dead thrips or mites were counted. Living thrips and mites were also counted and collected, and assessed again 24 hours after treatment for further mortalities. All treated thrips were individually inspected and any adult western flower thrips were identified and counted. The rest were classified as “mixed thrips”, belonging to various species and consisting of multiple instars.

3.2.5 Statistical analyses

Overall flower scores were analysed by a mixed model repeated measures ANOVA using Statistica, version 13 (Dell Statistica – data analysis software system, Dell Inc. 2016). The treatments and time in vase were included as fixed effects, and the flowers were included as random effects.

3.3 Results

3.3.1 Phytotoxicity immediately after CATTS treatment

Across all four commodities and between treatments, differences were observed in overall flower quality of stems assessed in vase life studies starting immediately after treatment.

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Leucospermum 'Veldfire' flowers exhibited severe and rapid pin wilting across all treatments (Fig 3.1A; Fig 3.4A). Bleaching and drying of leaf margins was also evident after treatments (Fig 3.1B). The more severe treatments (Treatment 3 and 4) that ramped to 50°C induced higher levels of leaf damage, maximizing the overall phytotoxicity score. The average overall flower scores for treated stems by Day 2 were 10.6 ± 0.52, 13.2 ± 0.79, 18.00 ± 0.00, and 18.00 ± 0.00 for Treatments 1 to 4 respectively, all already exceeding marketability and vase life limits (Fig 3.4A). Untreated control stems exhibited an average overall flower score of only 3.00 ± 1.0, maintaining overall quality significantly better than treated stems ($p < 0.01$). It appears that 'Veldfire' is not able to tolerate high temperatures in combination with controlled atmospheres, as in pilot trials, utilising the same temperature ramps under normal atmosphere, no pin wilting was observed (data not shown).

Figure 3.1: Phytotoxic damage observed in CATTS treated *Leucospermum* 'Veldfire' stems on Day 7 of vase life studies. Damage manifested as severe and complete wilting of styles (A) (Treatment 1 - 4 from left to right, untreated control far-right), with bleaching and drying of leaf margins evident (B) (Treatment 1 - 4 from left to right, untreated control far-right).

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Protea magnifica 'Barbi' flowers withstood both temperature ramps of 30°C/hr and 35°C/hr to 40°C (Treatments 1 and 2) and reached average overall flower quality scores of 6.4 ± 1.35 and 7 ± 1.05 respectively on conclusion of vase life assessment conducted immediately after treatment. These were comparable to, and did not differ

Figure 3.2: Phytotoxic damage observed in CATTS treated *Protea magnifica* 'Barbi' stems within 48 hours of treatment. Damage manifested as severe leaf blackening and discoloration of involucral (Treatment 3).

significantly from the untreated control score at 6.00 ± 1.25 (Fig 3.4B). The treatment ramped at 35°C/hr to 50°C (Treatment 3) resulted in significantly more damage to the flowers when compared to control stems and the treatments ramped to 40°C ($p <$ 0.01). Treatment 3 stems exhibited severe leaf blackening and discoloration of involucral bracts (Fig 3.2). With regard to limit of marketability, all treatments reached the limit of marketability before the untreated controls. Stems subjected to Treatment 3 breached limit of marketability immediately after treatment, with an average overall flower score of 5.4 ± 0.97. Both Treatments 1 and 2 did not differ significantly from, and maintained vase life as well as untreated controls, and did not breach limit of vase life by conclusion of the study ($p > 0.05$). Treatment 3 stems breached limit of

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vase life within 48 hours of treatment, exhibiting an average overall flower score of 8.00 ± 0.47.

Leucadendron 'Safari sunset' stems subjected to Treatments 1 and 2 also maintained quality comparable to the untreated controls ($p > 0.05$), exhibiting average overall flower scores of 1.7 ± 0.48 and 1.9 ± 0.32 respectively, with 1.9 ± 0.74 recorded for the control stems (Fig 3.3A; Fig 3.4C). Stems subjected to Treatments 3 and 4 did not perform as well by exhibiting average overall flower scores of 4.2 ± 0.92 and 4.9 ± 0.74 respectively, both being significantly above that of Treatments 1, 2 and controls ($p < 0.05$), by conclusion of the vase life study. Treatments 3 and 4 stems also breached both limit of marketability and limit of vase life by Day 3. Phytotoxic damage on Day 3 in Treatment 3 stems was seen as wilting and darkened foliage with desiccation of leaf tips (Fig 3.3B; 3.3C). By Day 3, Treatments 1 and 2 stems, together with controls, did not breach even the stricter limit of marketability.

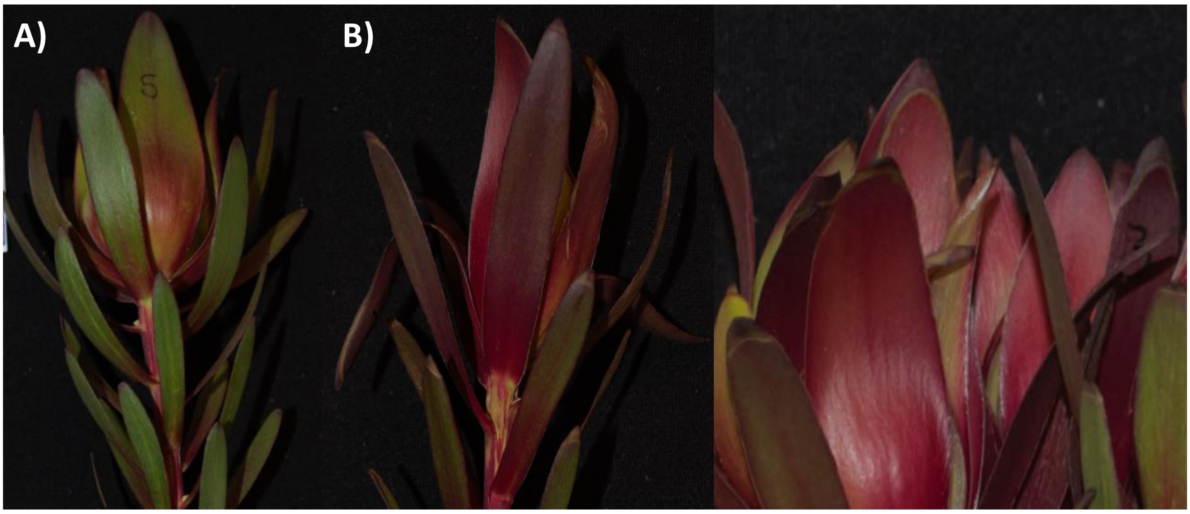

Figure 3.3: Phytotoxic damage observed in CATTS treated *Leucadendron* 'Safari sunset' stems on Day 3 of vase life studies. A) Treatment 1 stems (A) showed no discernible damage; B) Treatment 3 stems exhibited slight wilting and some darkening of foliage (B) and desiccation of leaf tips (C).

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Geraldton wax 'Ofir' withstood Treatment 1 and 2 equally well (Fig 3.4D), exhibiting average overall flower scores of 1.8 ± 0.42 and 1.9 ± 0.32 respectively by conclusion of the vase life study ($p > 0.05$). Overall flower quality was better in untreated control stems on conclusion of vase life study ($p < 0.05$), however neither Treatment 1 nor 2 reached limit of marketability by conclusion of the vase life study.

Of the four cultivars treated and assessed immediately after treatment, *Leucospermum* 'Veldfire' was the most susceptible by exhibiting the most notable damage in the form of severe style wilting, even when subjected to the mildest treatment. At the milder treatments (Treatments 1 and 2) the most tolerant cultivar was *Leucadendron* 'Safari sunset', as stems maintained quality on par with the untreated controls and did not breach the limit of marketability by the end of the evaluation period. The three cultivars subjected to the harsher treatments (Treatments 3 and 4) all reached the limit of vase life prior to the final vase life evaluation day. Again *Leucospermum* 'Veldfire' being the most susceptible reaching the limit before Day 2 whereas *Leucadendron* 'Safari sunset', the most tolerant, lasted up to Day 8 for Treatment 4 and Day 11 for Treatment 3.

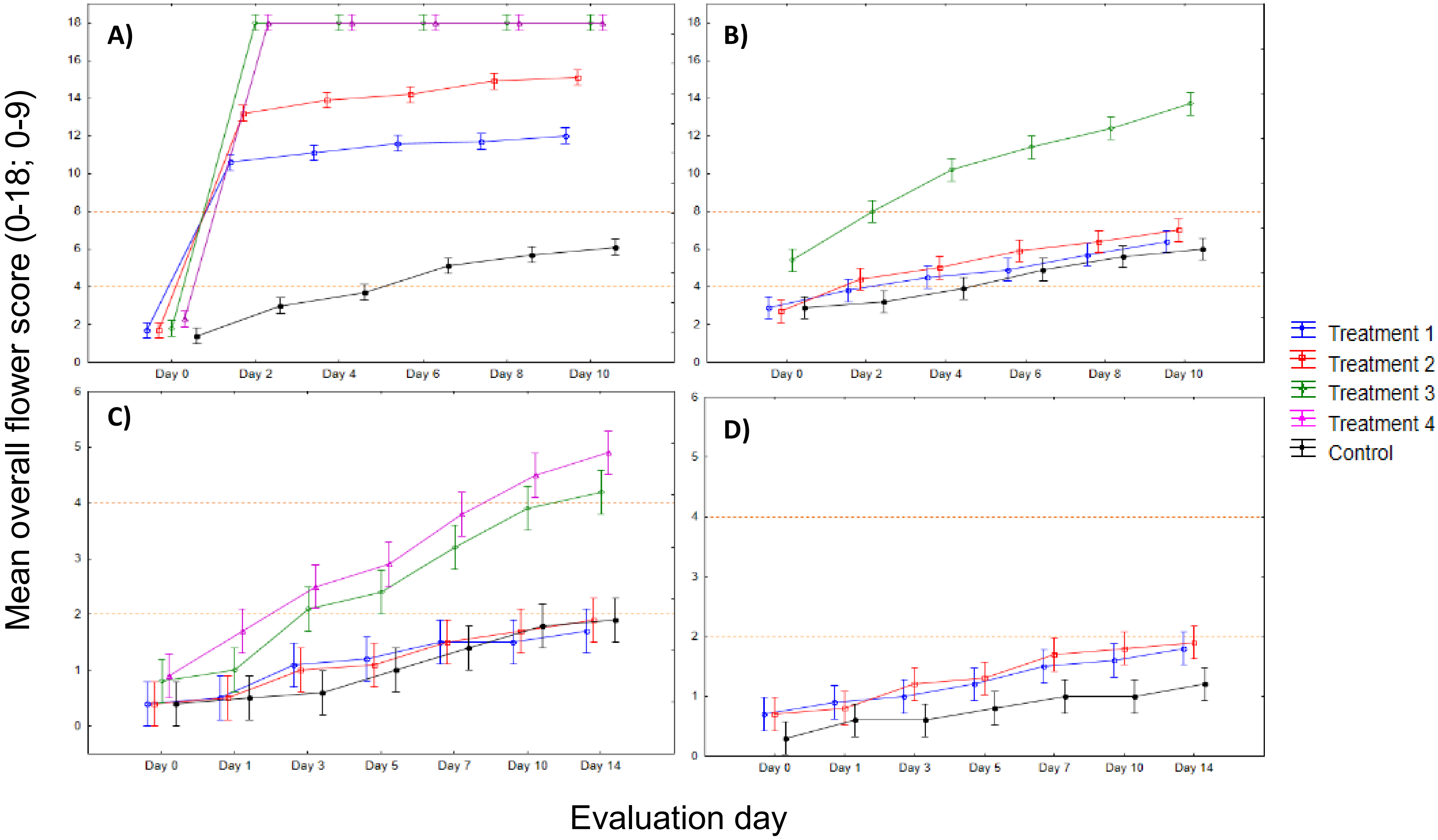

Figure 3.4: Mean overall flower scores of *Leucospermum* ‘Veldfire’ (A), *Protea magnifica* ‘Barbi’ (B), coneless *Leucadendron* ‘Safari sunset’ (C), and Geraldton wax ‘Ofir’ (D) as assessed immediately after CATTS treatments. Dashed yellow line (y=4; 2 in D) represents limit of marketability (point after which flower is no longer commercially sellable), and orange dashed line (y=8; 4 in D) represents limit of vase life (point at which flowers are no longer aesthetically pleasing). Vertical bars denote 0.95 confidence intervals.

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3.3.2 Phytotoxicity following CATTS treatment plus air-freight simulation

Leucadendron 'Safari sunset' stems withstood the combination of CATTS treatments and simulated air-freight, with overall quality in stems subjected to both Treatments 1 and 2 being comparable to untreated controls throughout the vase life period (Fig 3.6A). Neither limit of marketability nor vase life limits were reached by conclusion of vase life studies in treated and untreated stems. The average overall flower scores of stems subjected to Treatment 1, Treatment 2 and untreated control stems by the conclusion of the vase life study was 2.3 ± 0.67, 2.4 ± 0.97 and 2.0 ± 0.67 respectively, and did not differ significantly (p > 0.05).

Leucadendron 'Jade pearl' was similar to 'Safari sunset', able to withstand the combination of CATTS and air-freight simulation, with treated and control stems performing equally well, showing no significant difference in quality, until Day 8 of vase life studies. No discernible damage was observed in *Leucadendron* 'Jade pearl' in either Treatments 1 or 2 (Fig 3.5). Both treated and untreated control stems did

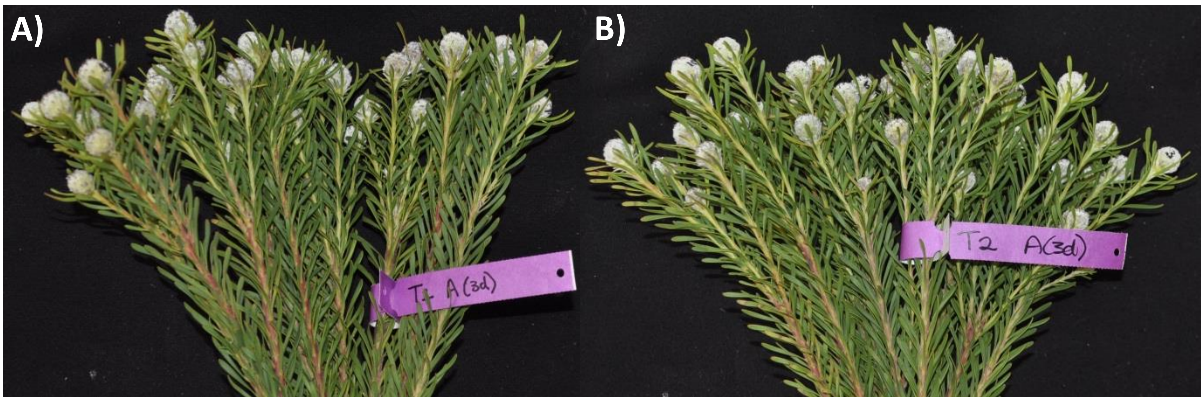

Figure 3.5: Phytotoxic damage in CATTS treated *Leucadendron* 'Jade pearl' stems following 3 days dry storage at 2°C and then 10 days in vase. No discernible damage was observed in Treatment 1 (A) or Treatment 2 (B).

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not reach limit of marketability after 10 days. The average overall flower scores of control stems and stems subjected to Treatment 1 and Treatment 2 did not differ throughout the vase life study until Day 10, when average overall flower scores of 1.5 ± 0.53, 2.2 ± 0.63 and 2.1 ± 0.74 respectively was recorded (Fig 3.6B).

Geraldton wax 'Ofir' withstood CATTS treatments and air-freight simulation, with overall flower scores in stems exposed to Treatment 1 rating comparable to untreated control stems (Fig 3.6C). Stems subjected to Treatment 2 did not maintain overall quality as well as those subjected to Treatment 1 and compared to untreated stems, and breached limit of marketability by the conclusion of the vase life study.

Apart from Geraldton wax 'Ofir' where stem just breached the limit of marketability by the end of the evaluation period, cultivars in general tolerated the CATTS treatment and air-freight simulation storage very well and were able to maintain marketable quality.

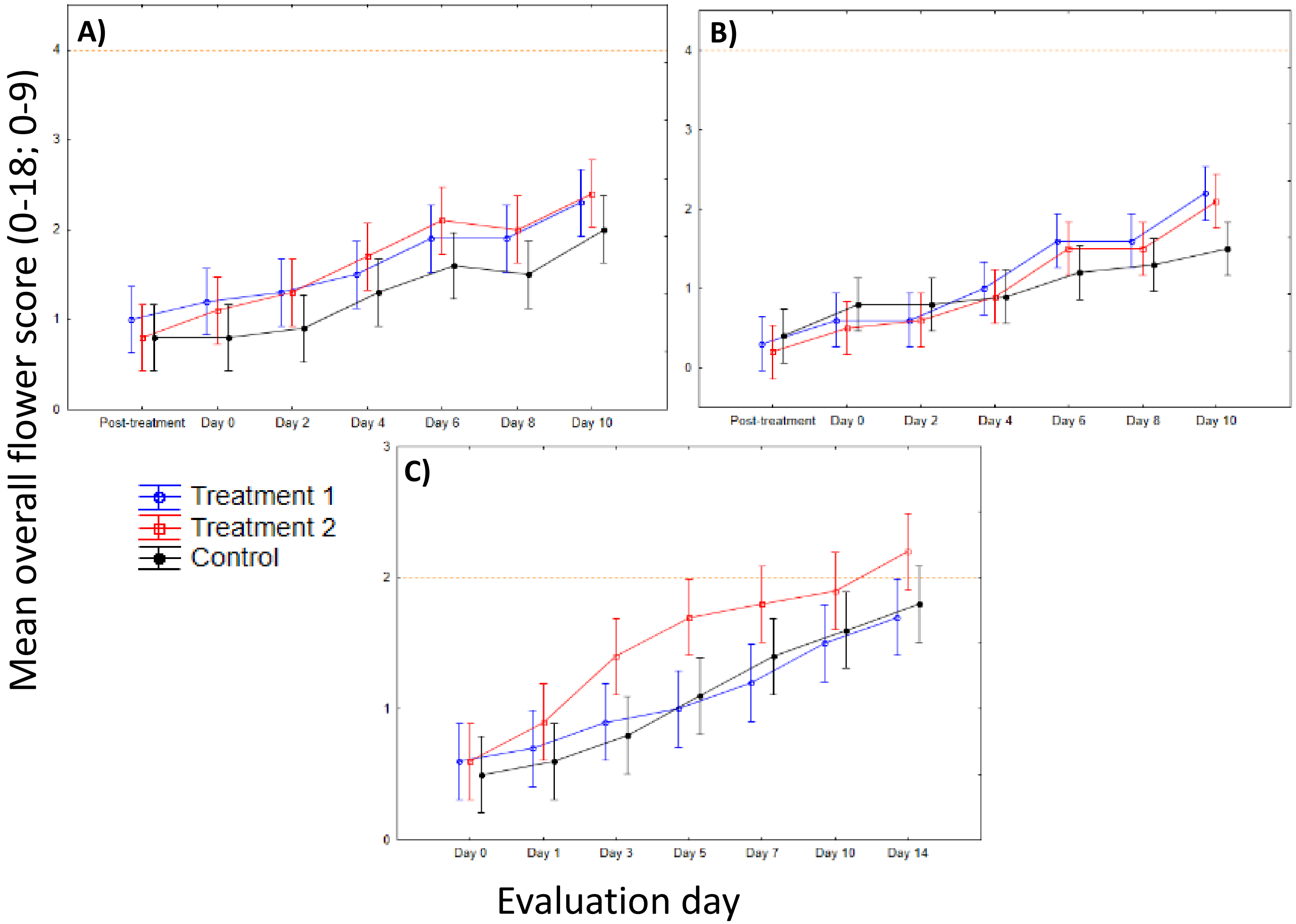

Figure 3.6: Mean overall flower scores of *Leucadendron* 'Safari sunset' (A), *Leucadendron* 'Jade pearl' (B), and Geraldton wax 'Ofir' (C) in after various CATTS treatments and simulated air-freight storage at 2°C for 3 days. Dashed yellow line (y=4; 2 in C) represents limit of marketability (point after which flower is no longer commercially sellable). Vertical bars denote 0.95 confidence intervals. Due to time constraints, post-treatment scores were recorded for Geraldton wax 'Ofir' (C).

3.3.3 Phytotoxicity and sea-freight simulations

Leucadendron 'Safari sunset' withstood the combination of CATTS treatments and simulated sea-freight (Fig 3.7A; 3.7B), with overall quality in stems subjected to both Treatment 1 and 2 being comparable to untreated controls throughout the vase life period (Fig 3.8A). Neither limit of marketability nor vase life limits were reached by conclusion of vase life studies in treated and untreated stems. The average overall flower scores of stems subjected to Treatment 1, Treatment 2 and untreated control stems did not differ significantly ($p > 0.05$) by the conclusion of the vase life study at 2.7 ± 0.67, 2.9 ± 0.57 and 2.7 ± 0.67 respectively.

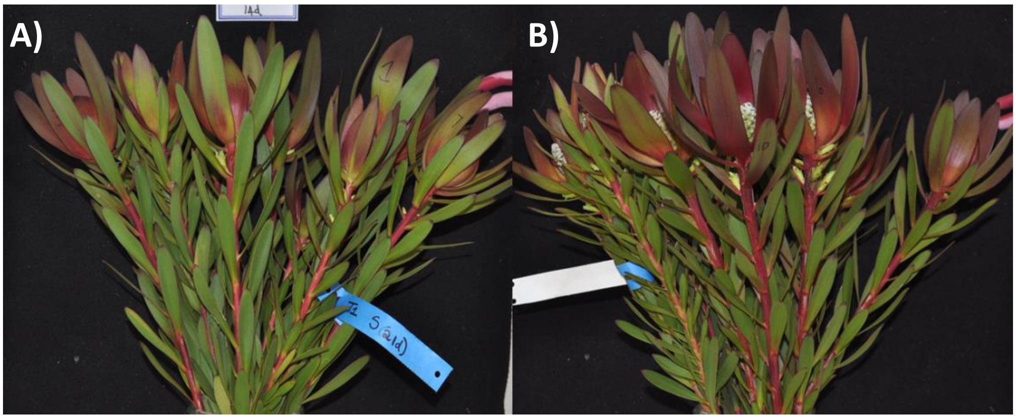

Figure 3.7: Phytotoxic damage in CATTS treated *Leucadendron* 'Safari sunset' stems following 21 days dry storage at 2°C and then 10 days in vase. No discernible damage was observed in Treatment 1 (A) or Treatment 2 (B).

Leucadendron 'Jade pearl' also withstood the combination of CATTS and sea-freight simulation, with stems subjected to Treatment 1 and 2 performing equally well throughout the vase life period ($p > 0.05$) with neither reaching limit of marketability by conclusion of the vase life period (Fig 3.8B). Untreated control stems and treated stems performed equally well until Day 8 of the vase life evaluation after storage, from which untreated control stems maintained quality better than treated stems of

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both Treatment 1 and 2, exhibiting average overall flower scores of 1.3 ± 0.48, 1.6 ± 0.52 and 1.5 ± 0.53 respectively.

Treated stems of both *Leucadendron* 'Safari sunset' and 'Jade pearl' after being subjected to 21 days of sea-freight simulation of dry storage at 2°C maintained overall quality and did not reach the limit of marketability by the end of the vase life period.

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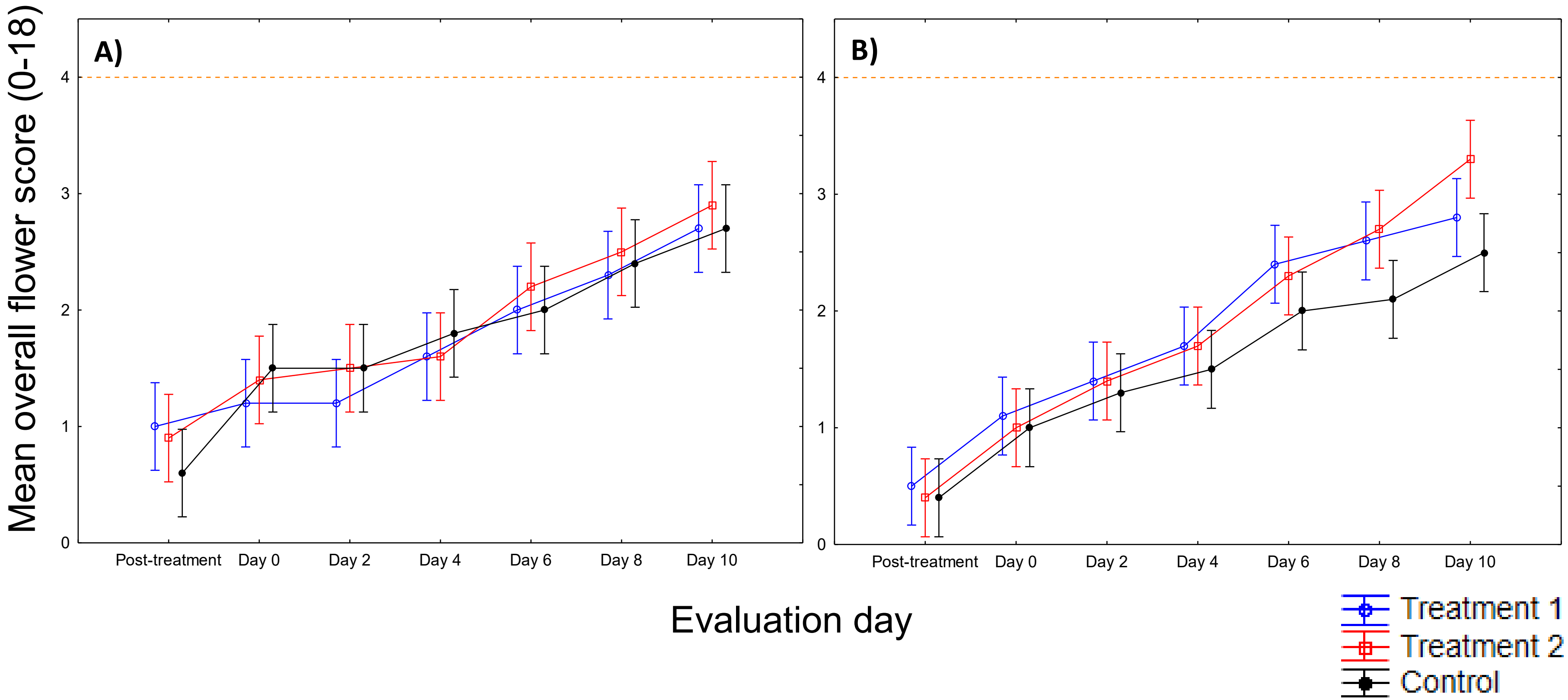

Figure 3.8: Mean overall flower scores of *Leucadendron* 'Safari sunset' (A) and *Leucadendron* 'Jade pearl' (B) after various CATTS treatments and simulated sea-freight storage at 2°C for 21 days. Dashed yellow line (y=4) represents limit of marketability (point after which flower is no longer commercially sellable). Vertical bars denote 0.95 confidence intervals.

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3.3.4 Invertebrate mortality trials

The total numbers of field collected thrips and mites subjected to Treatments 1 and 2 are given in Table 3.3. Post-treatment percentage invertebrate mortality as determined immediately after treatment and 24 hours later, are presented in Fig 3.9.

For Treatment 1, 97.17% mortality was achieved during the treatment for adult WFT, with 100% mortality within 24 hours of treatment. Similarly high mortality was achieved for mixed thrips, with 97.41% mortality during treatment, and 100% within 24 hours of treatment. Mortality at 100% was achieved in PIM during treatment. For Treatment 2, 100% mortality was achieved during the treatment for adult WFT, mixed thrips and PIM.

Table 3.3: Total number of western flower thrips (WFT), mixed thrips and protea itch mite (PIM) used to assess invertebrate mortality following CATTS treatments of 35°C/hr ramp to 40°C (Treatment 1) and 30°C/hr ramp to 40°C (Treatment 2) under a controlled atmosphere of 1% O_2, 15% CO_2 in N_2, directly after- and within 24 hours of treatment.

Assessment	Invertebrate species	Treatment 1		Treatment 2	
Prior to treatment	WFT	209		191	
	Mixed thrips	116		124	
	PIM	394		467	
Post-treatment mortality		0h	24h	0h	24h
	WFT	203 (97%)	209 (100%)	191 (100%)	191 (100%)
	Mixed thrips	113 (97%)	116 (100%)	124 (100%)	124 (100%)
	PIM	394 (100%)	394 (100%)	467 (100%)	467 (100%)

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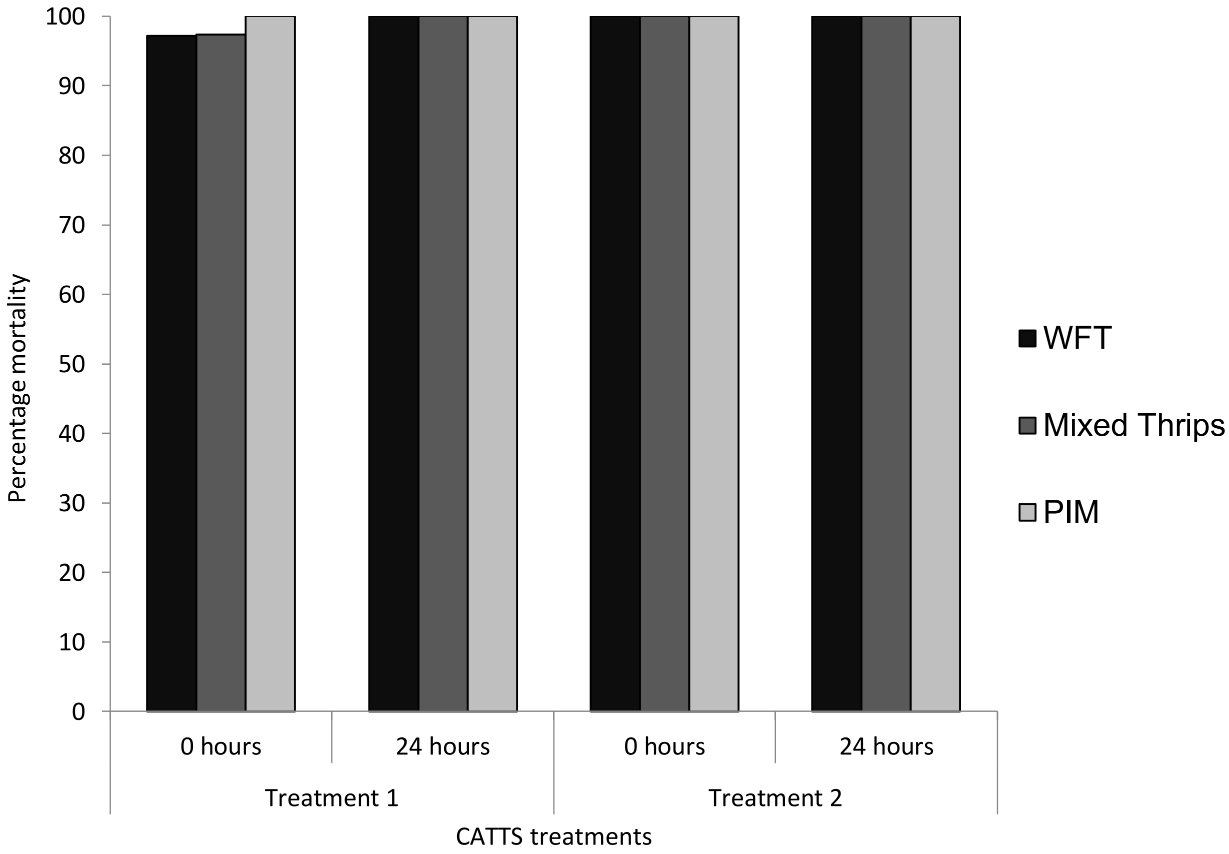

Figure 3.9: Percentage mortality of adult western flower thrips (WFT), mixed thrips and protea itch mite (PIM) directly after CATTS treatment and 24 hours after of treatment. Treatment 1: 35°C/hr ramp to 40°C; Treatment 2: 30°C/hr ramp to 40°C. Both treatments were performed in a controlled atmosphere of 1% O_2, 15% CO_2 in N_2.

3.4 Discussion

The rapid and effective control of WFT and PIM is promising. These invertebrates are commonly intercepted in export consignments, and regularly consist of high numbers of individuals. Reducing or eliminating the number of invertebrates will result in fewer consignment rejections, and exclude the need for secondary fumigations, further reducing economic and reputational losses as a result of phytosanitary insects. Although by far the most frequently intercepted pests, and the sheer magnitude of infestations being so high, future studies should include other less frequently intercepted pests and should also focus on pests which have the greatest potential for infestation abroad. One such pest, the

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banded fruit weevil (*Phlyctinus callosus*), is a South African endemic pest of various agricultural commodities and is an international concern with regard to phytosanitary control (Prinsloo and Uys, 2015). Another weevil, Fuller's rose weevil (*Naupactus godmanni*), is also intercepted in South African Proteaceae export consignments, and has proven to be a serious pest of Proteaceae plantations in both Hawaii and California (Wright, 2003). Additionally, various borers, particularly the larval stages of Lepidopteran species, have proven to be very important pests of cultivated Proteaceae (Wright, 2003). These include the Protea scarlet butterfly (*Capys alphaeus)*, Speckled stem borer (*Orophia spp*) and the American bollworm (*Helicoverpa armigera*). The cryptic nature of the boring larvae makes interception challenging, and their ability to drastically reduce productivity within Proteaceae plantations warrants research on their control utilising postharvest technologies.

Despite the success of the CATTS treatments tested here in controlling the insect pests, the use of CATTS technology is only a feasible option if flower quality is maintained after treatment. The Proteaceae commodities tested here exhibited diverse reactions to CATTS treatments. For most commodities, utilising CATTS treatments of 30°C/hr or 35°C/hr to 40°C did not reduce flower quality, marketability, or shelf life when compared to control stems, and far exceeded the commercially accepted vase life period of 7 days (Jones & Faragher, 1991). All treatments ramped to 50°C resulted in significant reduction in overall flower quality, marketability and shelf life of treated commodities, and exceeded temperatures needed to successfully control treated pests.

The heat damage observed in *Leucospermum* 'Veldfire' was severe. Leaves appeared bleached and changed to a lighter green hue with styles collapsing completely on the inflorescences, similar to the heat damage noted by Hara et al. (2003) when researching the potential of hot-air disinfestation *Leucospermum* 'High Gold'. In this study, treatments

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ramped to 40°C exhibited less foliage damage than when ramped to 50°C, but similar inflorescence damage in the way of severe and complete style wilting. When assessing the potential for vapour heat as a postharvest disinfestation technique, Hansen et al. (1992) noted that amongst the floricultural products tested, *Leucospermum* was one of the most susceptible to heat treatments. Likewise, Hara et al. (2003) noticed a tendency to heat sensitivity in *Leucospermum*. They also found that conditioning the stems at 39°C for 15 minutes and 41°C for 15 minutes prior to heat treatment of 44°C at 60% to 70% relative humidity, drastically reduced overall style wilting and resulted in overall flower quality comparable to control stems for treatments of up to 180 minutes. Preconditioning of *Leucospermum*, and perhaps other sensitive commodities, may reduce overall heat-stress phytotoxicity, and should be considered for future trials. Control stems breached the limit of marketability by Day 6 of the 10-day evaluation period, but this was likely due to the reflexing of styles once the stems were placed in vase solution. Had the control stems been stored dry for a period after treatment, the reflexing of styles would have been delayed, and overall flower scores would have been lower. In trials conducted on *Leucospermum* 'Kathryn', all treated and untreated inflorescences and foliage succumbed to severe fungal infection and deterioration, and they attributed the high relative humidity environment around the stems in vase as the cause of infection, most likely due to the very short stems and close proximity to vase solution (Hara et al. 2003). Hansen et al. (1992) also encountered severe mould infestation in treated *Protea* and *Leucospermum* commodities, and the high humidity conditions were also identified as the possible cause. It is therefore cautioned that treated stems should be dried off as much as possible prior to storage and freighting, and forced-air cooled to avoid onset of fungal attack.

In *P. magnifica* heat damage manifested as sporadic blackening of leaves and the browning of involucral bracts. Leaf blackening is a common and problematic postharvest disorder in a variety of *Protea* commodities, and one of the suggested reasons for

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occurrence is improper cooling (Van Doorn, 2001; Hoffman et al., 2018). Once again, the use of forced-air cooling directly after CATTS treatments could alleviate the leaf-blackening issues.

For both *Leucadendron* commodities tested, phytotoxicity was minimal in comparison with the other genera. In treatments ramped to 50°C, heat-stress phytotoxicity manifested as slight wilting along with the desiccation of leaf tips, a common disorder found when the commodity is stored and freighted for extended periods (Philosoph-Hadas et al. 2010). A slight browning of the petals was also noted by Hara et al. (2003) when testing hot air disinfestation 44°C for either 120 or 180 minutes. Once again, Hara et al. (2003) found that preconditioning the stems at 39°C for 15 minutes and 41°C for 15 minutes prior to heat treatment of 44°C at 60% to 70% relative humidity resulted in overall flower quality comparable to untreated control stems, with no observable damage manifested after the preconditioning treatment when simulating a freighting period of 3 days.

Finally, Geraldton wax 'Ofir' did not express any significant reduction in quality following treatment, and no distinguishable phytotoxic reaction was noted. Research on the potential of hot-water bath dips in *Proteaceae* found that Geraldton wax 'Purple pride' expressed significant damage when dipped for 20 minutes in 46°C water or 10 minutes in 56°C water (Seaton & Joyce, 1993). Damage was characterised by a change in flower colour, from deep purple to mauve, and a darkening of all the dipped foliage. Hot-water dips and vapour heat were suggested as being unsuitable for Geraldton wax 'Purple pride', although 'Ofir' did not express any damage following heat treatments in this study. As Geraldton wax products are the highest exported Cape flora greenery product, with an estimated 4 500 000 stems exported during the 2016/2017 season (www.capeflorasa.co.za/statistics), these results are very promising.

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Currently, a standardised scoring system for the grading of Proteaceae export goods does not exist. A standardised scoring system would allow for easier and more defined comparisons between research on postharvest treatments or disorders, and help improve communication between producers, exporters and overseas markets (Janick, 2007). Although the scoring system used in this study is concise and specific, the criteria might require adjustment between genera. This may particularly applicable when commodities exhibit drastically different growth forms (i.e. large sclerophyllous leaves on *Protea* and needle-like leaves on Geraldton wax and other Cape flora greenery). For example, *P. magnifica* control stems exhibited a marketable period of only 4 days, which reflects the need for genera specific alignment. The scoring system used here allows one to take into account the difference between foliage and inflorescence damage, which may be an important difference to note for products that are sold either as leafless or coneless/flowerless greenery stems. A total of 2 272 659 leafless *Protea* stems were exported in bouquets from South Africa in the 2016/2017 season (www.capeflorasa.co.za/statistics), and the demand for mixed and *Protea* bouquets continues to rise substantially for the export market (Reinten et al., 2011). Thus, a *Protea* specific scoring system, or alteration of marketability and vase life limits for *Protea* stems would result in improvement of calculated marketability and shelf life limits. Adjustments for other commodities should be also considered, as well as the time of export, as quality expectations vary considerably throughout the export season and with export volumes.

This study shows the potential for CATTS to be utilised fully as a postharvest disinfestation technology for various Proteaceae commodities. However, CATTS should not be utilised as a stand-alone technique. Integrating CATTS technology into the systems approach principle, where disinfestation and pest control is meticulously incorporated throughout the production and transport chains of export Proteaceae, will ultimately result in flowers which are mostly or entirely free of phytosanitary insect pests. Once refined for specific

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commodities, and used in combination with other disinfestation methods such as cold storage and controlled atmospheres during freighting, CATTS could be a very effective tool in the control of phytosanitary insects of export Proteaceae from South Africa.

3.5 References cited

Blend, J.R., & van Ravenswaay, E.O. (1999). Measuring consumer demand for ecolabeled apples. *American Journal of Agricultural Economics*, 81, 1072–1077.

Coetzee, J.H., Rust, D.J, & Latsky, L. (1985). Mites (Acari) on Proteas. In *1st International Protea Research Symposium* (pp. 247–252).

Follett, P.A., & Neven, L.G. (2006). Current trends in quarantine entomology. *Annual Review of Entomology*, 51, 359–385.

Hansen, J.D., Chan, H.T., Hara, A.H., & Tenbrink, V.L. (1991a). Phytotoxic reaction of Hawaiian cut flowers and foliage to hydrogen cyanide fumigation. *Hort Science*, 26, 53–56.

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.

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.

Hansen, J.D., Paull, R.E., Hara, A.H., & Tenbrink, V.L. (1991b). Predicting vase life in tropical cut flowers and foliage. *Florida State Horticultural Society*, 104, 61–63.

Stellenbosch University https://scholar.sun.ac.za

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

Hoffman, E.W., Windell, N.E., Vardien, W., & Jacobs, G. (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.

Janick, J. (2007). Proteaceous Ornamentals: *Banksia, Leucadendron, Leucospermum* and *Protea*. *Scripta Horticulturae*, 5, 1–161.

Jensen, S. (2000). Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integrated Pest Management Reviews*, 5, 131–146.

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.

Jones, R., & Faragher, J. (1991). Cold storage of selected members of the Proteaceae and Australian native cut glowers. *HortScience*, 26, 1395–1397.

Kirk, W.D.J., & Terry, L. I. (2003). The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology*, 5, 301-310.

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

Neven, L.G., & Johnson, S.A. (2018). Combination hot forced air treatments and controlled atmosphere treatments: CATTS — Controlled Atmosphere Temperature

Stellenbosch University https://scholar.sun.ac.za

Treatment System. In Novel Postharvest Treatments of Fresh Produce, S. Pareek, ed. (Boca Raton: Florida CRC Press).

Neven, L.G., Rehfield-Ray, L., & Obenland, D. (2006). Confirmation and efficacy tests against codling moth and oriental fruit moth in apples using combination heat and controlled atmosphere treatments. *Journal of Economic Entomology*, 99, 1620–1627.

Opara, L.U. (2003). Traceability in agriculture and food supply chain : A review of basic concepts, technological implications, and future prospects. *Food, Agriculture & Environment,* 1, 101–106.

Philosoph-Hadas, S., Perzelan, Y., Rosenberger, I., Droby, S., & Meir, S. (2010). *Leucadendron* “Safari Sunset”: Postharvest treatments to improve quality of cut foliage during prolonged sea shipment. *Acta Horticulturae*, 869, 207–217.

Prinsloo, G.L., & Uys, V.M. (Eds.). (2015). Insects of cultivated plants and natural pastures in Southern Africa. *Entomological Society of Southern Africa*. Pretoria: Southern Africa.

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.

Reitz, S.R. (2009). Biology and ecology of the Western flower thrips (Thysanoptera: Thripidae): The making of a pest. *Florida Entomologist*, 92, 7–13.

Roets, F., Dreyer, L.L., Geertsema, H., & Crous, P.W. (2006). Arthropod communities in Proteaceae infructescences : seasonal variation and the influence of infructescence phenology. *African Entomology*, 14, 257–265.

Stellenbosch University https://scholar.sun.ac.za

Rust, D.J., & Myburgh, A.C. (1975). Pests as a factor limiting the development of the protea industry. *Veld & Flora*, 61, 16–19.

Seaton, K.A., & Joyce, D.C. (1993). Effects of low temperature and elevated CO_2 treatments and of heat treatments for insect disinfestation on some native Australian cut flowers. *Scientia Horticulturae*, 56, 119–133.

Shellie, K.C., Neven, L.G., & Drake, S.R. (2001). Assessing "Bing" sweet cherry tolerance to a heated controlled atmosphere for insect pest control. *HortTechnology*, 11, 308–311.

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.

Taylor, J.E. (2000). Proteaceae pathogens: the significance of their distribution in relation to recent changes in phytosanitary regulations. *Acta Horticulturae*, 545, 253–264.

Tzortzakis, N.G. (2007). Maintaining postharvest quality of fresh produce with volatile compounds. *Innovative Food Science & Emerging Technologies*, 8, 111–116.

Van Doorn, W.G. (2001). Leaf blackening in protea flowers: Recent developments. *Acta Horticulturae*, 545, 197–204.

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

Stellenbosch University https://scholar.sun.ac.za

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., & Saunderson, M. (1995). Protea plant protection: From the African context to the international arena. *Acta Horticulturae 387*, 129-140.

www.capeflorasa.co.za/statistics/ Cape Flora SA statistics (Accessed October 2017).

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Chapter 4: Phytotoxic reaction of South African Proteaceae export commodities to ethyl formate fumigation

4.1 Introduction

Fumigation has been and still is the most commonly utilised postharvest disinfestation method for fresh produce (Taylor, 1994; Hansen and Hara, 1994; Mitcham, 2001; Maholtra and Ram, 2017). Methyl bromide fumigation was utilised internationally for the disinfestation of various agricultural goods, mainly due to its versatility with regard to pest control, but also the comparatively shorter treatment durations and low cost (Ducom & Banks, 2006). In accordance with the Montreal Protocol, methyl bromide is still being phased out in developing countries due to its ozone depleting factors and mammalian toxicity (Husain et al., 2017). Since the announcement of its phasing out, much research has gone into developing postharvest disinfestation methods which are as effective in pest control and maintaining produce quality, but simultaneously environmentally- and human health conscious (Mitcham, 2001; Follett and Neven, 2006; Tzortzakis, 2007).

The South African Proteaceae export industry is one of the many which will have to make use of alternative fumigants once methyl bromide is completely phased out, as the industry has historically relied on the fumigant for almost all of its disinfestation requirements (Malan, 2012). Insect pests of Proteaceae are diverse and numerous, and many pose significant phytosanitary barriers when exporting cut flowers to overseas markets (Wright and Saunderson, 1995; Taylor, 2000; Wright, 2003; Prinsloo and Uys, 2015). Major phytosanitary pests in export commodities of South African Proteaceae include various thrips, particularly the western flower thrips (*Frankliniella occidentalis*), as well as the protea itch mite (*Proctolaelaps vandenbergii*), as these invertebrates are regularly intercepted, and can occur in staggering numbers within a single box of export product.

Ongoing research on alternative fresh- and dried fruit fumigants revealed that certain naturally occurring plant volatiles exhibit repellent and insecticidal properties at higher concentrations, and could potentially replace methyl bromide as a fumigant (Simpson et al., 2007; Jayakumar et al., 2017). One such plant volatile is ethyl formate (EF), which occurs naturally in soil, water and plant matter, as well as raw and processed goods (Ducom & Banks, 2006). EF was historically used as a fumigant in the dried fruit industry (Cotton & Roark, 1928), but with the phasing out of methyl bromide, the volatile has received increased consideration as a potential postharvest fumigant replacement in other industries (Bell, 2000). One of the distinct advantages of EF fumigation is that it rapidly degrades in the presence of water, producing ethanol and formic acid and leaving residues on treated commodities in only trace amounts (Simpson et al., 2007). This promotes its use as an alternative fumigant, as consumers are demanding that agricultural commodities are free of pesticide residues and have minimal environmental impacts (Mitcham, 2001; Tzortzakis, 2007). Furthermore, EF has the GRAS (generally regarded as safe) status by the United States Food and Drug Administration, due to its prolonged use in the food industry (Mitcham, 2001; Ryan & De Lima, 2014).

EF is registered for use as a fumigant in certain countries in the form of VAPORMATE™, a formulation of EF (16.7% by weight) and liquid CO_2 (83.3% by weight) in pressurised cylinders (Grifin et al., 2013). The addition of CO_2 reduces the flammability of the product but also adds a synergistic stress for insect pests, which may improve mortality, although this is not always the case (Simpson et al., 2007). EF and VAPORMATE™ fumigation is highly effective in controlling a variety of major agricultural and phytosanitary pests, including mixed thrips (Simpson et al., 2007; van Epenhuijsen et al., 2007; Grifin et al.,

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2013; Pupin et al., 2013), Californian red scale (Simpson et al., 2007), two-spotted spider mites (Zhang & van Epenhuijsen, 2004) sawtoothed grain beetle and Indian mealmoth (Ryan & De Lima, 2014).

Both EF and VAPORMATE™ have been tested for use on a range of products, including table grapes (Simpson et al., 2007), citrus (Pupin et al., 2013), onions (van Epenhuijsen et al., 2007), persimmons (Lee et al., 2017) stored grains (Choi et al., 2017) and various cut flowers including Proteaceae (Weller & van Graver, 1998; Williams, 1998). Fresh produce and cut flowers exhibit various phytotoxic reactions to EF and VAPORMATE™ fumigation. Despite being highly successful in controlling pests, and the negligible phytotoxic reactions of some fruit and vegetable produce, cut flowers and particularly foliage tend to exhibit rapid and drastic deterioration in flower quality when exposed to the fumigants (Zhang & van Epenhuijsen, 2004). Of the Proteaceae commodities tested, *Protea* ‘Pink ice’, *Banksia coccinea* and a nameless *Leucadendron* product exhibited a rapid decline in flower and foliage quality following EF fumigation of $20g/m^3$ for 3 hours, but *Serruria florida* exhibited no phytotoxic damage when subjected to the same treatments (Weller & van Graver, 1998; Williams, 1998).

Due to its environmentally-friendly stature and relative success in other industries, as well as the existence of certain Proteaceae commodities withstanding fumigation with EF, this study aims to assess the phytotoxic reaction of untested Proteaceae and Myrtaceae commodities to EF fumigation, as well as the fumigant’s potential control of major phytosanitary pests of export Proteaceae.

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4.2 Materials and Methods

4.2.1 Flowers and pre-treatment handling

Freshly harvested flowers were procured from various commercial plantations within the Western Cape, South Africa. Floral commodities were selected according to availability, relative demand from overseas markets, as well as to include diverse product types and growth forms. A total of four Proteaceae commodities and one Australian wildflower species were tested: 1) *Protea magnifica* 'Barbi'; 2) *Leucospermum* 'Veldfire' (*L. conocarpodendron* x *L. glabrum*); 3) *Leucadendron* 'Safari sunset' (*L. salignum* x *L. laureolum*); 4) Geraldton wax (*Chamelaucium uncinatum*) 'Ofir'. Geraldton wax 'Ofir', although belonging to the floral family Myrtaceae, is an important component of the Cape flora greenery and bouquet filler material categories, and is sold as such.

The flowers were collected or delivered within 24 hours of harvesting, and then subjected to overnight storage at 4°C. Prior to treatment, floral stems were removed from overnight storage, the bottom 1cm of each stem was trimmed off at a 45° angle, and the stems were pulsed using various sugars. The *Protea* and *Leucospermum* commodities were pulsed with a 6% ($^{w}/_{v}$) sucrose solution, allowing each stem to absorb ~10mL and ~2mL for the respective genera. The *Leucadendron* commodities were pulsed with a 5% ($^{w}/_{v}$) glucose solution, until individual 'Safari sunset' stems absorbed ~2mL each. The Geraldton wax 'Ofir' was also pulsed using a 5% ($^{w}/_{v}$) glucose solution, until individual stems had absorbed ~2mL each. Once pulsing was completed, individual stem masses were determined. Prior to treatments, flower quality was evaluated according to a scoring system described below.

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4.2.2 Flower scoring system

The stems were subjected to evaluation, each receiving an individual score. Hereafter, foliage refers to the stems and leaves of the product, inflorescence refers to the flower head or bloom itself, and flower refers to the inclusive product as a whole.

The commodity quality and reaction to treatments was evaluated using a 2-part, 10-point scoring system. Each stem received individual foliage and an inflorescence scores ranging from 0 to 9 (Table 4.1). The scoring criteria used were adjusted from a version used in various other studies (Hansen et al., 1991a; Hansen et al., 1991b; Hansen et al., 1992). The individual foliage and inflorescence scores of each stem were summed to achieve an overall score of 0 to 18, representing an overall quality score for each stem. This scale of 0 to 18 was used to determine critical quality thresholds, where 0 = no damage, 4 = limit of marketability (i.e. lowest acceptable quality for commercial sale of product), 8 = limit of vase life (i.e. product deteriorates in vase to the point of being unpleasant aesthetically) and 18 = complete discoloration or destruction of both the foliage and inflorescence.

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Table 4.1: Description of scoring criteria for phytotoxicity ratings and overall flower score in Proteaceae products following ethyl formate fumigation treatments and vase life studies. Overall flower score equals the sum of individual inflorescence and foliage scores (except for Geraldton wax where overall flower score equals foliage score)

Rating	**Inflorescence score** *Protea*	*Leucospermum*	*Leucadendron*	**Foliage score/ Geraldton wax score/ Coneless *Leucadendron* score**
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

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4.2.3 Ethyl formate fumigation regimes and vase life studies

All flower commodities were fumigated in 14 litre glass desiccators at ambient room temperature (20°C ±2°C). To calculate the volume of liquid EF per treatment, 10 randomly selected stems were chosen to determine the average volume:mass ratio using water displacement for each commodity. Utilising the total mass of the stems used for each treatment, as well as the calculated average volume:mass ratio, the free space in each desiccator could be determined and head space concentrations of ethyl formate could be calculated for each treatment.

Fumigation regimes were computed using a Central Composite Design (CCD) analysis model, based on fumigation concentration and durations of VAPORMATE™ researched on Australian wildflowers and Proteaceae (Rigby et al., pers. comm.) and a pilot trial on South African Proteaceae using *P. magnifica* (T Grout, pers. comm.). The CCD model created a series of fumigation regimes with concentrations ranging from 18.53g/m^3 to 151.47g/m^3 EF, and durations ranging from 30 mins to 3 hours (Table 4.2). These treatments were used for trials on *Protea, Leucospermum* and *Leucadendron* commodities. For the subsequent trials on Geraldton wax 'Ofir', concentrations and durations were reduced to potentially reduce phytotoxic damage. The treatments used were 1) 10g/m^3 for 1 hour, 2) 10g/m^3 for 2 hours, 3) 20g/m^3 for 1 hour, 4) 20g/m^3 for 2 hours.

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Table 4.2: Ethyl formate (EF) fumigation treatments from central composite design (CCD) analysis model used to fumigate Proteaceae commodities in 14 L glass desiccators.

EF Treatment	EF concentration (g/m^3)	Fumigation duration (hours)
1	38	0.87
2	132	0.87
3	38	2.63
4	132	2.63
5	85	0.51
6	85	2.99
7	18.53	1.75
8	151.47	1.75
9	85	1.75
10	85	1.75

Each treatment consisted of 5 stem replicates, and 5 stems were used as controls for *Protea, Leucospermum* and *Leucadendron* trials. Geraldton wax trials consisted of 10 stem replicates per treatment, and 10 control stems.

Directly after treatment, the individual stem masses were determined to calculate mass loss during treatment, and flower quality was evaluated using the same scoring system. The flowers were immediately placed into vase containing a 2% ($^w/_v$) sucrose solution and subjected to vase life studies.

The vase life studies consisted of 10 days for *Protea*, *Leucospermum* and Geraldton wax commodities, and 14 days for *Leucadendron* commodities. The flowers were kept under a natural photoperiod of 12:12 hours, with a room temperature of ~20°C ±2°C. All treatments received 2% ($^{w}/_{v}$) sucrose solution in vase, with the solution being replaced on every evaluation day. Flower quality evaluations were performed every second day in *Protea, Leucospermum* and Geraldton wax trials (Day 2, 4, 6, 8, and 10) and every second day following day three for *Leucadendron* trials (Day 1, 3, 5, 7, 10, and 14). The marketability and vase life critical quality thresholds were estimated by plotting the average overall flower score for each commodity against the time elapsed since the treatments commenced. The critical intercepts on the y-axis were determined and rounded to the nearest full evaluation day. A value of 4 was established as the marketability limit, except for coneless 'Safari sunset' and Geraldton wax 'Ofir to which a value of 2 was allocated, whilst a value of 8 was assigned for vase life limit, except for coneless 'Safari sunset' and Geraldton wax 'Ofir' where the value of 4 was established

4.2.4 Insect mortality trials

Wild-growing *Trifolioum* and *Tropaeolum* species were field collected in Stellenbosch, South Africa throughout the month of October 2017. The collected plant material was shaken above sheets of white paper, dislodging any insects present on the plants. Noticeably living and free-running thrips were collected into a specimen jar using a pooter. The specimen jar was sealed with finely-meshed gauze and elastics, allowing for gas penetration but preventing the escape of any specimens. The collected thrips were immediately subjected to two different EF fumigation regimes; 1) 18.53g/m^3 for 0.5 hours, and 2) 18.53g/m^3 for 1.75 hours. Directly after treatment, the specimen jars were emptied onto a sheet of white paper and any living thrips were counted and collected with a pooter using a microscope. Dead thrips were counted individually, and pootered into a separate vial. The same process was repeated 24 hours later to determine overall mortality within 24 hours of treatment. After 24 hours, all treated thrips were individually inspected and any

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adult western flower thrips were identified and counted from amongst them. The rest were classified as "mixed thrips", belonging to various species and consisting of multiple instars.

Wild-growing *Protea neriifolia* visibly infested with the protea itch mite (*Proctolaelaps vandenbergii*) were collected from the outskirts of Stellenbosch, South Africa throughout the month of September 2017. The stems were gently knocked together above a white sheet of paper, dislodging the mites. The live mites were collected using a fine-gauge paintbrush and dislodged into specimen jars, the jars were sealed using a finely-meshed gauze and elastics, and the collected mites were immediately subjected to the same treatments of 18.53g/m^3 for 0.5 hours, or 18.53g/m^3 for 1.75 hours. Posttreatment mortality was calculated as before.

4.2.5 Statistical analyses

Overall flower scores were analysed by a mixed model repeated measures ANOVA using Statistica, version 13 (Dell Statistica – data analysis software system, Dell Inc. 2016). The treatments and time in vase were included as fixed effects, and the flowers were included as random effects.

4.3 Results

4.3.1 Phytotoxicity immediately after EF fumigation

Across all four commodities and between treatments, differences were observed in mean overall flower quality of stems assessed in vase life studies starting immediately after fumigation treatment.

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Leucospermum 'Veldfire' was one of the most susceptible commodities to EF fumigation. Rapid and severe declines in both flower- and foliage quality were witnessed across all treatments within 48 hours (Fig 4.1). Phytotoxic damage manifested as bleaching and a brittleness of the foliage, and complete drying and collapse of the styles. Inflorescences were also prone to snapping off of the stem after a week in vase due to the brittle nature of the product after fumigation. The least phytotoxic treatments by Day 2 (Treatment 5 and 7) resulted in overall flower scores of 3.4 ± 1.14 and 8.0 ± 0.71 respectively, with the latter already exceeding both marketability and vase life limits. By Day 4, all fumigation treatments resulted in overall flower scores exceeding both limits of marketability and vase life, whereas untreated stems exhibited a mean overall flower score of only 3.0 ± 0.71 ($p < 0.01$). By conclusion of the vase life study, mean overall flower scores were 17.8 ± 0.45 for Treatments 1, 2, 3 and 5, and 18.0 ± 0.00 for Treatments 4, 6, 7, 8, 9 and 10, while control stems exhibited a mean overall flower score of only 6.0 ± 0.71 ($p < 0.01$), still maintaining overall flower quality suitable for vase.

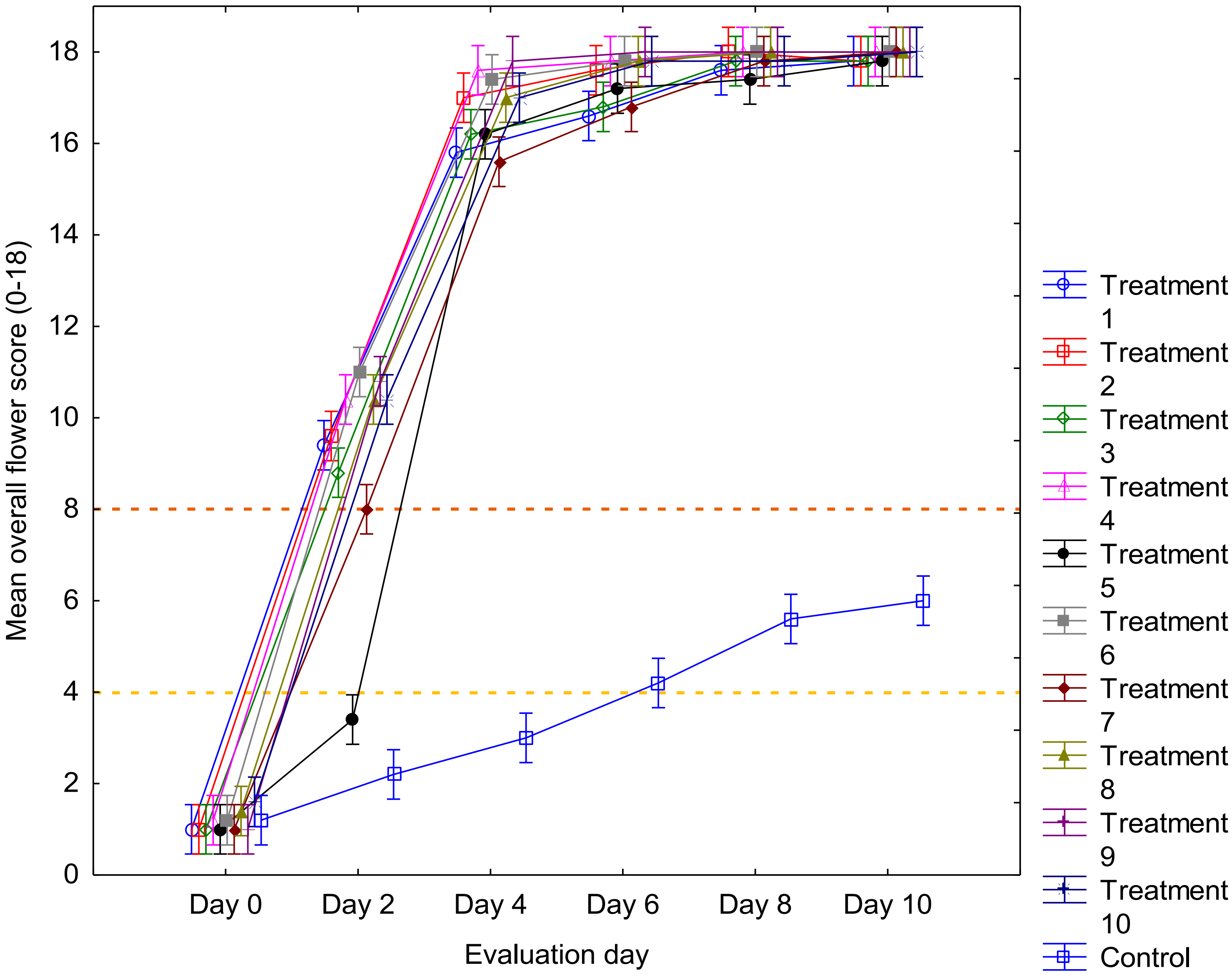

Figure 4.1: Mean overall flower scores of *Leucospermum* 'Veldfire' in vase following various EF fumigation treatments. Dashed yellow line (y=4) represents limit of marketability (point after which flower is no longer commercially sellable), and orange dashed line (y=8) represents limit of vase life (point at which flowers are no longer aesthetically pleasing). Vertical bars denote 0.95 confidence intervals.

Protea magnifica 'Barbi' exhibited varying phytotoxic reaction to EF fumigation (Fig 4.2) Phytotoxic damage manifested as rapid browning of both foliage (Fig 4.3A) and involucral bracts (Fig 4.3B), giving flowers a dried and withered appearance (Fig 4.3C). Treatment 7, utilising the lowest of all EF concentrations (18.53g/m^3 for 1.75 hours) was the only one to result in overall flower scores comparable to control stems throughout the vase life evaluations (p > 0.05). The mean overall flower score for Treatment 7 on conclusion of the vase life period was 8.4 ± 1.67, exceeding the vase life limit. Control stems exhibited a mean overall flower score of 6.2 ± 0.84 on conclusion of the vase life period, not differing significantly from Treatment 7 but also not exceeding the limit of vase life. Treatments 1, 3 and 5 exhibited comparable mean overall flower scores to Treatment 7 ($p > 0.05$), but were not comparable to control stems ($p < 0.01$). The remaining Treatments (2, 4, 6, 8, 9 and 10) exhibited severe decline in overall quality following treatment, all of which exceeded both limits of marketability and vase life within 48 hours and exhibiting significantly more damage than other treated stems and control stems ($p < 0.01$).

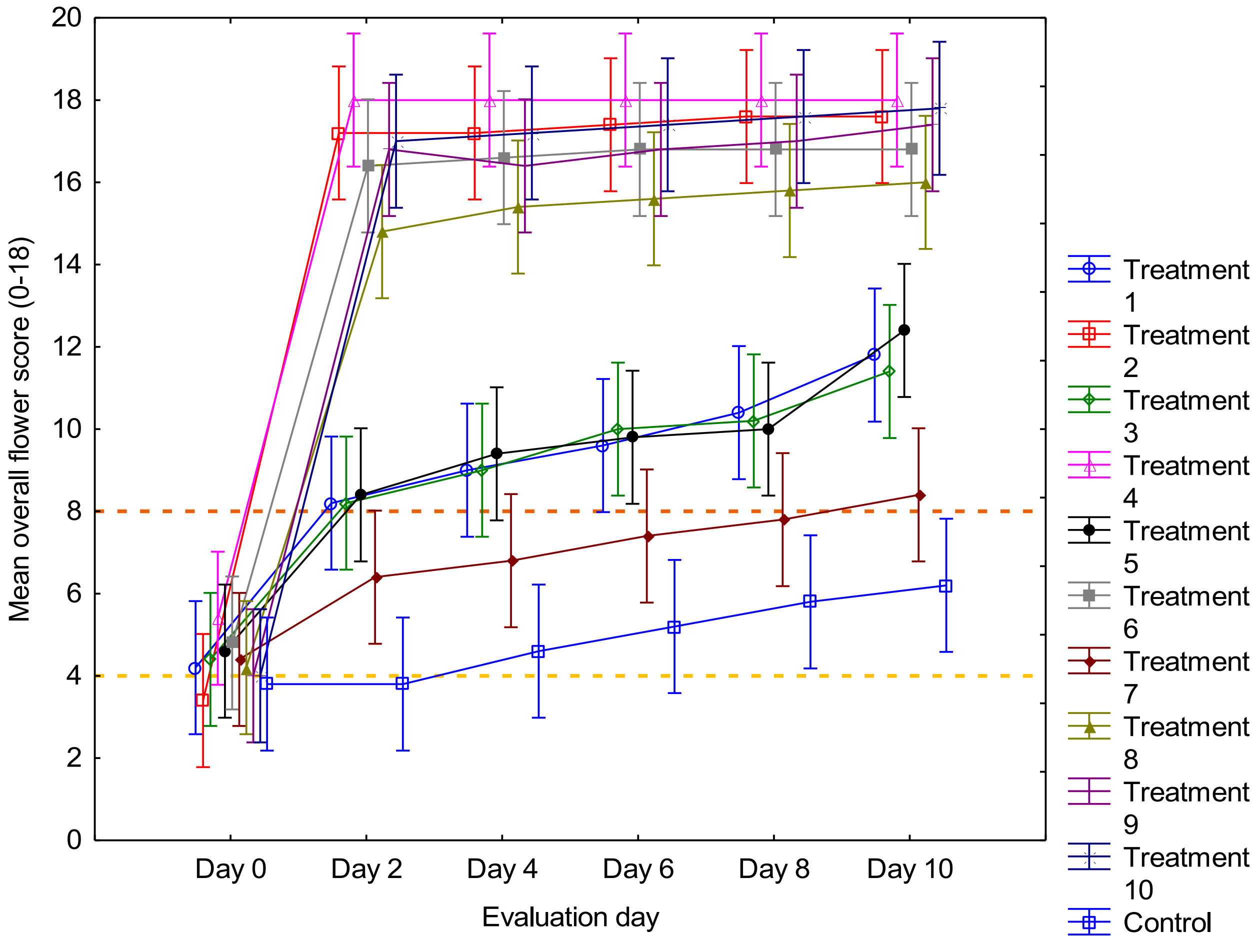

Figure 4.2: Mean overall flower scores of *Protea magnifica* 'Barbi' in vase following various EF fumigation treatments. Dashed yellow line (y=4) represents limit of marketability (point after which flower is no longer commercially sellable), and orange dashed line (y=8) represents limit of vase life (point at which flowers are no longer aesthetically pleasing). Vertical bars denote 0.95 confidence intervals

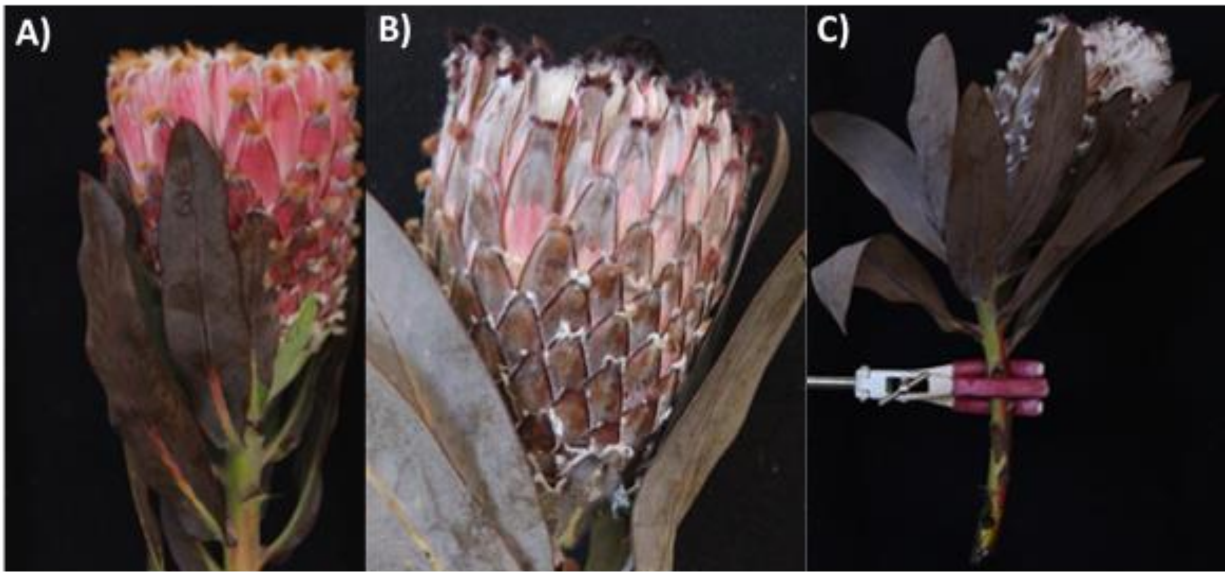

Figure 4.3: Phytotoxic reaction of *Protea magnifica* 'Barbi' to EF fumigation treatments. Damage manifested as severe blackening of leaves (treatment 5; A), browning of involucral bracts (Treatment 10; B), and overall reduction in quality, resulting in stems appearing dried and withered (Treatment 4; C).

Leucadendron 'Safari' sunset' experienced a rapid deterioration in overall flower quality following fumigation with EF. The treated stems did not exhibit significant reduction in overall quality immediately after treatments (Fig 4.4A), but all treated foliage appeared dried and withered within 24 hours of treatment (Fig 4.4B). All treated stems exceeded both limits of marketability and vase life within 24 hours (Fig 4.5). By conclusion of the vase life study, mean overall flower scores were 9.0 ± 0.00 for Treatments 1, 2, 3, 4, 5 8, 9 and 10, 8.6 ± 0.55 for Treatment 6, and 8.8 ± 0.45 for Treatment 7, with no significant

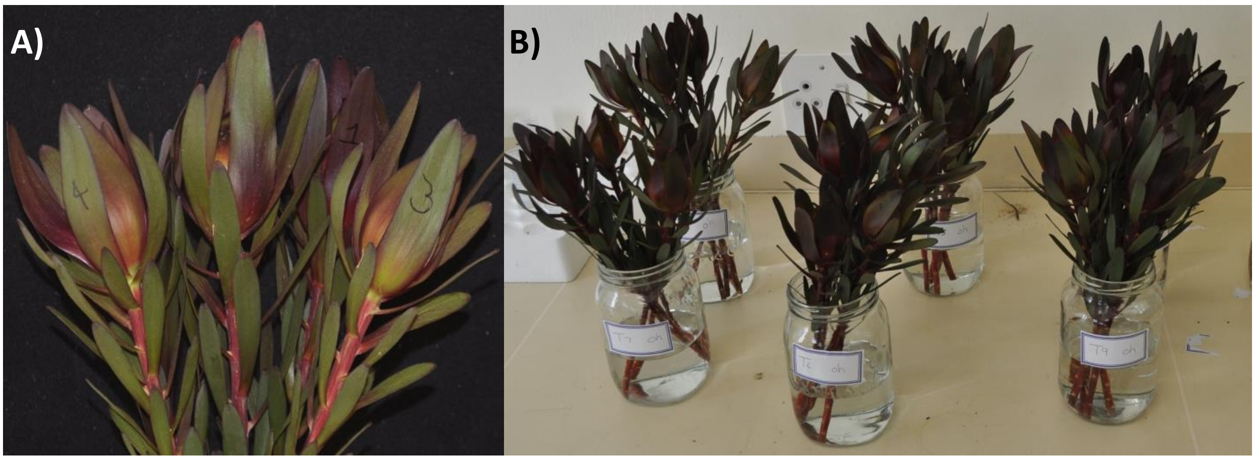

Figure 4.4: Phytotoxic reaction of *Leucadendron* 'Safari sunset' to EF fumigation. A) Foliage remains fairly unaffected by fumigations immediately after treatment, B) whereas all treated stems exhibited extreme darkening and discoloration of foliage within 24 hours of treatment.

difference between final scores by conclusion of the study ($p > 0.05$). Untreated stems exhibited a mean overall flower score of only 0.4 ± 0.55 within 24 hours of treatment, and a mean overall score of 1.2 ± 0.45 by conclusion of the vase life study, well within both the limits of marketability and vase life and maintaining quality far better than treated stems ($p < 0.001$)

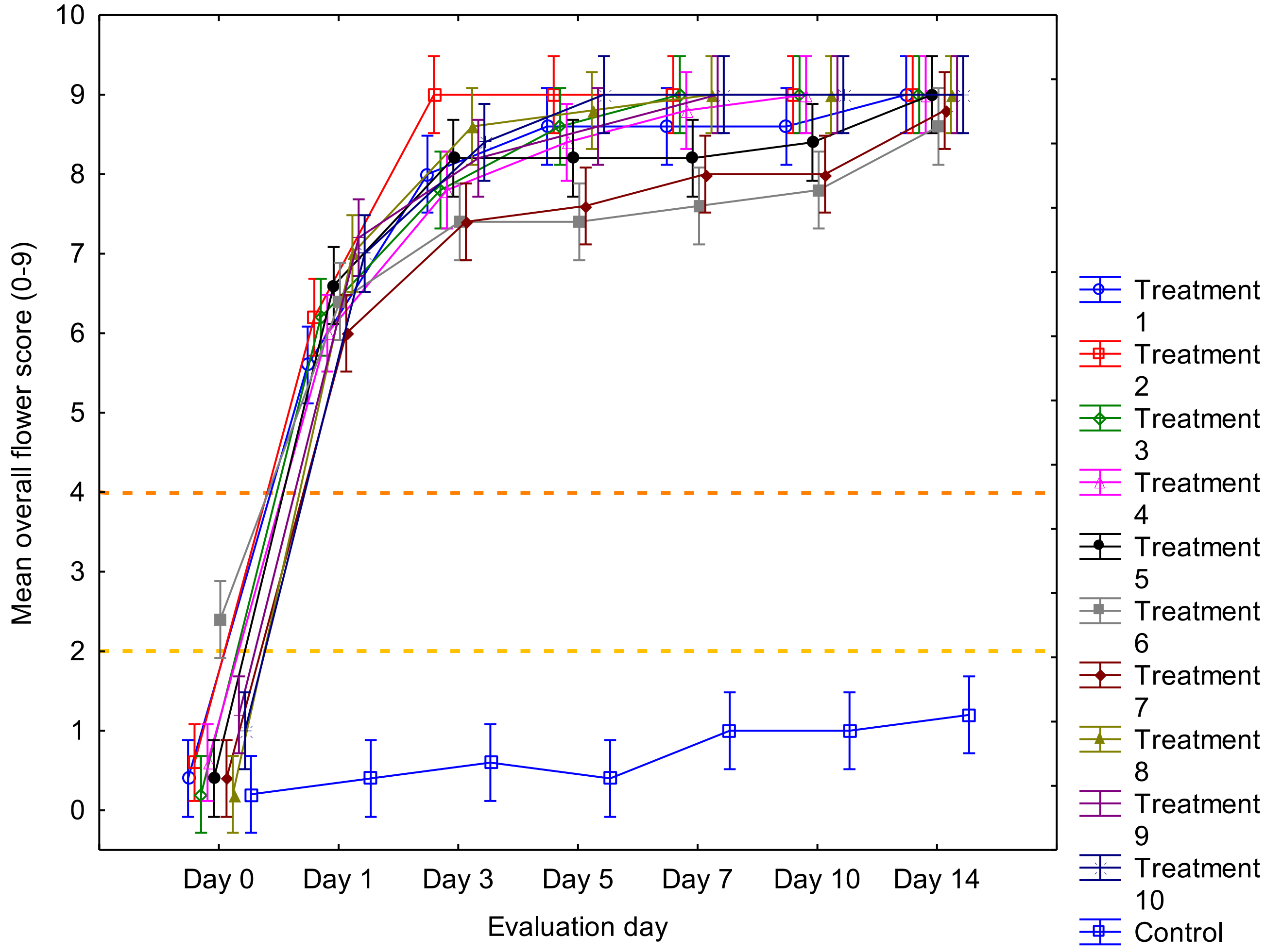

Figure 4.5: Mean overall flower scores of coneless *Leucadendron* 'Safari sunset' in vase following various EF fumigation treatments. Dashed yellow line (y=2) represents limit of marketability (point after which flower is no longer commercially sellable), and orange dashed line (y=4) represents limit of vase life (point at which flowers are no longer aesthetically pleasing). Vertical bars denote 0.95 confidence intervals.

Geraldton wax 'Ofir' did not withstand the altered fumigation regimes. Phytotoxic damage manifested as a browning of flowers and curling of petals (Fig 4.6A), rapid colour change in foliage from vibrant green to dull olive (Fig 4.6B), and an overall drying and browning of

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the treated stems resulting in a withered and brittle appearance. The average overall flower score for Treatments 1 and 2 after 48 hours was 3.1 ± 0.74 and 3.8 ± 1.14 respectively, both of which were within the limits of both marketability and vase life (Fig 4.7). Treatments 3 and 4 exhibited average scores of 4.6 ± 0.70 and 7.5 ± 1.27 within 48 hours, both of which exceeded the limit of marketability, but neither exceeding the limit of vase life. The untreated control stems exhibited overall flower scores of only 0.6 ± 0.52 after 48 hours, maintaining overall flower quality far better than all treated goods ($p < 0.001$). By day 4 of vase life studies, all treated stems exhibited an overall flower score of 18.0 ± 0.00, whereas untreated control stems exhibited an overall flower score of only 0.9 ± 0.57 ($p < 0.001$).

Figure 4.6: Phytotoxic reaction of Geraldton wax 'Ofir' to EF fumigation. A) Fumigation resulted in a rapid browning and curling of petals, B) and a rapid discolouration of foliage from vibrant green to drab olive (C) when compared to untreated control stems.

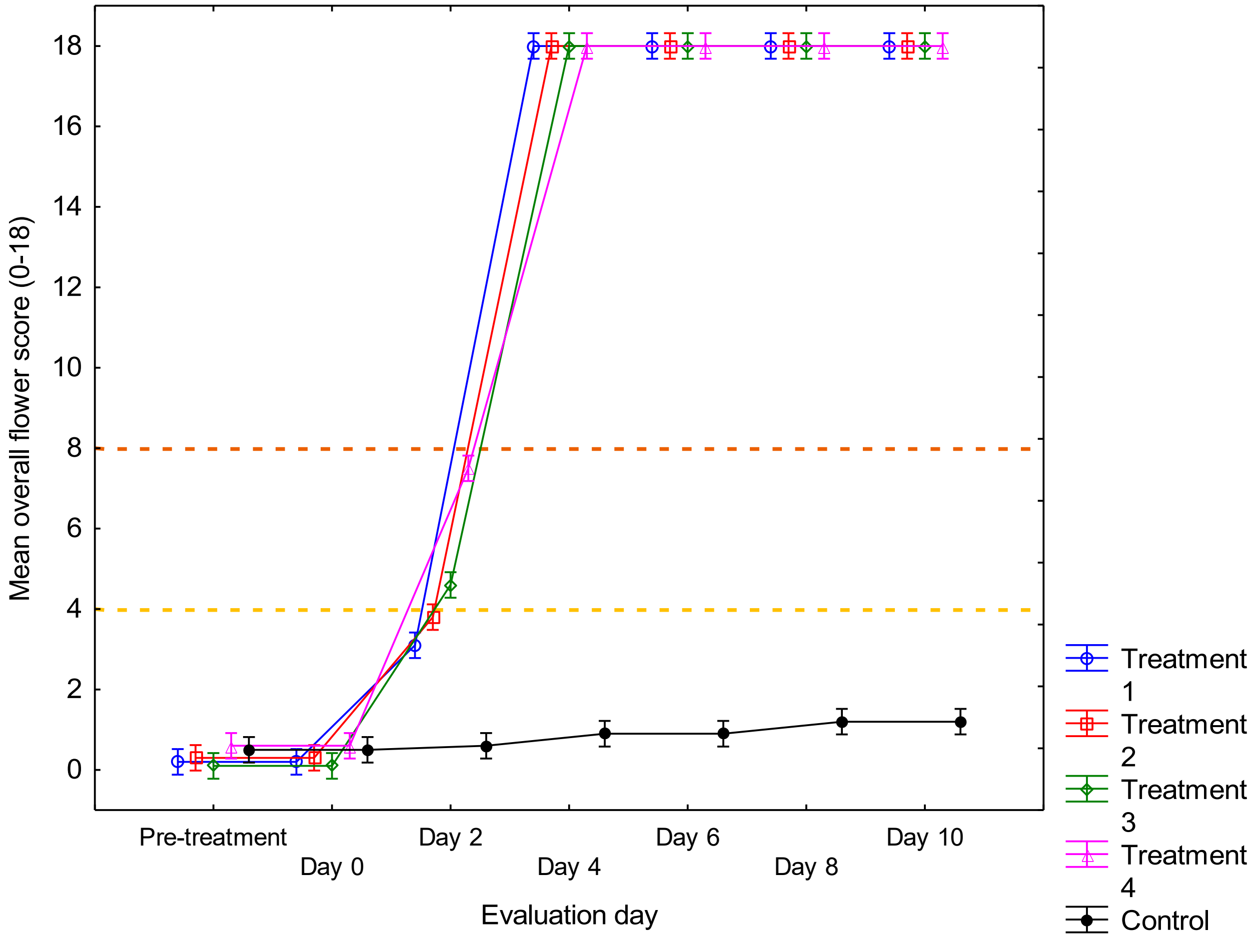

Figure 4.7: Mean overall flower scores of Geraldton wax 'Ofir' in vase following various EF fumigation treatments. Dashed yellow line (y=4) represents limit of marketability (point after which flower is no longer commercially sellable), and orange dashed line (y=8) represents limit of vase life (point at which flowers are no longer aesthetically pleasing). Vertical bars denote 0.95 confidence intervals.

4.3.2 Insect mortality trials

The total numbers of field collected thrips and mites subjected to altered Treatments 1 and 2 are given in Table 4.3. Post-treatment percentage invertebrate mortality as determined immediately after treatment and 24 hours later, are presented in Fig 4.8.

For the fumigation treatment of 18.53g/m3 for 0.5 hours, 91.04% mortality was achieved during the treatment for adult WFT, with 100% mortality within 24 hours of treatment. Similarly high mortality was achieved for mixed thrips, with 87.7% mortality during treatment, and 100% within 24 hours of treatment. 100% mortality was achieved in PIM during treatment. The same EF concentration applied for longer, 1.75 hours, resulted in

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100% mortality during the treatment for adult WFT, mixed thrips and PIM. For the fumigation treatment ramped of 18.53g/m^3 for 1.75 hours, 100% mortality was achieved during the treatment for adult WFT, mixed thrips and PIM during treatment

Table 4.3: Total number of western flower thrips (WFT), mixed thrips and protea itch mite (PIM) used to assess invertebrate mortality following EF fumigations of 18.53g/m^3 for 0.5 hours (Treatment 1) or 1.75 hours (Treatment 2) directly after- and within 24 hours of fumigation

Assessment	Invertebrate species	Treatment 1		Treatment 2	
Prior to treatment	WFT	268		245	
	Mixed thrips	187		133	
	PIM	422		251	
Post-treatment mortality		0h	24h	0h	24h
	WFT	244 (91%)	268 (100%)	245 (100%)	245 (100%)
	Mixed Thrips	164 (87%)	187 (100%)	133 (100%)	133 (100%)
	PIM	422 (100%)	422 (100%)	251 (100%)	251 (100%)

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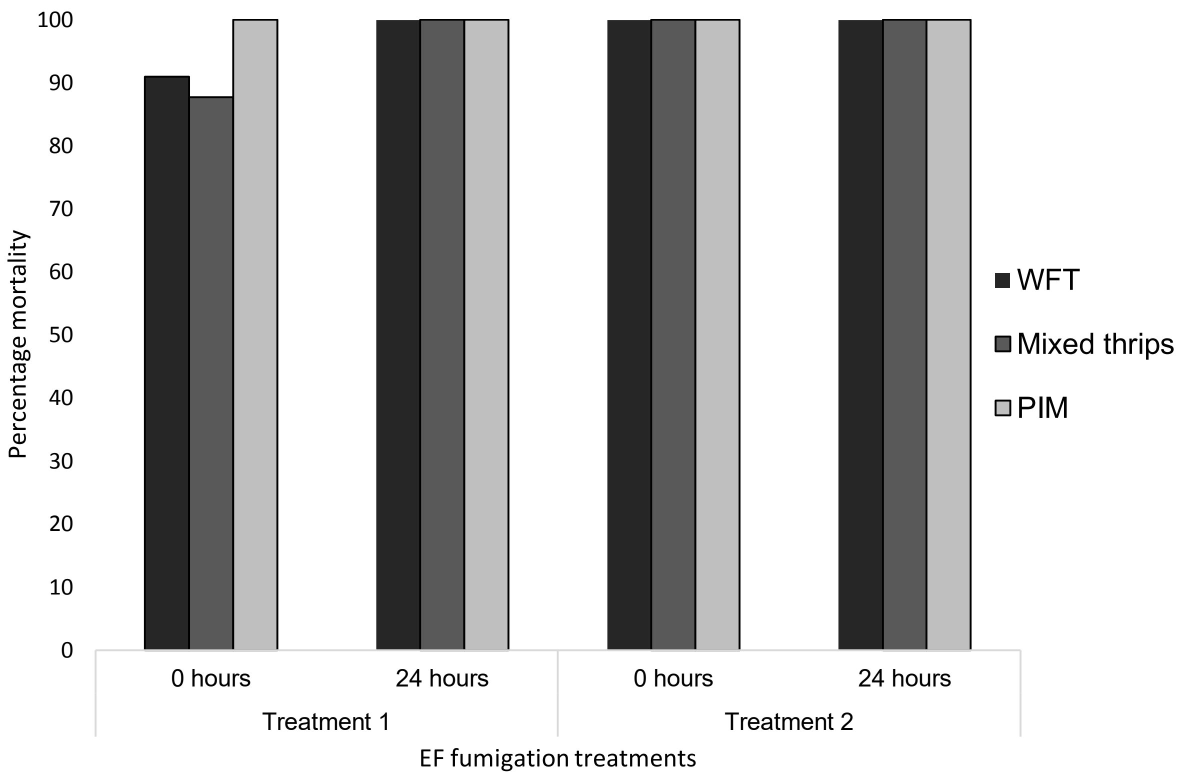

Figure 4.8: Percentage mortality in adult western flower thrips (WFT), mixed thrips and protea itch mite (PIM) directly after and within 24 hours of EF fumigation regimes. Treatment 1: 18.53g/m^3 for 0.5 hours; Treatment 2: 18.53g/m^3 for 1.75 hours.

4.4 Discussion

All tested phytosanitary pests were highly susceptible to EF fumigation. Various thrips are major agricultural pests internationally, and EF has proven effective against WFT (Simpson et al., 2007; Grifin et al., 2013; Pupin et al., 2013) and onion thrips (van Epenhuijsen et al., 2007). This study found similar results, as 100% mortality was achieved in both WFT and mixed thrips within 24 hours of fumigation at 18.53g/m3 for 0.5 hours, and 100% mortality was achieved at the same concentration, during treatment when treatment time was increased to 1.75 hours. The two-spotted spider mite is effectively controlled at 20-24/m3 for 1-2 hours, comparable to this study's findings (Grifin

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et al., 2013).This is the first recorded fumigation of *P. vandenbergii* with EF and 100% mortality was achieved during treatments applied here. The control of these and other pests bolster the potential use of EF as a postharvest fumigant in a multitude of industries.

However, for the cut flower industry EF fumigation may not be an easy replacement for methyl bromide. The results of this study echo those on EF fumigation of other cut flower commodities, where cut flowers and foliage exhibit severe and rapid deterioration in overall flower quality shortly after treatment (Weller and van Graver, 1998; Williams, 1998). Of the tested commodities, *P. magnifica* showed a slight resistance to EF fumigation at 18.53g/m^3 for 1.75 hours, the lowest of the tested concentrations for this commodity. Fumigations of 20g/m^3 for 3 hours by Weller and van Graver (1998) and Williams (1998) on *Protea* 'Pink ice' and *Banksia. coccinea* also noted unacceptable phytotoxic damage, whereas *Serruria florida* and roses experienced no noticeable damage under the same fumigation regimes. It appears that certain commodities are capable of withstanding EF fumigation, whereas the rest of the tested commodities experience rapid and severe decline in quality and vase life. Of the tested Proteaceae commodities, in both this study and others, only *S. florida* 'Blushing bride' seems capable of withstanding EF fumigation.

The range of concentrations and durations in this study were calculated using a CCD analysis model, calculated off of VAPORMATE™ trials conducted on both Australian and South African wildflowers (Rigby et al. pers. comm; T Grout pers. comm.). These differed drastically in both concentrations and durations, as well as phytotoxicity reactions. As a result of this disparity in concentrations, the CCD analysis model was skewed towards far higher concentrations (e.g. 85g/m^3-151.47g/m^3) than those seen in other studies on cut flower fumigations (Weller and van Graver, 1998; Williams, 1998). The lowest concentration and intermediate duration in this study of 18.53g/m^3 for 1.75 hours was comparable to both studies, and similar phytotoxic reactions were noted. Research on

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other cut flower commodities by Zhang and van Epenhuijsen (2004) also noted severe damage in *Sandersonia* and *Pittosporum* products and mild damage in Calla lily at 60g/m^3 VAPORMATE™, which is comparable to 18.53g/m^3 EF concentration used in this study (Grifin et al., 2013).

For the tested *P. magnifica*, stems exhibited no difference in overall flower quality when fumigated at 38g/m^3 for 0.87 hours and 2.63 hours, indicating that the concentration of EF may be a more determinant factor with regard to phytotoxic reaction. Studies suggest that treatments with lower concentrations for longer durations may be more effective in maintaining overall flower quality following fumigation (Zhang & van Epenhuijsen, 2004), a trend noted in phosphine fumigation of cut flowers as well (Williams & Muhunthan, 1996). Due to the highly volatile nature of EF and its tendency to break down into ethanol and formic acid in the presence of water and produce, headspace concentration of EF will decrease significantly in longer treatments, and the hydrolization of EF into formic acid and ethanol will have to be accounted for (Simpson et al., 2007; Grifin et al., 2013).

EF and VAPORMATE™ are promising alternative fumigants to methyl bromide, and have proven to be successful in the disinfestation of various agricultural crops, controlling many major pests while simultaneously maintaining product quality. This, coupled with the environmentally-friendly nature of the fumigant, bodes well for its use in other industries. Unfortunately, the fumigant results in rapid and severe phytotoxic reaction to cut flowers and Proteaceae flora, with the exception of only *S. florida*. More research is required but its use as a disinfestation fumigant for the South African Proteaceae export industry seems unlikely.

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4.5 References cited

Bell, C. H. (2000). Fumigation in the 21st century. *Crop Protection*, 19, 563–569.

Choi, H., Lee, B. H., Moon, Y. S., Kim, K., Lee, H. S., & Lee, S. E. (2017). Antifungal and antiaflatoxigenic effects of a fumigant, ethanedinitrile, on *Aspergillus flavus*. *Applied Biological Chemistry*, 60, 473–476**.**

Cotton, R. T., & Roark, R. C. (1928). Fumigation of stored–product insects with certain alkyl and alkylene formates. *Industrial & Engineering Chemistry*, 20, 380–382**.**

Ducom, F., & Banks, J. (2006). The Return of the Fumigants. *9th International Working Conference on Stored Product Protection*, October, 510–516.

Follett, P. A., & Neven, L. G. (2006). Current trends in quarantine entomology. *Annual Review of Entomology*, 51, 359–385.

Grifin, M. J., Jamieson, L. E., Chhagan, A., Page-Weir, N. E. M., Poulton, J., Davis, V. A., Zulhendri, F., Connolly, P. G. (2013). The potential of ethyl formate + carbon dioxide to control a range of horticultural pests. *New Zealand Plant Protection*, 66, 54–62.

Hansen, J. D., Chan, H. T., Hara, A. H., & Tenbrink, V. L. (1991a). Phytotoxic Reaction of Hawaiian Cut Flowers and Foliage to Hydrogen Cyanide Fumigation. *Hort Science*, 26, 53–56.

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.

Stellenbosch University https://scholar.sun.ac.za

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.

Hansen, J. D., Paull, R. E., Hara, A. H., & Tenbrink, V. L. (1991b). Predicting vase life in tropical cut flowers and foliage. *Florida State Horticultural Society*, 104, 61–63.

Husain, M., Sukirno, S., Mehmood, K., Tufail, M., Rasool, K. G., Alwaneen, W. S., & Aldawood, A. S. (2017). Effectiveness of carbon dioxide against different developmental stages of *Cadra cautella* and *Tribolium castaneum*. *Environmental Science and Pollution Research*, 24, 12787–12795.

Jayakumar, M., Arivoli, S., Raveen, R., & Tennyson, S. (2017). Repellent activity and fumigant toxicity of a few plant oils against the adult rice weevil *Sitophilus oryzae* Linnaeus 1763 (Coleoptera : Curculionidae). *Journal of Entomology and Zoology Studies*, 5, 324–335.

Lee, B.-H., Park, C.-G., Yang, J.-O., Kim, K., & Lee, S.-E. (2017). Concurrent application of ethyl formate and 1-methylcyclopropene to control *Tetranychus urticae* on exported sweet persimmons (*Diospyros kaki* Thunb. “Fuyu”). *Entomological Research*, 1–5.

Malhotra, S.K. & Ram, L. (2017). Advances in Floriculture and Landscape Gardening. Central Institute of Horticulture, Medziphema, Dimapur, Nagaland, India, 1-321.

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

Mitcham, E. (2001). Quarantine issues in 2000. *Acta Horticulturae*, 553, 451–455.

Stellenbosch University https://scholar.sun.ac.za

Prinsloo, G. L., & Uys, V. M. (Eds.). (2015). Insects of cultivated plants and natural pastures in Southern Africa. *Entomological Society of Southern Africa*.

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., Gallagher, E., & Collins, A. (n.d.). Vapormate ® phytotoxicity trials on Australian Wildflowers. *Cedar Hill Flowers & Foliage Pty Ltd*, 1–20.

Ryan, R., & De Lima, C. P. (2014). Ethyl formate fumigation an overview update. In *11th International Working Conference on Stored Product Protection* (pp. 969–978).

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.

Taylor, J. E. (2000). Proteaceae pathogens: the significance of their distribution in relation to recent changes in phytosanitary regulations. *International Protea Research Symposium*, 545, 253–264.

Taylor, R. W. D. (1994). Methyl bromide-Is there any future for this noteworthy fumigant? *Journal of Stored Products Research*, 30, 253–260.

Tzortzakis, N. G. (2007). Maintaining postharvest quality of fresh produce with volatile compounds. *Innovative Food Science & Emerging Technologies*, 8, 111–116.

Stellenbosch University https://scholar.sun.ac.za

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.

Weller, G. L., & van Graver, J. E. (1998). Cut flower disinfestation: Assessment of replacement fumigants for methyl bromide. *Postharvest Biology and Technology*, 14, 325–333.

Williams, P. (1998). Fumigating postharvested wildflowers for export. *Rural Industries Research and Development Corporation.*

Williams, P., & Muhunthan, M. (1996). Fumigants for the postharvest control of insect pests of cut flowers. *International Postharvest Science Conference*, 96, 291–296.

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., & Saunderson, M. (1995). Protea plant protection: From the African context to the international arena. In *III International Protea Research Symposium*, 387, 129-140.

Zhang, Z., & van Epenhuijsen, C. W. (2004). Improved Envirosol fumigation methods for disinfesting export cut flowers and foliage crops. *New Zealand Institute for Crop and Food Research Limited*, (September).

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Chapter 5: General discussion

South African Proteaceae floral products have been used to create an internationally renowned indigenous cut flower industry. Naturally-occurring Proteaceae associates with a considerable entomofauna, many of which have become serious pests of cultivated Proteaceae. The presence of phytosanitary pests within export consignments of Proteaceae cut flowers to international markets remains a considerable barrier to the industry, as stricter international markets may mandate additional disinfestations, reducing overall quality of the fresh produce, or reject consignments entirely, which has negative economic- and reputational implications for the industry itself. Understanding the key phytosanitary pests within export consignments and developing effective postharvest disinfestation techniques for those pests, while simultaneously maintaining postharvest flower quality, storage capability and ensuring viable vase life of treated goods would help reduce interceptions, promoting the export of fresh Proteaceae cut flowers from South Africa.

Chapter 2 utilised a qualitative survey of consignment inspections destined for strict international markets to determine the most intercepted phytosanitary pests, as well as to determine the frequency of interceptions. Inspections were conducted over a two year period, utilising the same techniques as those done under official capacity, and encompassing as many products, variants and cultivars as possible. A total of 82 interceptions were made, all of which would have required phytosanitary action in order to export the goods. These interceptions consisted of 8 insect orders and 26 families. The majority of interceptions were as a result of singular representatives of various families, referred to colloquially as ‘hitchhikers’. However, various interceptions consisted of a large

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number of a singular phytosanitary insect pest species, such as the western flower thrips (*Frankliniella occidentalis*), the banded fruit weevil (*Phlyctinus callosus*) and Fuller rose weevil (*Naupactus godmanni*). These insects were not only frequently intercepted in a variety of commodities, but also often occurred in very large numbers. Although not an insect, interceptions of the protea itch mite (*Procotolaelaps vandenbergii*) were also frequent, and also consisted of a large number of individuals. Field-harvested and bouquet filler material most often contained insects and invertebrates, followed by commercially-grown *Leucospermum*, *Protea* and *Leucadendron* products. Although most of the interceptions consisted of solitary individuals, many interceptions also consisted of major phytosanitary pests and serious pests within the international Proteaceae production industry. Due to the frequency at which they are intercepted across a variety of export commodities, as well as the number of individuals found within each interception, both the western flower thrips (*F. occidentalis*) and protea itch mite (*P. vandenbergii*) were considered target phytosanitary pests for postharvest control.

Chapter 3 tested the efficacy of Controlled Atmosphere and Temperature Treatment System (CATTS) technology as a postharvest disinfestation technique for various fresh Proteaceae cut flower products, and also tested its efficacy in the control of key pests within the industry. The tested commodities included *Leucospermum* cultivar 'Veldfire', *Protea magnifica* 'Barbi', *Leucadendron* cultivar 'Safari sunset' and 'Jade pearl' and Geraldton wax *Chamelaucium uncinatum* 'Ofir'. CATTS treatments utilised were temperature ramps of 35°C/hour and 30°C/hour temperature ramp rate from 23°C to 40°C, with a 15 min soaking period at 40°C, and 35°C/hour and 30°C/hour temperature ramp rate from 23°C to 50°C, with a 15 min soaking period at 50°C, with all treatments applied under a controlled atmosphere of 1% O_2, 15% CO_2 in N_2. Treated goods were subjected to immediate vase life studies after treatments, as well as simulated air- and sea freight storage of 3 and 21 days of storage at 2°C prior to vase life studies. Overall flower quality

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evaluations were performed prior to treatments, directly after treatments, and throughout the vase life period. *Leucospermum* 'Veldfire' did not withstand treatments, exhibiting severe style wilting immediately after treatment. *Protea* 'Barbi' withstood treatments and maintained quality in vase comparable to control stems. *Leucadendron* 'Safari sunset' and 'Jade pearl' withstood treatments and maintained quality in vase comparable to controls following both air- and sea freight simulations. Geraldton wax 'Ofir' withstood treatments and maintained quality in vase immediately after treatment and following airfreight simulations. CATTS treatments were very effective in controlling two major phytosanitary pests, western flower thrips (*Frankliniella occidentalis*) and protea itch mite (*Proctolaelaps vandenbergii*) , as 100% mortality was achieved within 24 hours of treatments. CATTS technology appears to be capable of controlling two major phytosanitary pests, while maintaining overall flower quality in treated *Protea, Leucadendron* and Geraldton wax products, and commercial-scale testing should be undertaken before CATTS technology can be considered a viable disinfestation method, especially if incorporated into current disinfestation techniques.

Chapter 4 assessed the efficacy of ethyl formate (EF) fumigation as a potential disinfestation technique for fresh Proteacae cut flowers, as well as the efficacy of its control of two major phytosanitary pests of the industry. The treated goods were *Leucospermum* cultivar 'Veldfire', *Protea magnifica* 'Barbi', *Leucadendron* cultivar 'Safari sunset' and Geraldton wax *Chamelaucium uncinatum* 'Ofir'. Fumigation regimes were compiled using a Central Composite Design (CCD) analysis model, with concentrations ranging from 18.53g/m^3 to 151.47g/m^3 EF, and durations ranging from 30 mins to 3 hours for *Leucospermum*, *Protea* and *Leucadendron* commodities. Treatments were altered for Geraldton wax 'Ofir' to potentially decrease phytotoxic damage, with treatments of 10g/m^3 and 20g/m^3 EF for 1 and 2 hours. The overall flower quality of treated goods was assessed prior to and directly after treatment, as well as throughout the vase life period.

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Fumigation with EF resulted in rapid and drastic reduction in overall flower quality across treated goods, particularly manifesting as severe damage to foliage in the form of discolouration, withering and brittleness. Inflorescences also rapidly discoloured, and became brittle to the touch. EF fumigation is highly effective in controlling two major pests, western flower thrips (*F. occidentalis*) and protea itch mite (*P. vandenbergii*), as fumigation at 18.53g/m^3 for 1 and 2 hours achieved 100% mortality within 24 hours of treatment. Despite the rapid and effective control of two major phytosanitary pests within the industry, EF fumigation results in severe reduction in the quality of treated fresh goods, particularly foliage, and seems unsuitable for use within the industry as a postharvest disinfestation fumigant.

The South African Proteaceae fresh cut flower industry has to reliably and consistently supply insect-free goods to overseas markets to remain competitive in the ever-expanding international industry. The local industry has to do so despite the considerable and diverse entomofauna that associates with its export goods. The development of alternative postharvest disinfestation technologies could alleviate the phytosanitary pressures currently facing the industry, reducing export consignment interceptions and rejections, allowing for easier access to the stricter international markets and further market expansion, and ultimately bolstering the reputation of the South African Proteaceae export industry.

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Appendix

List of publications from MSc study

Popular articles

Huysamer, A. (2016). New tools to bash bugs. *Innovate magazine*, Klein Karoo Media CC**.** (Copywriter for magazine).

Huysamer, A. (2017). New tools to bash bugs. *Agri Kultuur*, 53, 66-70. (republished courtesy of The Post-Harvest Innovation Programme, INNOVATE magazine).

Huysamer, A., Johnson, S., & Hoffman, L. (2018). Mere weeds no more: the rise of the South African protea industry. *Veld & Flora*, 104, 30-33.

Semi-scientific article

Huysamer, A., Bezuidenhout, K., and Hoffman, L. (2016). Cape Flora – a hidden treasure of the Cape Floristic Kingdom claiming its place as exciting international floricultural products. *Chronica Horticulturae*. 56, 22–27.

Scientific peer-reviewed articles / conference proceedings

Huysamer, A., Hoffman, E. W., & Johnson, S. (2017). Novel technologies for the postharvest treatment of Cape Flora to control phytosanitary insect pests. *Acta Horticulturae*, 1201, 427-434.

Presented at the VII International Conference on Managing Quality in Chains and II International Symposium on Ornamentals (2017) – Stellenbosch, South Africa. (Received the ISHS Young Minds Award for best student oral presentation)

Huysamer, A., Hoffman, E.W. & Johnson, S. (2018). Postharvest insect pest control for Western flower thrips, *Frankliniella occidentalis*, in export Proteaceae cut flowers. *Acta Horticulturae*, (*In press*).

Presented at the 30th International Horticultural Conference and 11th International Symposium on Postharvest Quality of Ornamental Plants (2018) – Istanbul, Turkey. (Received the ISHS Young Minds Award for best student oral presentation)