Cape Peninsula
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PROPAGATION PROTOCOL FOR *LEUCADENDRON ELIMENSE* E.PHILLIPS SUBSP. *ELIMENSE* FROM THE AGULHAS PLAIN, SOUTH AFRICA

by

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DECLARATION

I, Jenny Liedtke, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

26 July 2021

Signed **Date**

ABSTRACT

The purpose for this study was to investigate whether *Leucadendron elimense* subsp*. elimense* was suitable for cultivation and to determine whether different rooting auxins would have an effect on the vegetative growth of the plant. The experiment was conducted over a period of 18 weeks. Two hundred plants were cultivated from different mother plants obtained from the Elim church ground. Three treatments at varying strengths were evaluated with 10 sample replicates. Treatments were made up of 3 different rooting auxins, namely: Indole acetic acid (IAA), Naphthalene acetic acid (NAA) and Indole-3-acetic acid (IBA) at three different strengths.

Chapter 2 reviewed the danger of extinction of *L. elimense* subsp. *elimense* and its viability as a cut flower. It was found that *L. elimense subsp. elimense* has economical potential as a cut flower and as a feature plant in a garden. It was also made clear that the natural habitat of the plant is shrinking and therefor it is necessary to act in preserving the species.

In chapter 3 it was seen that the various treatments had significant effects in terms of plant rooting, namely root length and number of roots. Treatment IAA at 4000 ppm showed the highest individual mean value for root growth in female plants. The lowest individual value for root growth was observed in treatment NAA 6000 ppm. Overall treatments with IAA 4000 ppm had better root growth.

In chapter 4 treatments showed significant effects on the wet weight and flowering percentage. It was observed that IAA at 4000 ppm had gained the most weight and flowers in female plants, while NAA at 4000 ppm showed the best results in male plants.

Chapter 5 investigated a propagation protocol for the rooting and flowering of *L. elimense* subsp. *elimense.*

Overall, this study has found that *L. elimense subsp. elimense* can be grown vegetatively, and that different auxins at different strengths had an impact on the rooting and growth of the plant. This research has shown that some auxins at certain strengths had more desirable results in terms of vegetative growth of the plant.

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CHAPTER ONE:

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PROBLEM STATEMENT, AIMS, HYPOTHESIS AND OBJECTIVES

1.1 General introduction

Leucadendron elimense E.Phillips subsp. *elimense,* a Proteaceae species from the Agulhas Plain, is classified as endangered on the South African Red Data List. The attractive female cones and male flowers of *L. elimense* subsp. *elimense* continue to be popular for the indigenous cut flower industry. The species is endangered as flowers are continually harvested from the species natural habit which is also being transformed into agricultural land. It has become vitally important to research a successful propagation technique and cultivation schedule for the species before all its natural resources are completely diminished. In this study the rooting and flowering potential of *L. elimense* subsp. *elimense* was researched. Essential misting, bottom heat and a naturally ventilated greenhouse provided a successful growing environment.

1.2 Structure of the thesis

The thesis is drafted differently to the alternative of a traditional format for a thesis. The article-format thesis examples of published, co-published and/or “ready-for-publication” articles were prepared during candidature and applies to the format prescribed by CPUT for 100% master's studies which complies to the following principles:

1. The overriding principle of the thesis is that it remains an original contribution to the discipline or field by the candidate.

2. Chapters containing the journal articles form a coherent and integrated body of work, which focused on a single project or set of related questions or propositions. All journal articles form part of the sustained thesis with a coherent theme.

3. The study does not include work published prior to commencement of the candidature.

4. The number of articles included depending on the content and length of each article and take full account of the university’s requirements for the degree as well as the one article already published or “ready-for-publication” expected for a master’s degree in this discipline.

5. The thesis should be examined in the normal way and according to the normal requirements as set out by the “Guidelines for Examiners of Dissertations and Theses” (using form HDC 1.7).

The thesis consists of the following chapters which are concisely discussed as:

Chapter One: Problem statement, aims, hypothesis and objectives

This chapter provides an overview of the research problem, aims and hypotheses of the research topic.

Chapter Two: *Leucadendron elimense* E.Phillips subsp. *elimense* the threatened endemic species of the Proteaceae family on the Agulhas Plain: A review.
This chapter provides background information on what has been researched already on the topic.

Chapter Three: Rooting responses of female and male cuttings of *Leucadendron elimense* E Phillips subsp. *elimense* to different rooting auxin treatments.
This chapter provides the first experimental results, investigating the rooting potential of *L. elimense* subsp. *elimense.*

Chapter Four: The effects of different rooting auxins on the wet weight and flowering percentage of *Leucadendron elimense* E.Phillips subsp. *elimense.*
This chapter provides the results of wet weight, flowering percentage, and chlorophyll content of *L. elimense* subsp. *elimense.*

Chapter Five: A propagation protocol for *Leucadendron elimense* E.Phillips subsp. *elimense* for conservation and cut flower production.
This chapter provides a recommended propagation schedule for separate male and female plants of *L. elimense* subsp. *elimense.*

Chapter Six: General discussion, conclusion and recommendations.

This chapter deals with the general discussion which connects the previous chapters and is followed by the conclusions of the study. Recommendations are made for further work; to introduce future research topics.

Chapter Seven: References

1.3 Problem statement

The Proteaceae family is a major plant family of the Cape Floral Region in the Western Cape province of South Africa. Many species are threatened due to agriculture, climate change, alien vegetation and illegal harvesting of flowers (Hall *et al*., 1980; Winter & Botha, 1994; Rebelo, 2001; Younge & Fowkes, 2003; Bomhard *et*

al., 2005). The Cape Floristic Kingdom consists of 65% of endemic plants (Hall *et al*., 1980; Cowling & Richardson, 1995; Goldblatt, 1997; Younge & Fowkes, 2003; Rhode, 2004; Manning, 2007).

Almost all species in the family have potential to be used in the landscaping and cut flower industry (Brits *et al.*, 1983). Considering the endangered and endemic status of *Leucadendron elimense* E.Phillips subsp. *elimense* it is also important to consider the species commercial value and availability for the landscaping and horticultural industry. The distribution of the species stretches from Gansbaai to Bredasdorp in the South Western Cape where only a small population of 2000 plants remains (Rebelo, 2001; Carolus, 2008). According to the Red List, the species is listed as endangered (Rebelo, 2001). The fact that the species is insect-pollinated and endemic to that area means that many insects are living in cohabitation and that both plant and insect species are dependent for their continual existence (Rebelo, 2001; Carolus, 2008).

L. elimense subsp. *elimense* flowers from July to September, which could give the landscape some colour during the cold winter months when not much else is flowering and in February the fruits develop (Rebelo, 2001; Protea Atlas, 2008; Protea Atlas Project, 2019). The leaf size does vary in a population, the ones from the south and south east have larger leaves, while the ones from the north and east have smaller leaves (Rebelo, 2001). The plants grow in shallow soils and need little maintenance (Rebelo, 2001; Carolus, 2008).

L. elimense subsp. *elimense* has potential to be used as an ornamental plant in the horticultural industry and the fact that populations are declining in nature necessitate the importance to research an optimal method of propagating for the species to enhance its future both in conservation and commercial industries.

1.4 Aims

The aim of this study was to determine the vegetative growth of *L. elimense* subsp. *elimense* in response to different growth auxins to establish an optimal growth protocol.

1.5 Hypothesis

It is hypothesised that the different rooting auxins will increase the rooting and have an effect on the flowering and chlorophyll potential of *L. elimense* subsp. *elimense*.

1.6 Objectives of the research

1.6.1 Main Objective

To assess the effects of different rooting auxins on the rooting, flowering and chlorophyll content of *L. elimense* subsp. *elimense*.

1.6.2 Specific objectives

- To evaluate the percentage of callusing of *L. elimense* subsp. *elimense* cuttings in response to different rooting auxins.
- To evaluate the percentage of rooting of *L. elimense* subsp. *elimense* cuttings in response to different rooting auxins.
- To evaluate the root length of *L. elimense* subsp. *elimense* cuttings in response to different rooting auxins.
- To evaluate the increase in wet weight of *L. elimense* subsp. *elimense* cuttings in response to different rooting auxins.
- To evaluate the flowering of *L. elimense* subsp. *elimense* cuttings in response to different rooting auxins.
- To evaluate the number of roots of *L. elimense* subsp. *elimense* cuttings in response to different rooting auxins.
- To evaluate the chlorophyll content of *L. elimense* subsp. *elimense* cuttings in response to different rooting auxins.
- To evaluate the optimal response of *L. elimense* subsp. *elimense* from data gathered in sub-problems 1–7 in order to establish an optimal propagation protocol for producing high quality *L. elimense* subsp. *elimense* plants.

1.7 References

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CHAPTER TWO:

LEUCADENDRON ELIMENSE E.PHILLIPS SUBSP. *ELIMENSE* - THE THREATENED ENDEMIC SPECIES OF THE PROTEACEAE FAMILY ON THE AGULHAS PLAIN: A REVIEW

LEUCADENDRON ELIMENSE E.PHILLIPS SUBSP. *ELIMENSE* - THE THREATENED ENDEMIC SPECIES OF THE PROTEACEAE FAMILY ON THE AGULHAS PLAIN: A REVIEW

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2.1 Abstract

The review evaluates the species *Leucadendron elimense* E.Phillips subsp. *elimense*, a threatened endemic species of the Proteaceae family on the Agulhas Plain. The aim of the study is to evaluate the need to conserve the endangered *L. elimense* subsp. *elimense* because it is an area-specific species and no scientific experiments have been recorded on the successful propagation of the species. The Cape Floral Kingdom stretches over 90 000 square kilometres which makes up one of the six floral kingdoms of the world. Many habitat areas of fynbos species are being used for agricultural purposes and urban expansion, reducing the natural habitat for already endangered species. Illegal harvesting of commercial flowers causes a further decline of the species. Evidence from this study was conclusive that there is a need for propagation techniques to be developed to restore the species in its natural environment and to improve future cut flower cultivation potential to contribute to the export cut flower market.

2.2 Introduction

Leucadendron elimense E.Phillips subsp. *elimense* is at risk of becoming extinct in the wild with possibly 2000 plants remaining in their natural habitat (Mustard *et al*., 1997; Protea Atlas, 2008; SANBI, 2010). The species was already red listed as 'Endangered' in 1980 (Rebelo, 2001; Carolus, 2008). By 1996 the status improved to 'Vulnerable', where the risk of extinction is slightly lower (Hilton-Taylor, 1996; SANBI, 2010), however by 2009 the species was placed back in the 'Endangered' category due to a further decline of population numbers (Raimondo *et al*., 1980; Hall & Veldhuis, 1985). The status was based on recordings of eight populations occurring in four areas with a couple of hundred plants in heavily threatened habitats in the coastal lowlands (Hall *et al*., 1980; Hall & Veldhuis, 1985).

Before 1999 only 10 000 plants were counted, shortly thereafter the individual plant numbers dropped to 7000 plants in eight populations (Brown, 1999). This amounts to only 10% of populations that are conserved while 51% of the species are already lost (Protea Atlas, 2008). Nine percent of populations are found in nature reserves, which are not conserved well while 53% have an extensive natural habitat, with 25% growing on road verges, 20% on agricultural islands and 1% in naturally fragmented habitats (Protea Atlas, 2008). Interesting to note that the Protea Atlas (2008) reported "for every live plant found, there were nine dead, and many looked unhealthy and yellow" and "where there are as many live ones as dead, while others reported more dead than live ones".

The reasons for declining numbers are threats from alien invasive species such as exotic *Acacia, Myrtus* and *Pinus* species with records showing populations where 2% have dense alien vegetation, 20% have abundant amounts, 63% have sparse aliens and only 15% are alien free (Hall & Veldhuis, 1985; Protea Atlas, 2008; Protea Atlas Project, 2019). Other reasons for decline of population numbers are grazing and trampling by cattle, too frequent fires, ploughing, quarrying and illegal flower picking (Hall & Veldhuis, 1985; Grootbos Foundation, 2019). While a study by Laubscher and Ndakidemi (2009) reported that 97% of farmers revealed that they were not harvesting *L. elimense* subsp. *elimense* from natural areas the study revealed that the main reasons for decline in numbers of Red listed species on the Agulhas Plain are contributed to expansion of agriculture and lack of knowledge and propagation skills of these species. The urgency status for conservation remain of a high priority (Hall & Veldhuis, 1985). *L. elimense* subsp. *elimense* only grow on the "Elim Flats" where it is up to the local community of Elim and the surrounding farmers to protect the species from extinction (Protea Atlas, 2008; Protea Atlas Project, 2019). This study thus aimed to review the current status of *L. elimense* subsp. *elimense* and to evaluate its ecological and economic importance as a *Protea* species to support its conservation and cultivation potential.

2.3 The Fynbos Habitat of Proteaceae

The Cape Floral Kingdom (CFK) stretches from the southern and south-western coast of Africa over 90 000 square kilometres from Niewoudtville in the Western Cape to Grahamstown in the Eastern Cape (Cowling & Richardson, 1995; Low & Rebelo, 1996; Rhode, 2004; Born *et al*., 2007; Manning, 2007) (Figure 2.1). In this region a total of 8600 plant species are found of which 5800 are endemic (Hall *et al*., 1980; Winter & Botha, 1994; Cowling & Richardson, 1995; Low & Rebelo, 1996; Goldblatt, 1997; Pauw & Johnson, 1999; Tansley & Brown, 2000; Rouget *et al*., 2003a; Rouget

et al., 2003b; Rhode, 2004; Manning, 2007; Reinten *et al*., 2011; Manning & Goldblatt, 2012). Most of these Proteaceae species can be found growing on foothills and mountainous areas in nutrient-poor soils and where they enjoy misty environments (Lamont *et al*., 1985; Rebelo, 2001). More than two-thirds of the plants grow on mountainous sandstone slopes from Ceres to Cape Agulhas (Rhode, 2004).
The CFK is one of the world's six floral Kingdoms, being the smallest and the only one that is found entirely in one country where many of its species are threatened (Hall *et al*., 1980; Low & Rebelo, 1996; Younge & Fowkes, 2003; Reinten *et al*., 2011). The CFK is only 1% of southern Africa but includes 65% of the southern African threatened and rare plants (Hall *et al*., 1980; Low & Rebelo, 1996). The Cape Province region stretches to 25% of southern Africa, however it harbours 78% of the plant species diversity of the continent (Hall *et al*., 1980). In the CFK there is little structural diversity as three plant families dominate the area, namely the Proteaceae, Ericaceae and the Restionaceae families (Lamont *et al*., 1985; Pauw & Johnson, 1999; Tansley & Brown, 2000; Born *et al*., 2007).

According to the International Union of Conservation of Natural resources two taxa are extinct, 36 endangered, 41 vulnerable, and 73 are naturally rare (Brown, 1999). The reasons for plants to be naturally rare are when they have small populations, meaning five or less (Brown, 1999). Or species may occur in a small area of 5 square kilometres or less or they have few plants remaining meaning 5000 or less which are often dioecious plants and 20% are threatened (Winter & Botha, 1994; Brown, 1999). The reasons for a threatened status are changes in the natural fire regime, invasive species such as *Pinus* sp., *Acacia* sp., and *Hakea* sp., habitat conversion, pathogens such as *Phytophthora cinnamomi*, overgrazing and illegal harvesting of cut flowers, as well as agriculture, roads and urban development (Hall *et al*., 1980; Winter & Botha, 1994; Low & Rebelo, 1996; Rebelo, 2001; Rouget *et al*., 2003a; Bomhard *et al*., 2005; Laubscher & Ndakidemi, 2009).

Many nature reserves have earmarked mainly wild animals for conservation, while not enough emphasis has been placed on the conservation of the floral species of the region (Winter & Botha, 1994). For this reason, landowners, officials and scientists have the responsibility to practice conservation management skills in protecting the vegetation (Hall *et al*., 1980). Every plant species of the CFK needs to be maintained as it contributes to an important part of this unique biodiversity. An understanding of ecological requirements of Proteaceae are important to aid in the conservation of its natural habitat.

Proteas are an iconic symbol of South Africa with the king protea, *Protea cynaroides,* celebrated as the national flower of South Africa since 1976 and are proudly carried by the national cricket team as their emblem (Shales, 2018). The Proteaceae family consists of 1400 species of which 800 species of 45 genera occur in Australia, while Africa has 400 species and of which 330 are found in the Western Cape in South Africa (Brown, 1999; Leonhardt & Criley, 1999; Paterson-Jones, 2000; Tansley & Brown, 2000; Walker, 2018). The rest of the species of this family can be found in Central and South America, on islands of New Guinea and New Caledonia (Leonhardt & Criley, 1999; Paterson-Jones, 2000; Walker, 2018). Proteaceae species have for many years dominated the indigenous cut flower industry of the South African export market. More species however need to be studied to expand its unique international cut flower potential.

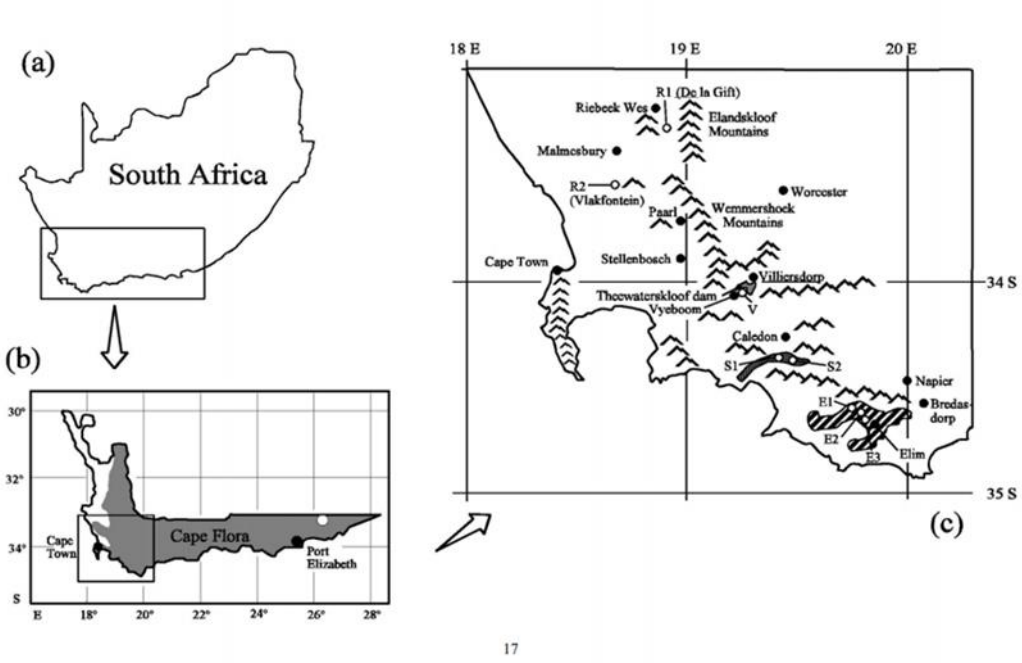

Figure 2.1 The Cape Floristic Region of South Africa (a) The region shows the most southern point of Africa (b) Bottom right enlarged area from Cape point to Cape Agulhas (c). (Source: Tansley & Brown, 2000)

2.4 *Leucadendron* as a member of the Proteaceae family

There are around sixty species of *Leucadendron*, which are also known as Cone bushes (Leonhardt & Criley, 1999; Baessler, 2019). The name *Leucadendron* comes from the silvertree (*Leucadendron argenteum*) as it is descriptive of the species, the word ‘Leuca’ means white and ‘dendron’ means tree (Carolus, 2008). *Leucadendron* species are dioecious, the female produces a woody seed head, known as a cone with spirally arranged floral bracts (Lamont *et al*., 1985; Leonhardt & Criley, 1999;

Rhode, 2004; Ben-Jaacov & Silber, 2006). The male flowers only last two weeks and are inconspicuous, the plants flower from June to September and they produce the first flowers after three to four years (Leonhardt & Criley, 1999; Rhode, 2004). In general, male plants are more branched, slightly larger and have smaller leaves and flower heads (Ben-Jaacov & Silber, 2006). The main pollinators of the genus are rodents, birds, insects and more dominant wind pollination (Lamont *et al*., 1985; Leonhardt & Criley, 1999). *Leucadendron* species are adapted to the fynbos biome where all but three species are found in the CFK (Ben-Jaacov & Silber, 2006). Two species are extinct, while eight are vulnerable and seventeen are naturally rare (Ben-Jaacov & Silber, 2006). It is alarming to see how the Red List status of this genus continues to further decline.

The species preferred soil is sandstone or quartzite, granite or limestone with some humus, light, well-draining and with an acidic pH, below five, with low concentrations of dissolved salts (Eliovson, 1965; Rousseau, 1970; Pienaar, 1991; Cowling & Richardson, 1995; Leonhardt & Criley, 1999; Brown & Duncan, 2006; Manning, 2007; Andrews, 2018). The midsummer temperature is preferred on an average 28 °C but can drop to 4 °C and rise to 43 °C (Ben-Jaacov & Silber, 2006). The midwinter temperature averages at 17 °C but can drop to -5 °C or rise to 30 °C (Ben-Jaacov & Silber, 2006). Most *Leucadendron* species grow in winter rainfall areas where annual rainfall ranges between less than 250 mm and 3000 mm (Eliovson, 1965; Rousseau, 1970; Pienaar, 1991; Cowling & Richardson, 1995; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Manning, 2007; Andrews, 2018). Periodic fires are essential for the genus' natural reproduction where intervals of between ten to fourteen years are optimal (Cowling & Richardson, 1995; Paterson-Jones, 2000; Brown & Duncan, 2006; Manning, 2007; King, 2017). Veld fires recycle nutrients back into the soil, returning fertility requirements and a burnt canopy allows light to reach the soil surface for seeds to germinate (Manning, 2007). During periods of no fire of within 45 years the plant dies (Cowling & Richardson, 1995) with a resultant species diversity decline. *Leucadendron* is the second largest commercially produced genus, but also the least studied of the Proteaceae family with little, solid, scientifically based information being recorded (Rhode, 2004; Ben-Jaacov & Silber, 2006). Due to this reason, the lack of research could be advanced with further studies to collect data on the vulnerability and loss of species diversity within this genus.

2.5 *Leucadendron* uses as commercial products

Proteas are perennial, woody evergreens which dominate their natural habitat with bright floral displays (Leonhardt & Criley, 1999; Rhode, 2004). While many species grow as groundcovers, some are vertical spreading and others grow to upright trees (Lamont *et al*., 1985; Leonhardt & Criley, 1999; Rhode, 2004). Most flowering stems are either upright or drooping and vary in length and diameter (Rhode, 2004) and hard leathery leaves constructed to survive windy, hot and dry summers, which are present in the area (Leonhardt & Criley, 1999; Rhode, 2004). These sclerophyllous leaves prevent water loss while their lignified tissue keeps them from collapsing when there is little water available (Leonhardt & Criley, 1999; Rhode, 2004). The plants continue to photosynthesize where other plants would wilt during these drought stress periods (Rhode, 2004). Proteas are mostly pest-free because of the high leaf carbon to nitrogen ratio (Leonhardt & Criley, 1999). Proteoid roots form a dense cluster of hairy rootlets with a 2 to 5 cm thick mat which increases nutrient uptake for the plants (Leonhardt & Criley, 1999). Fire has been recorded as a stimulus for reproduction and regeneration as it assists in the evolution of reproduction of species (Rhode, 2004).

South African garden cultivation of Proteaceae began in the late 18th to early 19th century when these flowers also gained popularity in Europe (Eliovson; 1965; Brits *et al*., 1983). By 1913 the National Botanic Gardens at Kirstenbosch began with experimental cultivation studies of Proteas (Brits *et al*., 1983; Malan, 2012). By the 1960's many cultivation requirements were identified, classified and described through experimental research work and plant response observations studies (Brits *et al*., 1983; Malan, 2012). Already at this time it was known that Proteas have economic potential as cut flowers and therefore their cultivation was further studied to become flowering ornamentals of the horticulture industry with mainly export of fresh flowers for a European market (Brits *et al*., 1983).

As South African Protea cut flowers reached the European flower market in 1980, about eighty percent of the flowers exported were directly harvested from their natural habitat (Brits *et al*., 1983; Malan, 2012). Even though cut flower Proteas have been transformed to an orchard cultivation production industry in more recent years, many flowers are still illegally picked, and overharvesting continues from their natural habitat in order to be sold on local and international markets (Laubscher & Ndakidemi, 2009).

These practises continue to lead to the threat of losing many of the iconic species of this important plant family (van Deventer *et al*., 2003). Considering the importance of

Proteaceae more efforts should be made to conserve and cultivate *Protea* species in South Africa. The genus *Leucadendron* are most suitable for the cut flower industry because of their long stems, long-lasting foliage or flowers, dominate colour feature plant in the landscaping industry, as potted plants and dried flower products (Leonhardt & Criley, 1999; Rhode, 2004; Reinten *et al*., 2011). Most species have bright colourful foliage, for example green, red, orange, gold, silver, bright yellow or soft yellow (Rhode, 2004; Ben-Jaacov & Silber, 2006). Female plants are in higher demand because of their longer vase life, as fillers in floral bouquets and because their cones are favoured in the dry flower industry (Rhode, 2004; Ben-Jaacov & Silber, 2006). The male flowers are desirable for their flowers, because they are either white-silver, red or yellow, but only have a short market season of two to three weeks of the year (Rhode, 2004; Ben-Jaacov & Silber, 2006).

Annually 100 million cut stems of Proteaceae are produced around the world, of those, half are *Leucadendron* sp. (Ben-Jaacov & Silber, 2006). As *Leucadendron* flowers gained popularity especially on the Dutch market, their cultivation advanced as most species are the easiest to adapt and can be propagated easily and grow vigorously (Rhode, 2004). There are 91 different species of *Leucadendron*, however only 22 species are used commercially (Rhode, 2004). The cultivars that are used, mainly flower from late winter to early spring in the CFK (Rhode, 2004). Many species have been genetically crossed for named cultivars, one of the most popular hybrids, *Leucadendron* 'Safari Sunset' has been bred in New Zealand and has dominated the cut flower market for over forty years, with over 90% of its production in Israel (Ben-Jaacov & Silber, 2006). The genus has great potential for the cut flower industry and therefore more species should be studied further to improve their cultivation practices and optimise their potential.

2.6 *Leucadendron elimense* E.Phillips subsp.*elimense*

L. elimense subsp. *elimense* is also known as the Elim conebush, Bergkatjiepiering and Elim se mense, grows up to 1.5 m tall and with a single-stemmed bush with few branches (Mustard *et al*., 1997; Rebelo, 2001; Carolus, 2008; Protea Atlas, 2008; Protea Atlas Project, 2019) (see Figure 2.2). This species belongs to the category ventricosa, also known as the crown cone bush with flowers borne in clusters (Rhode, 2004; Ben-Jaacov & Silber, 2006). The specific epithet name "*elimense*" comes from the name Elim, a village and old German Moravian mission station where the plants occur naturally (Carolus, 2008). According to Hall *et al*. (1980) the plant was categorised to be from the Caledon area and not Bredasdorp as thought in 1999, however it occurs naturally from Gansbaai to Bredasdorp (Brown, 1999). Today it can

be found, especially on the road from Elim to Bredasdorp in the Overberg region where it is endemic to the region (Mustard *et al*., 1997; Rebelo, 2001; Carolus, 2008).

Figure 2.2 *L. elimense* subsp. *elimense* in its natural habitat showing a single stemmed branched plant (Source: Liedtke, 2019).

L. elimense subsp. *elimense* grows inland, meaning at least two kilometres from the coast at an altitude of 20 to 200 metres (Protea Atlas, 2008). It grows in shallow soils which have acid sulphate over clay (Rebelo, 2001). Documented by the Protea Atlas (2008), the soils are deep and have a gentle incline, can either be loamy, clayey or sandy and some are gravelly. The soil colour is mostly found to be grey or brown and the geology is shale, silicrete or ferricrete, while some can be found on sandstone (Protea Atlas, 2008). Most plants grow on south facing slopes but can also be found on north and west facing slopes while few occur on east facing slopes (Protea Atlas, 2008). The plants are mostly found in shrubland and only some are found on agricultural land (Protea Atlas, 2008).

The flower buds appear from June to August and flowering occurs from August to September and the cones form in February in the southern hemisphere (Hall & Veldhuis, 1985; Rebelo, 2001; Protea Atlas, 2008; Protea Atlas Project, 2019). The most growth occurs in March, but it also grows in the months from November to May (Protea Atlas, 2008). The fact that both male and female flower heads have a pungent smell indicates that they are insect pollinated, for example by flies and beetles (Carolus, 2008; Protea Atlas, 2008).

The plant is dioecious and the leaves on the male plant are red and elliptic with a length of 13–49 mm and 5–19 mm wide (Rebelo, 2001; Carolus, 2008), while the female plant has a hairless, narrow, firm, but flexible margin with closely-spaced leaves with a length of 14–57 mm and a width of 7–21 mm (Rebelo, 2001; Carolus, 2008). Yellow involucral leaves, which overlap the base of the flowerhead are found

on both male and female plants (Mustard *et al*., 1997; Carolus, 2008). The male flower head is 46 mm across and 19 mm long and the flower head has a pungent smell and a depressed, round shape (Rebelo, 2001; Carolus, 2008). The female flower head is 17 mm long and 21 mm across, it also has a pungent smell and has the flowers closely packed to the upper surface and the sides closely overlapped by recurved bracts and leaves (Rebelo, 2001; Carolus, 2008). The cones that form are globe-shaped and 35 mm in diameter (Mustard *et al*., 1997; Rebelo, 2001). The numerous bracts are oval, with no hairs on top, but with some below (Rebelo, 2001) (see Figure 2.3). It is critical to understand the natural habitat of the species and the ecological requirements to ensure successful cultivation.

Figure 2.3 Female cone (left) male flower (right) (Source: (left) Carolus, 2008 (right) Laubscher, 2018)

2.7 Cultivation of *Leucadendron* species

Growing *Leucadendron* sp. from seed is the most used method of propagation of the genus in South Africa (Ben-Jaacov & Silber, 2006). *L. elimense* subsp. *elimense* falls under the group crown conebushes which release their fruit after five months of ripening (Rebelo, 2001). The section Alatosperma means the seeds are flattened and remain within the cone for one year or more, this means the seeds are not spontaneously released and the cones can be collected from the previous year and be stored in a warm, dry place (Brits, 1986; Carolus, 2008; Reinten, 2014). Seeds are normally collected and sown in autumn in the winter rainfall area where fluctuating temperatures between day and night activate seed germination, with night temperatures drop between 5 to 8°C and day temperatures are between 15 to 20°C (Eliovson, 1965; McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Carolus, 2008; Malan, 2012; Reinten, 2014). Dormancy of seed can also be broken when seed are soaked in sulphuric acid for five to ten minutes and then rinsed

in clean water (McLennan, 1993; Malan, 2012). Another way to break dormancy is by mechanically scarifying the seed with sandpaper, to increase the oxygen supply to the embryo (Brown & Duncan, 2006; Malan, 2012). The seeds can be treated with smoke or with a smoke derivative and scarified to break dormancy (Brown & Duncan, 2006; Malan, 2012). A fourth option is to soak the seed in 1% hydrogen peroxide with smoke primer paper for 24 hours (Brits, 1986; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012). Older seeds lose their viability and can be soaked in a solution containing gibberellin (Brown & Duncan, 2006). The seeds should be treated with a fungicide to prevent post-emergence infection (Brown & Duncan, 2006). It is also recommendable to use 500 ml plastic seedling bags as they provide improved results compared to using seedling trays (Oertel & Oertel, 2018b). Seedling plastic bags should be prepared with two parts coarse river sand, two parts peat and one part of vermiculite or perlite with a pH of 5.5, as this medium provides sufficient drainage and aeration to prevent rotting of seed in a too wet medium (Eliovson, 1965; McLennan, 1993; Carolus, 2008; Oertel & Oertel, 2018b). After the first watering the medium should be drenched with a fungicide (McLennan, 1993). The seedling bags should be placed under mist sprayers in a warm environment with sufficient ventilation (McLennan, 1993). After one to three months, germination will take place and the roots should be strengthened with an application of kelp, liquid fertiliser (McLennan, 1993; Brown & Duncan, 2006; Carolus, 2008; King, 2018). When two to six true leaves have formed the seedlings are ready to be pricked out into individual bags, where they are kept for one year before being planted into the garden with the following rainy season (Eliovson; 1965; Carolus, 2008). When the seedlings reach a height of 15 to 30 cm, they are ready to be transplanted into the open ground (Eliovson, 1965).

Many species of the *Leucadendron* genus have been propagated successfully in raising clones for cut flower orchards plantings. In the southern hemisphere the best time for cuttings is from March to April (Eliovson; 1965; McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014). Terminal or sub-terminal cuttings should be made early in the morning to avoid heat and drought stress and should be 12 cm long and have a diameter of 8 mm (Eliovson; 1965; Ben-Jaacov *et al*., 1986; McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014; Oertel & Oertel, 2018a; ACS Distance Education; 2019). The leaves on the lower half of the cutting should be removed (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a).

Several studies reported that indole butyric acid (IBA) at 0.1% can be used to stimulate root growth, at a concentration of 2000 ppm for Proteaceae (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). The options would be to dip the cuttings in IBA powder at 4 g per litre dissolved in 50% ethanol for five to ten seconds (Brown & Duncan, 2006). Liquid IBA has shown best results, the cuttings should be placed in the liquid for ten seconds at a depth of one to two centimetres (McLennan, 1993). When powder IBA is used, it can burn the cutting if too much is applied (McLennan, 1993). Seradix 2 or liquid Dip and Grow can also be used as a rooting stimulant (Brown & Duncan, 2006; Malan, 2012). The best results for Proteas have been to use 4000 ppm of IBA, while 2000 ppm of IBA is better for cone bushes (Ben-Jaacov & Silber, 2006). Indole-3-acetic acid (IAA) had a positive effect on the rooting of *L. laxum* in a shaded tunnel environment (Laubscher & Ndakidemi, 2008b).

Cutting length of 10 mm should be dipped into the correct strength for ten seconds (Ben-Jaacov & Silber, 2006). The growing medium can be in transparent growing bags and can either be sand, peat, polystyrene in equal amounts or two to three parts river sand with one-part peat, (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). In comparison with other growth media tested, a bark and polystyrene medium was more effective in the stimulation of rooting, survival of cuttings, root length and number of roots formed for *L. laxum* (Laubscher & Ndakidemi 2008a).
The cuttings should be one third deep in the medium and have good aeration and be misted (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). The air temperature should be at 26°C, while the root zone should be 18°C (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012). They should be placed on hotbeds and every hour the cuttings should receive a mist spray, but only during the day, and receive a spray programme against diseases (Eliovson, 1965; Ben-Jaacov *et al*., 1986; McLennan, 1993; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a; ACS Distance Education, 2019).

After six to sixteen weeks have passed the cuttings will begin to form roots and are ready to be planted out, once the roots are well-developed and appear discoloured on the sides of the bag (Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018). The plants are ready to be planted out into well-drained, acidic soil that has not been fertilised (Reinten, 2014). Research in Israel has shown that *L*. 'Safari Sunset' can be grown in three to five months by using 15 cm long cuttings, dipped in 4000 ppm of IBA and are grown in a Styrofoam-peat medium under intermittent mist with 25%

reduced natural light (Ben-Jaacov *et al*., 1986; Leonhardt & Criley, 1999). Cuttings will root in a period of four weeks (Leonhardt & Criley, 1999). Although many of these propagation techniques are being used successfully, it remains uncertain if this methodology could be used for *L. elimense* subsp. *elimense,* as no evidence could be found to support this. It is therefore important to study the vegetative propagation of the species to increase plant numbers.

2.8 Conclusion

It has been shown that several species from the Proteaceae family have the potential to be cultivated commercially to save natural populations from overharvesting in the wild. Species such as *L. elimense* subsp. *elimense* have reached 'Endangered' Red Listed levels due to continued habitat destruction with no evidence that population numbers have recovered to repair the natural biodiversity. The study reported that a demand for cut flowers from female plants are the highest while there has been no record of the vegetative propagation of the species. Future research should focus on the cultivation of *L. elimense* subsp. *elimense* to establish different propagation methodology that are used to propagate the species vegetatively. Efforts should be aimed towards finding solutions for propagating dioecious plants by correctly outlining the unknown factors that effects rooting inhibition of the species. This can lead to increased clonal production through improved methodology in advancing commercial cultivation for local and international supplies and support restoration of wild populations.

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CHAPTER THREE:

ROOTING RESPONSES OF FEMALE AND MALE CUTTINGS OF *LEUCADENDRON ELIMENSE* E.PHILLIPS SUBSP. *ELIMENSE* TO DIFFERENT ROOTING AUXIN TREATMENTS

ROOTING RESPONSES OF FEMALE AND MALE CUTTINGS OF *LEUCADENDRON ELIMENSE* E.PHILLIPS SUBSP. *ELIMENSE* TO DIFFERENT ROOTING AUXIN TREATMENTS

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3.1 Abstract

Female and male plants of difficult-to-root species, *Leucadendron elimense* E.Phillips subsp. *elimense* was investigated for callusing and rooting potential in relation to three different rooting auxins namely Indole-3-acetic acid (IAA), 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA) at three different concentrations of 2000 ppm, 4000 ppm and 6000 ppm. Male plants boast an upright growing shrub with beautiful yellow flowers compared to female plants with striking cones, both with enormous commercial cut flower potential. Callusing commenced from 6 weeks while rooting determination ended over 18 weeks with the longest rooting lengths measured. Essential misting, bottom heat and a naturally ventilated greenhouse provided a successful rooting environment. Callusing percentage and rooting success, root number and root length proved significant with IAA at 4000 ppm on female cuttings. In comparison neither female or male cuttings showed specific significance in rooting, however the combination of gender and rooting auxin were highly significant in adventitious root development. These results proved the success of IAA in rooting for *Leucadendron* species with new evidence to propagate both female cones and male flowers of *L. elimense* subsp. *elimense* to support the future cultivation of the species for the export Proteaceae cut flower industry.

Key words: commercial potential, cone bush, cut flower, Fynbos, Red List

3.2 Introduction

3.2.1 *Leucadendron elimense* E.Phillips subsp. *elimense* male and female plants

L. elimense E.Phillips subsp. *elimense* male and female flowers both have potential as commercial cut flowers. Field harvesting of this species is illegal since it is on the Red List and its status is endangered, therefore is has become important to investigate its potential with vegetative propagation (Rebelo, 2001; Carolus, 2008). The species is dioecious, with a clear distinction between separate male and female

plants. Female plants have only a few branches to a growth height of 1.5 m on single stems compared to more multi branched stems from male plants (Rebelo, 2001; Carolus, 2008). The leaves of species are elliptic and slightly larger in female plants reaching a length of 14–57 mm and width of 7–21 mm, whereas leaves from male plants grow up to 13–49 mm long and 5–19 mm wide (Rebelo, 2001). Both male and female plants have yellow involucral leaves which overlap the base of the flower head, flowers appear from July to September and the fruits and cones develop in February (Hall & Veldhuis, 1985; Mustard *et a*l., 1997; Rebelo, 2001; Carolus, 2008; Protea Atlas, 2008; Protea Atlas Project, 2019) (Figures 3.1 and 3.2).

Figure 3.1 *Leucadendron elimense* subsp. *elimense* female flower (left) and cone (right) (Source: Carolus, 2008)

Figure 3.2 *Leucadendron elimense* subsp. *elimense* male flower
(Source: (left) Turner, 2010 (right) Laubscher, 2018)

The species mainly reproduces from seed in its natural habitat. The pungent smell of the male and female flower heads indicates that they are insect pollinated by flies and beetles, the main pollinator is the Monkey Beetle (*Clania glenlyonensis*) (Carolus, 2008; Protea Atlas, 2008). *L. elimense* subsp. *elimense* also known as the Elim

conebush belongs to the crown conebushes, subsection Ventricosa (Mustard *et al*., 1997; Rebelo, 2001; Rhode, 2004; Ben-Jaacov & Silber, 2006; Carolus, 2008; Protea Atlas, 2008; Protea Atlas Project, 2019). The crown cone bushes have hairless seeds that bulge outward on one side and go inward on the other, they have a ridged perimeter (Rebelo, 2001). The cone bracts are free from one another, all species in this group release their seeds after five months of ripening (Mustard *et al*., 1997; Rebelo, 2001; Rhode, 2004; Ben-Jaacov & Silber, 2006; Carolus, 2008; Protea Atlas, 2008; Protea Atlas Project, 2019) (Fig 3.3).

Germination of the seed is fire dependant to ensure that the species reproduction cycle is completed. Proteaceae fynbos must burn at an age of 6 to 45 years in order to propagate (Low & Rebelo, 1996; Pauw & Johnson, 1999). Some species are either resprouters or reseeders, meaning they either grow again from an existing plant or they grow from seed after a fire (Low & Rebelo, 1996; Pauw & Johnson, 1999). The reseeders either keep their seeds safe in a cone until a fire or they drop them after a few months, and ants bury them in the ground to keep them safe from rodents and fire (Low & Rebelo, 1996; Pauw & Johnson, 1999).

Figure 3.3 *L. elimense* subsp*. elimense* cone and seeds which develop from the female flowerhead. (Source: Liedtke, 2020)

3.2.2 Natural fynbos habitat

L. elimense subsp. *elimense* is an endemic species of the Proteaceae family found along the road from Elim to Bredasdorp in the Overberg region and more specifically the town Elim, arising to the epithet name of the species (Mustard *et al*., 1997; Rebelo, 2001; Carolus, 2008). The habitat of *L. elimense* subsp. *elimense* in the

south western part of the Western Cape Province fynbos biome encompasses fynbos and renosterveld species. This biome has little structural diversity as mainly three plant families dominate the area, namely the Proteaceae, Ericaceae and the Restionaceae families (Lamont *et al*., 1985; Low & Rebelo, 1996; Pauw & Johnson, 1999; Tansley & Brown, 2000; Born *et al*., 2007). Over 7000 plants can be found in the fynbos biome, of which over 80% are endemic to the region mostly growing on well-leached Cape sandstone soil (Low & Rebelo, 1996).

L. elimense subsp. *elimense* grows inland, meaning at least two kilometres from the coast at an altitude between 20 to 200 metres (Protea Atlas, 2008). It grows in shallow soils which have ferricrete over clay (Rebelo, 2001). According to Protea Atlas (2008) the soil is deep and has a gentle incline and can either be loamy, clayey or sandy and some are gravelly. The Elim ferricrete Fynbos can be found in low-lying areas from Botrivier to De Hoop and remain threatened, critically endangered due to alien invasive species, land transformation and poor fire regimes (Grootbos Foundation, 2019). *L. elimense* subsp. *elimense* mostly grows on south facing slopes but can also be found on north and west facing slopes and few grow on east facing slopes (Protea Atlas, 2008).

3.2.3 Vegetative propagation of female and male plants

Considering the economic potential of male and female cut flowers, there has been little attention to cultivate these sexes individually. Earlier reports from Laubscher and Ndakidemi (2008a) indicated that there was no scientific evidence that *L. elimense* subsp. *elimense* propagates clonally as no previous studies have been recorded on the vegetative propagation of this species. A cultivation protocol to propagate these individual gender plants could support the commercial cultivation of the species and further reduce the species red listed status. This study therefore aimed to investigate the vegetative propagation of *L. elimense* subsp. *elimense* female and male plants to establish which auxin treatment would be most suitable for both female and male plants.

3.3 Materials and methods

3.3.1 Greenhouse experiment

The experiment was conducted from the 23 May until 24 September 2019 in the propagation greenhouse on the Cape Peninsula University of Technology, Bellville campus. This part of the greenhouse is a semi-controlled environment. The sides of the greenhouse are made of shade netting to allow for natural air flow and moisture

penetrating the building, however the roof prevented rain from above. Temperatures ranged between 15 and 26 °C while the relative humidity (RH) ranged between 90 to 96% as measured on the environmental control system, by the researcher, according to McLennan (1993), Ben-Jaacov and Silber (2006), Brown and Duncan (2006) and Malan (2012) this was optimal. During the rooting period plant bags were placed on hot beds with a temperature of 22 °C and received intermitted mist irrigation alternating every 40 minutes for 21 seconds (Eliovson, 1965; McLennan, 1993; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018b; ACS Distance Education, 2019).

The amount of water received per interval of irrigation measured 500 ml during the rooting stage. Once the cuttings had rooted, they were moved to a hardening off section where they received a mist spray every 50 minutes for 18 seconds, the amount of water received per interval of irrigation measured 150 ml.

3.3.2 Plant material collection and cutting preparation

The plant material of *L. elimense* subsp. *elimense* was collected with permission on the land of the Elim church, a German Mission Station in the Western Cape Province of South Africa (Schoeman & Visagie, 2014). To ensure sustainable harvesting, the plant material was collected from randomly selected mother plants to collect cuttings from 50 female and 50 male plants. The plant material was stored in moist newspaper overnight to ensure they do not dry out. The following morning the cuttings were taken to the Cape Peninsula University of Technology Bellville campus, South Africa at GPS coordinates - 33º 55’45.53S, 18º 38’ 31. 16E..The terminal cuttings were cut into uniform lengths of 15 cm for female plants and 10 cm for male plants. The bottom 1/3 of the leaves were removed and the treatment was administered. These methodologies were recommended for collection of cuttings during the early hours in the morning to avoid heat stress, should be of terminal or sub-terminal with a length of 12 cm with a diameter of 8 mm and the lower half of the leaves removed (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018b). The cuttings were taken just below the node with a straight cut. The cuttings were then sprayed with Captab at 4 g/l. Two hundred terminal cuttings were then planted into perforated, transparent, bags 125 x 190 mm, obtained from Packit 354 Voortrekker Road, Maitland, Cape Town, Western Cape, with a mix of two parts coarse river sand (2 mm diameter), one part coco peat and one part perlite (2:1:1) (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a;). The rooting medium should preferably be a well-draining, acidic medium which has not been

fertilised (Reinten, 2014). The cuttings should be one third deep in the medium and have good aeration (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018b). The plants were then placed on the hot beds in the greenhouse and watered thoroughly.

Figure 3.4 Female cutting (left) and male cutting (right) indicating approximate length of 15 cm and 10 cm respectively using standard ruler for measuring. (Source: Liedtke)

3.3.3 Experimental treatments

The treatments consisted of three different rooting auxins, namely Indole-3-acetic acid (IAA), 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA) at concentrations of 2000 ppm, 4000 ppm and 6000 ppm. The treatments were laid out in a randomised block design with ten female and ten male plants to allow for 10 repetitions for each treatment in female and male plants. The auxins were ordered from Sigma Aldrich, Unit 4 Aviation Park, 17 Pomona Road, Kempton Park. Treatments were done with cuttings dipped in the liquid for ten seconds at a depth of one to two centimetres (McLennan, 1993). Rooting auxins IAA, NAA and IBA, at different strengths were prepared by using 0.5 g in 250 ml of water, 1 g in 250 ml of water and 1.5 g in 250 ml of water to make the concentrations. Repetitions were labelled accordingly:

IAA2F = Indole Acetic Acid at 2000 ppm on cuttings of female plants
IAA2M = Indole Acetic Acid at 2000 ppm on cuttings of male plants
IAA4F = Indole Acetic Acid at 4000 ppm on cuttings of female plants
IAA4M = Indole Acetic Acid at 4000 ppm on cuttings of male plants

IAA6F = Indole Acetic Acid at 6000 ppm on cuttings of female plants
IAA6M =Indole Acetic Acid at 6000 ppm on cuttings of male plants
NAA2F = 1-Naphthaleneacetic acid at 2000 ppm on cuttings of female plants
NAA2M = 1-Naphthaleneacetic acid at 2000 ppm on cuttings of male plants
NAA4F= 1-Naphthaleneacetic acid at 4000 ppm on cuttings of female plants
NAA4M = 1-Naphthaleneacetic acid at 4000 ppm on cuttings of male plants
NAA6F = 1-Naphthaleneacetic acid at 6000 ppm on cuttings of female plants
NAA6M = 1-Naphthaleneacetic acid at 6000 ppm on cuttings of male plants
IBA2F = Indole-3-butyric acid at 2000 ppm on cuttings of female plants
IBA2M = Indole-3-butyric acid at 2000 ppm on cuttings of male plants
IBA4F = Indole-3-butyric acid at 4000 ppm on cuttings of female plants
IBA4M = Indole-3-butyric acid at 4000 ppm on cuttings of male plants
IBA6F = Indole-3-butyric acid at 6000 ppm on cuttings of female plants
IBA6M = Indole-3-butyric acid at 6000 ppm on cuttings of male plants
CF = Control no auxin treatment on cuttings of female plants
CM = Control no auxin treatment on cuttings of male plants

3.3.4 Care during rooting period of the cuttings

Cuttings were continuously monitored for stress of overwatering and treated weekly with a preventative fungicide spray (Eliovson, 1965; McLennan, 1993; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a; ACS Distance Education, 2019). A ratio 1:1 of a mixture of Captab (4 g/l) and Kelpak (5 ml/l) was sprayed on the cuttings at 12 pm weekly. Overwatering, signs of blackening on leaves and stems and rotting were monitored during the rooting period and yellow and dead leaves were removed from cuttings.

Figure 3.5 A randomised block layout of the experimental layout showing repetitions of cuttings clearly and individually labelled. (Source: Liedtke, 2019)

3.3.5 Care during the hardening-off and growing period

On 20 August 2019 (Week 13) the cuttings were moved to the hardening off section, where they received 150 ml of water via mist sprayers every 50 minutes for 18 seconds. The plants were sprayed weekly with a mixture of Captab (4 g/l) and Kelpak (5 ml/l).

3.3.6 Determination of plant growth

On the 24 September 2019 (Week 18) the cuttings were removed from the growing medium; the growing media was carefully rinsed off the roots and measurements were done.

3.3.6.1 Callusing percentage

Callusing percentage was determined on the number of cuttings that developed successful callus tissue of parenchyma cells which covered the wounded area at the basal cut end and node of each cutting. All cuttings that callused rooted, therefor Table 3.1 shows the results for callusing and rooting percentage.

3.3.6.2 Rooting percentage

Rooting success was determined on the number of cuttings which developed adventitious roots from the callused tissue at the basal end of each cutting. The longest roots were measured from each cutting at week 18. Rooting time could vary but cuttings should root between six to sixteen weeks and develop roots which discolour on the sides of the bag as an indication that they are ready to be planted out (Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018).

3.3.6.3 Root length

To determine the root length the cuttings were removed from the growing medium and rinsed in water, then the root length was measured with a standard ruler in mm.

3.3.6.4 Number of roots

To determine the number of roots the cuttings were removed from the growing medium and rinsed in water, then the number of roots were counted from each cutting.

3.3.7 Statistical analysis

The data was analysed by using two-way analysis of variance (ANOVA), with the computing software program STATISTICA 13. The occurrence of statistical difference was determined by using the Fisher Least Significance Difference (L.S.D.) at values of P ≤0.05; P ≤0.01 and P ≤0.001 levels of significance (Steel & Torrie, 1980).

3.4 Results

The experiment was conducted to determine which rooting auxin at what concentration would give significant results. The results showed that callusing and rooting showed statistically significant variance in the auxin treatment and the combination of the treatment with the gender female cuttings.

3.4.1 Callusing percentage

Effects of auxin treatments

The auxin IAA at 4000 ppm (IAA4F) treatment was highly successful with an 80% callusing success rate and statistically significant different (P ≤ 0.001) compared to the control treatment (CF) for *L. elimense* subsp. *elimense* at the 18th week period of the experiment. The control showed no callusing in the cuttings. The means varied between 80% and 0% (Table 3.1). The effects of the auxin IAA were more stable in callusing compared to the control and other auxin treatments of NAA and IBA (Table 3.1).

Comparisons between the female and male gender cuttings

The effects of auxin treatments were not significant on the callusing of female and male gender cuttings. Male cuttings of *L. elimense* subsp. *elimense* treated with IAA at 4000 ppm, NAA at 4000 ppm and IBA at 6000 ppm showed the highest callusing percentage at 70% while the effects of the auxin IAA at 4000 ppm proved a higher callusing percentage of 80% in the female cuttings (Table 3.1). Both female and male cuttings had 50 deaths and 50 live cuttings.

Interactions between auxin and gender

The effects of auxin treatments in conjunction with gender on callusing of *L. elimense* subsp. *elimense* cuttings were highly significant at P ≤0.01 compared to the control (CF/CM). The highest individual mean percentage (80%) for callusing was observed

in treatment IAA4F (see Table 3.1 and Figure 3.11) *L. elimense* subsp. *elimense*. The combination of the 4000 ppm IAA and the female cuttings proved to be more successful in callusing than the control and the male cuttings in the experiment (Table 3.1). The female and male cuttings both had 50 healthy and 50 dead cuttings.

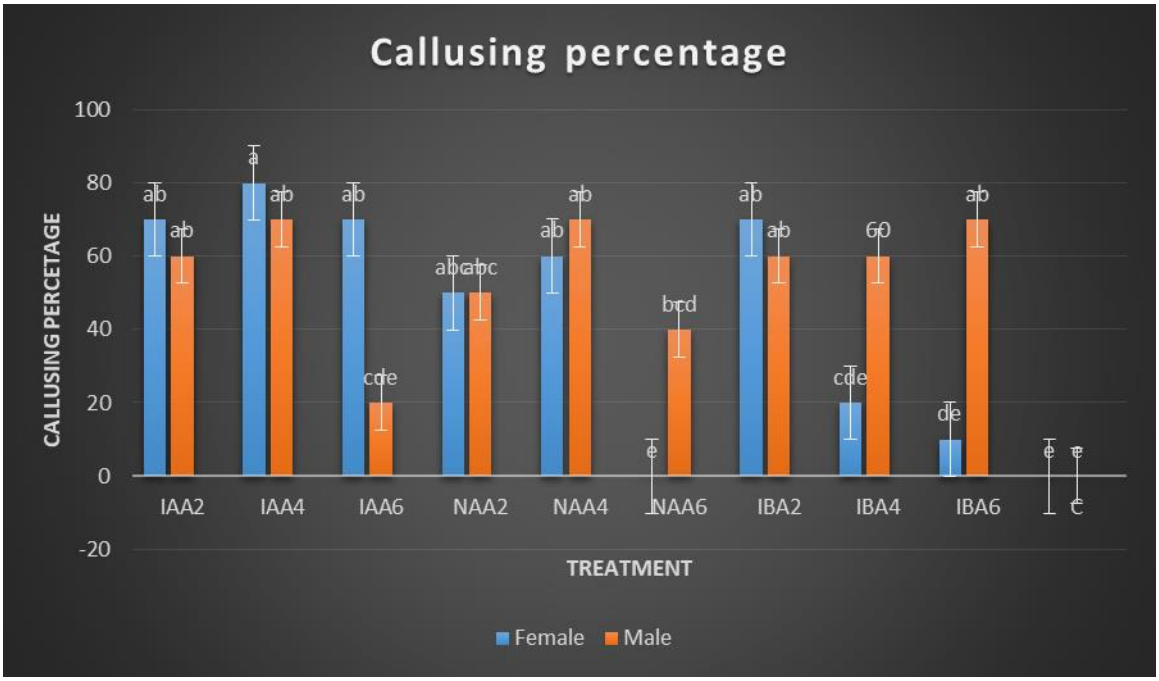

Figure 3.6 The effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the rooting success of female and male cuttings of *L. elimense* subsp. *elimense*.

3.4.2 Adventitious rooting

Effects of auxin treatments

Rooting results of *L. elimense* subsp. *elimense* showed a highly significant difference (P≤ 0.001) in the auxin IAA at 4000 ppm (IAA4F) with a mean value of 8.00 compared to the control (CF) at 0.00 over 18 weeks. The auxin treatments IAA at 4000 ppm, NAA at 4000 ppm and IBA 6000 ppm showed the highest results for the rooting percentage at 7.00, for male cuttings was not significant in rooting results (Table 3.1). When comparing the results with the root length and number of roots it can be seen that IAA at 4000 ppm had the best results for female cuttings as well as NAA at 4000 ppm in the male cuttings compared to the control.

Comparisons between the female and male gender cuttings

The effects of auxin treatments were not significant (ns) on the rooting of female and male gender cuttings. Male cuttings of *L. elimense* subsp. *elimense* treated with IAA at 4000 ppm, NAA at 4000 ppm and IBA at 6000 ppm showed the highest rooting measurements in male cuttings, while the effects of the auxin IAA at 4000 ppm on female cuttings proved a higher rooting percentage compared to the male cuttings. (Table 3.1). When comparing the results with the root length and number of roots it can be seen that IAA at 4000 ppm had the best results for female cuttings as well as NAA at 4000 ppm in the male cuttings compared to the control.

Interactions between auxin and gender

Over 18 weeks, the effects of auxin treatments in conjunction with gender on rooting of *L. elimense* subsp. *elimense* cuttings were highly significant at P ≤0.01 compared to the control (CF/CM). The highest individual mean 8.00 for rooting was observed in treatment IAA4F (see Table 3.1 and Figure 3.11) for *L. elimense* subsp. *elimense*. The combination of the 4000 ppm IAA and the female cuttings proved to be more successful in rooting compared to the control and the male cuttings in the experiment (Table 3.1).

Figure 3.7 Rooting of IAA 4000 ppm female cutting at week 7 showing healthy down wards roots protruding on the side of the clear bag.

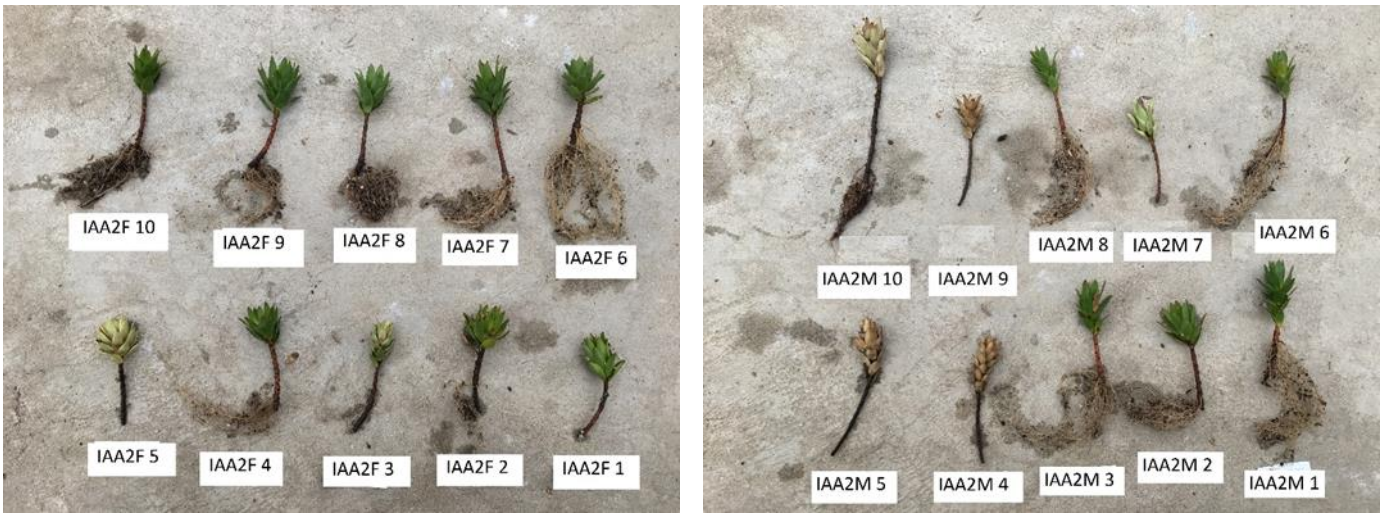

Figure 3.8 Female cuttings (left) treated with IAA 2000 ppm show healthy roots and green leaves in 7 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with IAA 2000 ppm show healthy roots with green leaf colour in 5 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

Figure 3.9 Female cuttings (left) treated with IAA 4000 ppm show healthy roots and green leaves in 8 out of the ten cuttings in week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with IAA 4000 ppm show healthy roots with green leaf colour in 7 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

Figure 3.10 Female cuttings (left) treated with IAA 6000 ppm show healthy roots and green leaves in 7 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with IAA 6000 ppm show healthy roots with green leaf colour in 2 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

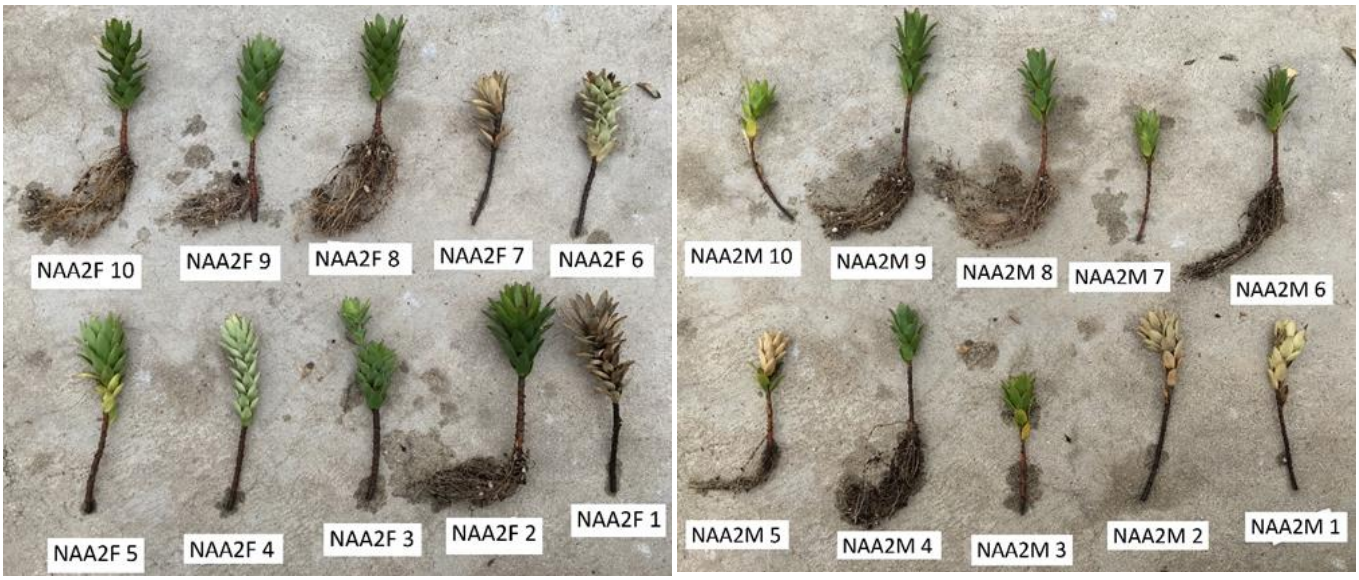

Figure 3.11 Female cuttings (left) treated with NAA 2000 ppm show healthy roots and green leaves in 4 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with NAA 2000 ppm show healthy roots with green leaf colour in 4 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

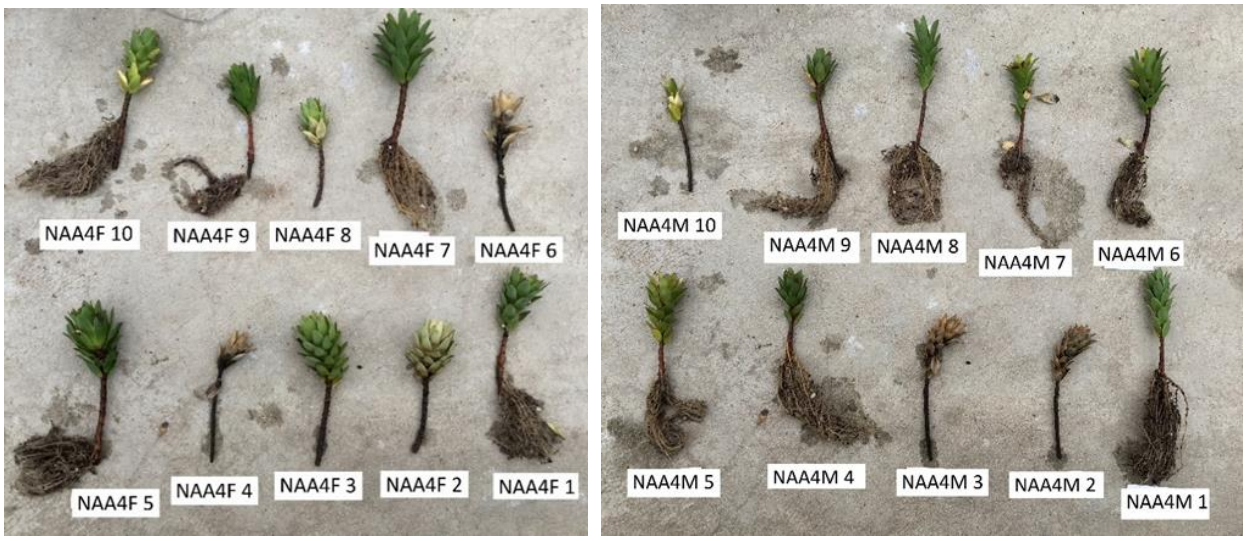

Figure 3.12 Female cuttings (left) treated with NAA 4000 ppm show healthy roots and green leaves in 5 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with NAA 4000 ppm show healthy roots with green leaf colour in 7 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

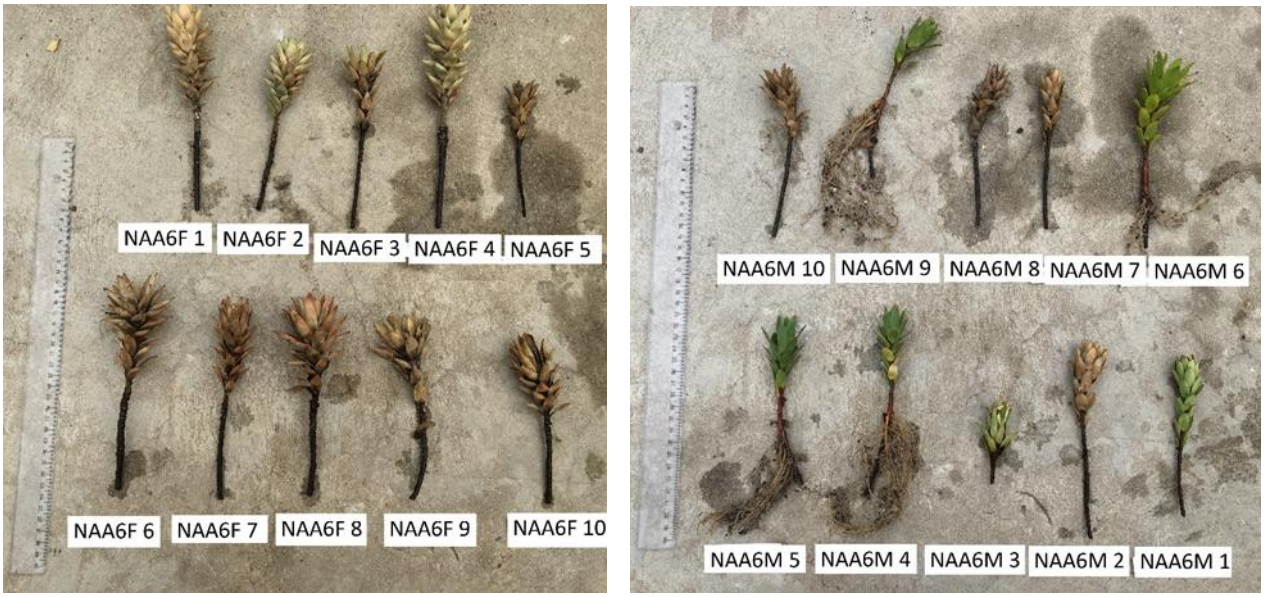

Figure 3.13 Female cuttings (left) treated with NAA 6000 ppm show healthy roots and green leaves in 0 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with NAA 6000 ppm show healthy roots with green leaf colour in 4 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

Figure 3.14 Female cuttings (left) treated with IBA 2000 ppm show healthy roots and green leaves in 7 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with IBA 2000 ppm show healthy roots with green leaf colour in 6 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

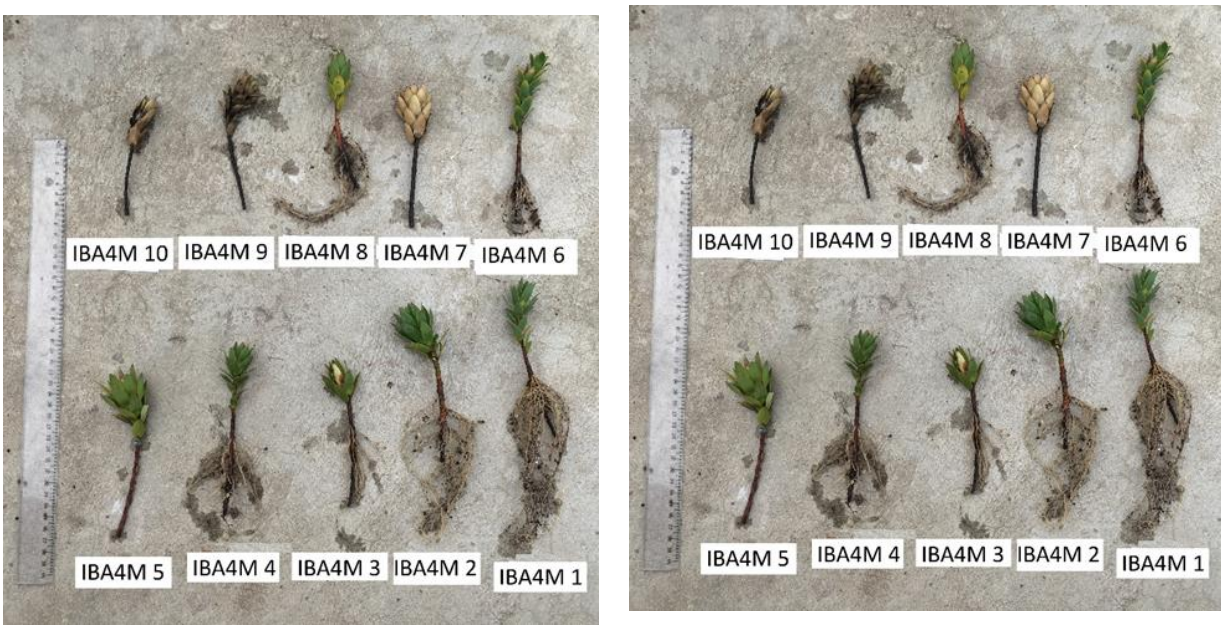

Figure 3.15 Female cuttings (left) treated with IBA 4000 ppm show healthy roots and green leaves in 2 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with IBA 4000 ppm show healthy roots with green leaf colour in 6 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

Figure 3.16 Female cuttings (left) treated with IBA 6000 ppm show healthy roots and green leaves in 1 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) treated with IBA 600pp00 ppm show healthy roots with green leaf colour in 6 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense.*

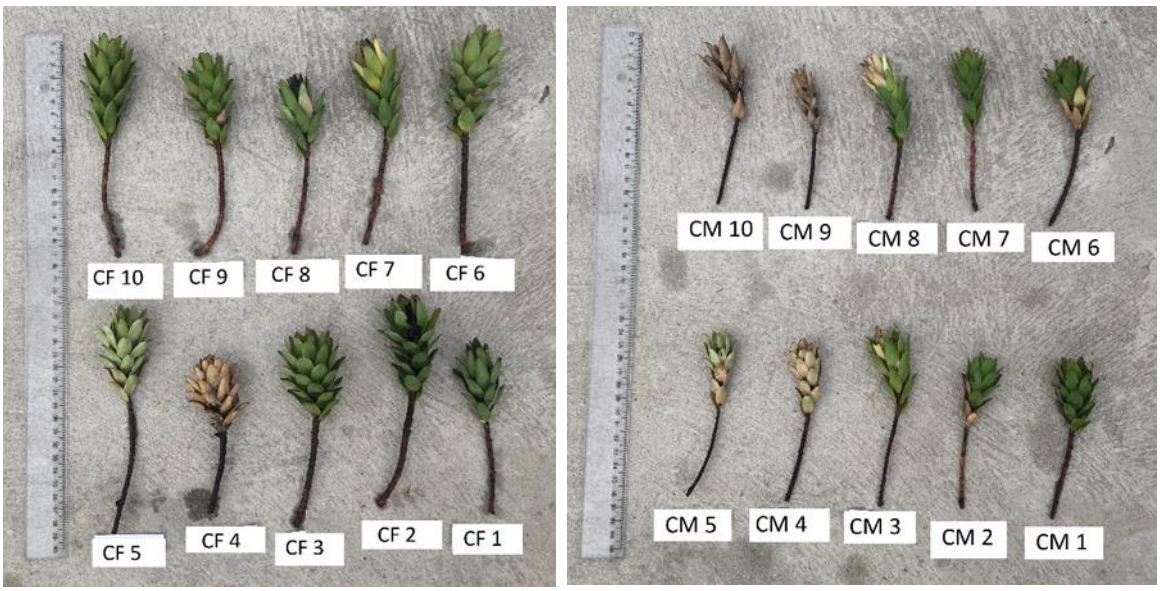

Figure 3.17 Female cuttings (left) not treated show healthy green leaves in 7 out of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*. Male cuttings (right) show healthy green leaf colour in 3 of the 10 cuttings at week 18 of *L. elimense* subsp. *elimense*.

Table 3.1

Mean squares from the analysis of variance for the effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the rooting success of female and male cuttings of *L. elimense* subsp. *elimense*.

Treatment			**Rooting Success (N=10)**	
Code	**Auxin**	**Gender**	**Mean**	**Standard Error + Mean Group**
IAA2F	IAA 2000 ppm	F	7.00	±15.28ab
IAA2M	IAA 2000 ppm	M	6.00	±16.33ab
IAA4F	IAA 4000 ppm	F	8.00	±13.33a
IAA4M	IAA 4000 ppm	M	7.00	±15.28ab
IAA6F	IAA 6000 ppm	F	7.00	±15.28ab
IAA6M	IAA 6000 ppm	M	2.00	±13.33cde
NAA2F	NAA 2000 ppm	F	5.00	±16.67abc
NAA2M	NAA 2000 ppm	M	5.00	±16.67abc
NAA4F	NAA 4000 ppm	F	6.00	±16.33ab
NAA4M	NAA 4000 ppm	M	7.00	±15.28ab
NAA6F	NAA 6000 ppm	F	0.00	±0e
NAA6M	NAA 6000 ppm	M	4.00	±16.33bcd
IBA2F	IBA 2000 ppm	F	7.00	±15.28ab
IBA2M	IBA 2000 ppm	M	6.00	±16.33ab
IBA4F	IBA 4000 ppm	F	2.00	±13.33cde
IBA4M	IBA 4000 ppm	M	6.00	±16.33ab
IBA6F	IBA 6000 ppm	F	1.00	±10de
IBA6M	IBA 6000 ppm	M	7.00	±15.28ab
CF	Control female	F	0.00	±0e
CM	Control male	M	0.00	±0e
Two-way ANOVA F-Statistics				
Auxin			5.4419***	
Gender			1.2493ns	
Auxin*Gender			2.6091**	

Mean values ± Standard Error are shown in columns. The mean values followed by different letters are significantly different at P ≤0.01 (**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. IAAF=Indole acetic acid female cutting, IAAM=Indole acetic acid male cutting, NAAF=1-Naphthaleneacetic acid female cutting, NAAM=1-Naphthaleneacetic acid male cutting, IBAF=Indole butyric acid female cutting, IBAM=Indole butyric acid male cutting, CF= control female cutting, CM=control male cutting.

3.4.3 Root length

Effects of auxin treatments

Root length results of *L. elimense* subsp. *elimense* showed a highly significant difference (P≤ 0.001) in the auxin IAA at 2000, 4000 and 6000 ppm in the female plants (IAA 2F, IAA4F, IAA6F) with a mean value of 103 mm compared to the control (CF) at 0 mm over 18 weeks. The auxin treatments IAA at 2000 ppm in female cuttings showed the highest results for the root length of 103 mm, for male cuttings the best results were with the auxin NAA at 4000 ppm with a root length mean of 101.12 mm (Table 3.2). When comparing the results with the rooting percentage and number of roots it can be seen that IAA at 2000 and IAA at 4000 ppm had significant results for female cuttings as well as NAA at 4000 ppm in the male cuttings compared to the control.

Comparisons between the female and male gender cuttings

The effects of auxin treatments were not significant (ns) on the root length of female and male gender cuttings. Female cuttings of *L. elimense* subsp. *elimense* treated with IAA at 2000 ppm, IAA at 4000 ppm and IAA at 6000 ppm showed the highest root length measurements in female cuttings, while the effects of the auxin NAA at 4000 ppm and IBA at 2000 ppm on the male cuttings proved more successful (Table 3.2).

Interactions between auxin and gender

Over 18 weeks, the effects of auxin treatments in conjunction with gender on root length of *L. elimense* subsp. *elimense* cuttings was significantly different at P ≤0.05 compared to the control (CF/CM). The highest individual mean 103 mm for root length was observed in treatment IAA4F (see Table 3.2 and Figure 3.11) for *L. elimense* subsp. *elimense*. The combination of the 2000 ppm IAA and IAA 4000 ppm and the female cuttings proved to be more successful in root length compared to the control and the male cuttings in the experiment (Table 3.1).

Table 3.2

Mean squares from the analysis of variance for the effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the root length of female and male cuttings of *L. elimense* subsp. *elimense.*

Treatment			**Root length (mm)**	
Code	**Auxin**	**Gender**	**Mean**	**Standard Error + Mean Group**
IAA2F	IAA 2000 ppm	F	103.00	±23.11a
IAA2M	IAA 2000 ppm	M	85.40	±24.18a
IAA4F	IAA 4000 ppm	F	96.30	±16.37a
IAA4M	IAA 4000 ppm	M	92.90	±21.39a
IAA6F	IAA 6000 ppm	F	91.00	±20.44a
IAA6M	IAA 6000 ppm	M	24.20	±16.74cd
NAA2F	NAA 2000 ppm	F	49.80	±20.45abcd
NAA2M	NAA 2000 ppm	M	69.10	±23.33abc
NAA4F	NAA 4000 ppm	F	70.10	±23.22abc
NAA4M	NAA 4000 ppm	M	101.20	±22.22a
NAA6F	NAA 6000 ppm	F	0.00	±0d
NAA6M	NAA 6000 ppm	M	61.00	±25.34abc
IBA2F	IBA 2000 ppm	F	90.00	±20.49a
IBA2M	IBA 2000 ppm	M	82.90	±22.68ab
IBA4F	IBA 4000 ppm	F	29.40	±19.71bcd
IBA4M	IBA 4000 ppm	M	82.80	±24.59ab
IBA6F	IBA 6000 ppm	F	16.40	±16.4cd
IBA6M	IBA 6000 ppm	M	84.30	±25.41ab
CF	Control female	F	0.00	±0d
CM	Control male	M	0.00	±0d
Two-way ANOVA F-Statistics				
Auxin			4.5906***	
Gender			2.3668ns	
Auxin*Gender			2.1408*	

Mean values ± Standard Error are shown in columns. The mean values followed by different letters are significantly different at P ≤0.1 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. IAAF=Indole acetic acid female cutting, IAAM=Indole acetic acid male cutting, NAAF=1-Naphthaleneacetic acid female cutting, NAAM=1-Naphthaleneacetic acid male cutting, IBAF=Indole butyric acid female cutting, IBAM=Indole butyric acid male cutting, CF= control female cutting, CM=control male cutting.

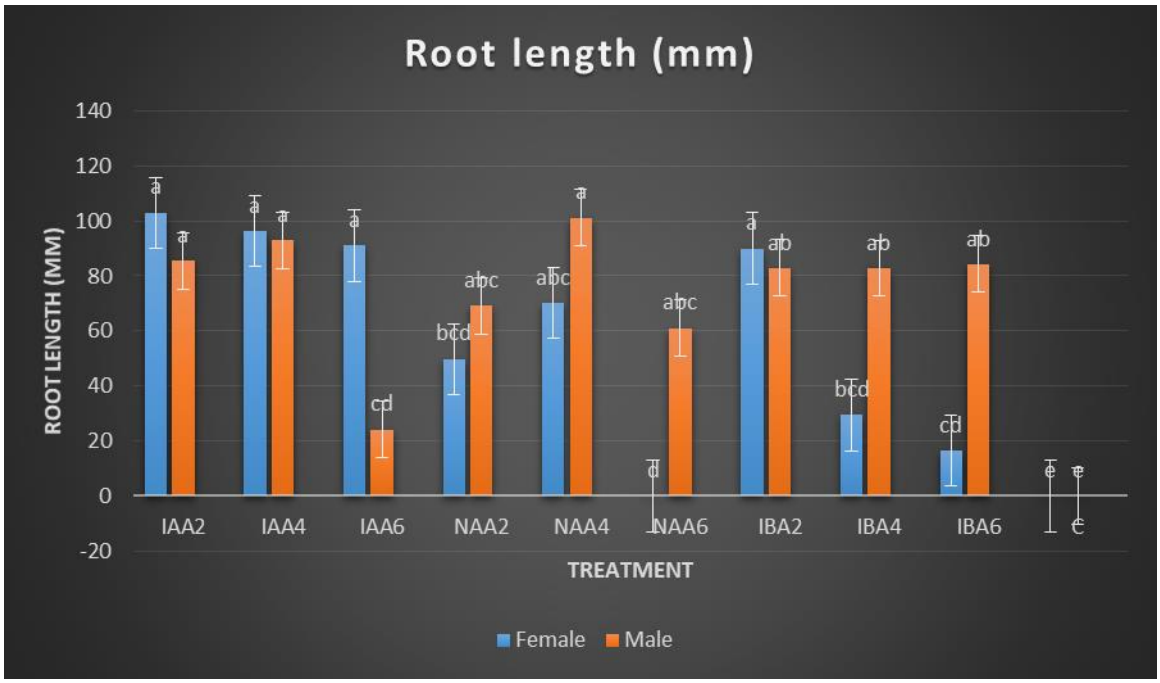

Figure 3.18 The effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the root length of female and male cuttings of *L. elimense* subsp. *elimense.*

3.4.4 Number of roots

Effects of auxin treatments

Number of roots results of *L. elimense* subsp. *elimense* showed a highly significant difference (P≤ 0.001) in the auxin IAA at 4000 ppm in the female plants (IAA4F) with a mean value of 31.3 compared to the control (CF) at 0 mm over 18 weeks. The auxin treatments IAA at 4000 ppm in female cuttings showed the highest results for the number of roots of 31.3, for male cuttings the best results were with the auxin NAA at 4000 ppm and IBA 2000 ppm with a root number mean of 17.8 and 17.4 (Table 3.3). When comparing the results with the rooting percentage and root length it can be seen that IAA at 2000 and IAA at 4000 ppm had the most successful results for female cuttings as well as NAA at 4000 ppm in the male cuttings compared to the control.

Comparisons between the female and male gender cuttings

The effects of auxin treatments were not significant (ns) on the number of roots of female and male gender cuttings. Female cuttings of *L. elimense* subsp. *elimense* treated with IAA at 4000 ppm showed the highest root number measurements in

female cuttings, while the effects of the auxin NAA at 4000 ppm on the male cuttings proved more successful. (Table 3.3).

Interactions between auxin and gender

Over 18 weeks, the effects of auxin treatments in conjunction with gender on root length of *L. elimense* subsp. *elimense* cuttings was significant difference at P ≤0.05 compared to the control (CF/CM). The highest individual mean 31.3 for number of roots was observed in treatment IAA4F (see Table 3.3 and Figure 3.11) for *L. elimense* subsp. *elimense*. The auxin IAA 4000 ppm and the female cuttings proved to be more successful in root length compared to the control and the male cuttings in the experiment (Table 3.1).

Table 3.3 Mean squares from the analysis of variance for the effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the root number of female and male cuttings of *L. elimense* subsp. *elimense*.

Treatment			**Root Number**	
Code	**Auxin**	**Gender**	**Mean**	**Standard Error + Mean Group**
IAA2F	IAA 2000 ppm	F	16.10	±4.04bcde
IAA2M	IAA 2000 ppm	M	13.30	±3.82bcdef
IAA4F	IAA 4000 ppm	F	31.30	±5.83a
IAA4M	IAA 4000 ppm	M	16.70	±3.77bcd
IAA6F	IAA 6000 ppm	F	23.10	±6.30abc
IAA6M	IAA 6000 ppm	M	2.00	±1.37ef
NAA2F	NAA 2000 ppm	F	15.60	±6.62bcde
NAA2M	NAA 2000 ppm	M	15.00	±5.58bcde
NAA4F	NAA 4000 ppm	F	25.40	±13.61ab
NAA4M	NAA 4000 ppm	M	17.80	±4.83abcd
NAA6F	NAA 6000 ppm	F	0.00	±0f
NAA6M	NAA 6000 ppm	M	4.80	±2.15def
IBA2F	IBA 2000 ppm	F	20.30	±5.28abc
IBA2M	IBA 2000 ppm	M	17.40	±5.78abcd
IBA4F	IBA 4000 ppm	F	2.00	±1.46ef
IBA4M	IBA 4000 ppm	M	14.40	±4.20bcde
IBA6F	IBA 6000 ppm	F	5.90	±5.9def
IBA6M	IBA 6000 ppm	M	8.80	±2.31cdef
CF	Control	F	0.00	±0f
CM	Control	M	0.00	±0f
Two-way ANOVA F-Statistics				
Auxin			4.7899***	
Gender			1.6416ns	
Auxin*Gender			1.7416*	

Mean values ± Standard Error are shown in columns. The mean values followed by different letters are significantly different at P ≤0.1 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. IAAF=Indole acetic acid female cutting, IAAM=Indole acetic acid male cutting, NAAF=1-Naphthaleneacetic acid female cutting, NAAM=1-Naphthaleneacetic acid male cutting, IBAF=Indole butyric acid female cutting, IBAM=Indole butyric acid male cutting, CF= control female cutting, CM=control male cutting.

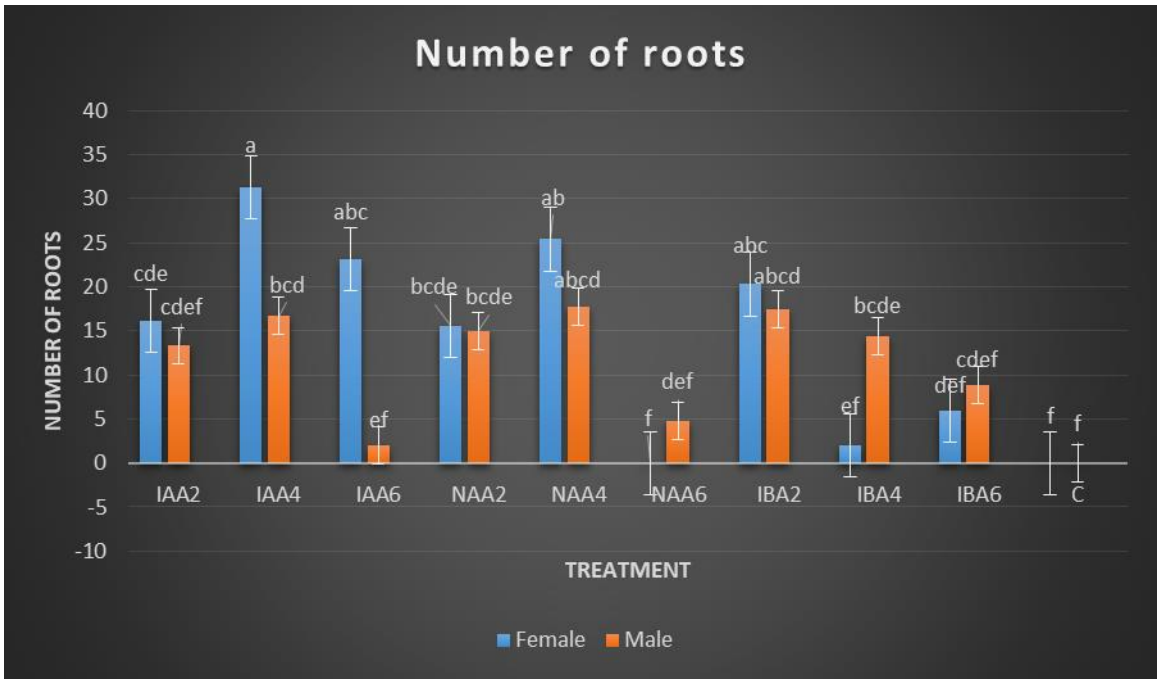

Figure 3.19 The effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the root number of female and male cuttings of *L. elimense* subsp. *elimense*.

3.5 Discussion

Callusing

Callusing was significant in the IAA 4000 ppm treatment on female cuttings. This plant auxin at this concentration proved to encourage parenchyma cells to differentiate to promote callus formation of *L. elimense* subsp. *elimense* and concur with results of callusing reported by Laubscher and Ndakidemi (2008a) when tested on *L. laxum*. Both the environment and watering contributed to the success of callusing of the cuttings which allowed free air movement and kept cuttings cool and preventing overwatering.

Rooting

The results for using IAA at 4000 ppm remained consistently in combination with the female cuttings to prove the most significant results in rooting of *L. elimense* subsp. *elimense*. These results agree with Laubscher and Ndakidemi (2008a) who reported significant callusing with 4000 ppm of IAA on *L. laxum*, while Hartmann *et al*. (2002) reported the benefits of IAA for rooting purposes. According to Gouws *et al*. (2015) auxins such as IAA are absorbed within the first seconds of application through the basal cut surface of the cutting. It is speculated that the correct strength of auxin is absorbed while depending on species rooting time is regulated for easy to difficulty to

root species as in this study for significant rooting to happen over an extended period. Proteaceae is seen as a difficult to root species compared to several other fynbos species. Ludwig-Müller (2003) investigated rooting potential in relation to peroxidase activity where difficult to root Protea showed a higher peroxidase (POX) activity in the leaves and middle stem part and although there was no difference in the uptake of IAA, the auxin was faster taken up in easier rooted species while being exposed over a longer period of time. This could possibly explain why callusing of *L. elimense* subsp. *elimense* was slow but improved over an extended rooting period of 16 to 18 weeks.

In this study the male gender cuttings showed a higher success rate in the NAA auxin treatments, however the results showed that NAA was not significant in rooting. These results concur with earlier reports by Perry and Trueman (1999) in rooting *Conospermum mitchellii* that NAA should be avoided to prevent death in cuttings. Similar results were confirmed by Laubscher & Ndakidemi (2008b) when *L. laxum* was tested for vegetative rooting. Gouws *et al*. (2015) reported that NAA resulted in tissue die-back at the basal cut surface of *Protea* species. Results from this study showed that NAA is not suitable for rooting of *L. elimense* subsp. *elimense* and should preferably be avoided. A possible concentration strength applied could be sufficient for root development, however over applications especially powder preparations can cause burning of the parenchyma tissue with delay in rooting and or death of the cutting (McLennan, 1993).

IBA has been reported as the most popular auxin used for general cutting propagation with various degrees of success documented in Proteaceae. This study however found that IBA was not significant in rooting *L. elimense* subsp. *elimense* and therefore remain in contrast with several other studies which recommended that a concentration of 0.1% IBA will stimulate root growth more specifically a concentration of 2000 ppm is recommended for *Leucadendron* species (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). Although most successful results for cone bushes were reported by Ben-Jaacov and Silber (2006) using 2000 ppm of IBA and later confirmed by Laubscher & Ndakidemi (2008b) with 1000 to 2000 ppm IBA to be most significant in rooting for *L. laxum*. They also confirmed that higher concentrations of 4000 ppm inhibit rooting in cuttings. Rodríguez-Pérez *et al.* (2003) reported after 8 weeks experimentation with IBA at 4000 ppm cuttings showed satisfactory results, while IBA at 2000 ppm showed the best results after 20 weeks. Peña-Baracaldo *et al.* (2018) discovered that IBA had the best results and according to Ben-Jaacov *et al.* (1986) the auxin IBA at 2000 ppm should be used for best results in *Leucadendron* species.

As several studies indicate that IBA at 4000 ppm or 2000 ppm being the better option for *Leucadendron* species, this study found that IBA at 4000 ppm to be more superior but showed no significance in rooting of *L. elimense* subsp. *elimense*.

Number of roots and rooting length

As can be seen from the above results IAA at 4000 ppm was consistently under the best results for the female cuttings. These results support Laubscher & Ndakidemi (2008a) who observed significant difference in the root length and number of roots with 4000 ppm of IAA. The male cuttings did not show consistent results, the only certain result was that NAA was the best auxin for the male cuttings, as it showed good results in three of the four categories. In previous research by Laubscher & Ndakidemi (2008b) it was shown that IBA at concentrations of 4000 ppm and higher supressed rooting, similar results were shown with all auxins used in the above experiment.

The results do not agree with Rodríguez-Pérez *et al.* (2009) who found that IBA at 4000 ppm gave best rooting results for the propagation of Protea Hybrid 'Susara'. According to Pérez-Francés *et al.* (2001) the cuttings of *L. discolor* and *L*. 'Safari Sunset' should be treated with 4000 ppm of IBA for best results. Rodríguez-Pérez *et al.* (2001) found that 4000 ppm of IBA showed best results compared to 2000 ppm IBA. On the other hand, Laubscher & Ndakidemi (2008) found that IBA at 1000 ppm showed best results in the propagation of *L. laxum* and Worrall (1976) found that 4000 ppm of IBA gave best rooting results initially, but a delayed toxicity was evident, therefore a pre-treatment of 2000 ppm IBA is recommended.
As reported here there have been several studies on using IBA rooting auxin, however most of these disagree with the concentration and type that should be used. This study is however in agreement with studies which reported success in using IAA at 4000 ppm in rooting *Leucadendron* species and other members of the Proteaceae family.

Due to availability and access to cutting material it was difficult to complete this experiment during autumn as recommended by Malan (2012) and Reinten (2014). The collection and rooting of cuttings for this study which were done during late autumn early winter were highly significant. Although seasons were not compared in the study, it is interesting to note that the collection of cuttings later in the season provided an extended period for cutting propagation of *Leucadendron* species.

3.6 Conclusion and recommendations

This study reported that *L. elimense* subsp. *elimense* can be successfully propagated vegetatively from cuttings using IAA 4000 ppm rooting auxin. These results for this species would greatly contribute to develop a cultivation protocol for *L. elimense* subsp. *elimense* which could aid in saving the species in its natural habitat and support restoration ecology. Additionally, the success could also provide commercial cut flower growers and farmers the opportunity to propagate more female and male plants for their cut flower potential, respectively. The commercial value of this species has further potential for the cut flower industry as much of its potential remain unexplored mainly due to its unavailability of saleable plants. Future propagation of *L. elimense* subsp. *elimense* could be explored with using IAA auxin concentrations for in-vitro culture purposes.

3.7 Acknowledgements

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CHAPTER FOUR:

THE INFLUENCE OF ROOTING AUXINS ON FLOWERING AND CHLOROPHYLL CONTENT OF *LEUCADENDRON ELIMENSE* E.PHILLIPS SUBSP. *ELIMENSE*

THE INFLUENCE OF ROOTING AUXINS ON FLOWERING AND CHLOROPHYLL CONTENT OF *LEUCADENDRON ELIMENSE* E.PHILLIPS SUBSP. *ELIMENSE*

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4.1 Abstract

Flowering control in Proteaceae could be regulated by auxin applications to advanced flowering pot plant production. Three auxins Indole-3-acetic acid (IAA), 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA) at different concentrations of 2000 ppm, 4000 ppm and 6000 ppm were tested during *L. elimense* subsp*. elimense* female and male cuttings to measure flowering percentage, chlorophyll content and wet weights. Simultaneously essential misting, bottom heat and natural ventilation was supplied under semi-controlled greenhouse conditions. The wet weight and chlorophyll content increases showed significant results with a significant increase in flowering percentage of female cuttings using IAA at 4000 ppm while NAA was more successful on the male cuttings. The flowering results provided new evidence for both female and male plants of the species with consistency between chlorophyll content and weight to increase flowering percentage. These results provide relevance in cultivating *L. elimense* subsp*. elimense* as a flowering potted plant or cut flower for the ornamental pot plant and cut flower industry where both female cones and male flowers have already gained popularity. Additionally, the results provided new evidence for an endemic and endangered species to be cultivated and conserved in a declining habit.

Keywords: commercial potential, cone bush, cut flowers, Proteaceae

4.2 Introduction

It is well documented that auxin play an important part as growth regulator in many plant growth development stages (Benkova *et al.,* 2003; Reinhardt *et al.,* 2000; Krizek, 2011). While most experimental studies on Proteaceae have involved testing auxin in rooting applications for propagation purposes, auxin regulation in flower development of *Leucadendron* is relatively unknown. According to Cheng and Zhao, (2007) and Sundberg and Ostergaard, 2009) auxins specify the site of flower initiation which could continue to regulate plant organ growth patterns in future reproduction

phases. Krizek (2011) stated that auxin distribution in particular around the area of the inflorescence development as well as auxin distribution levels in plants can vary greatly within different plant regions. These auxins are able to specify the site for floral meristem initiation at the tips of developing flower organ primordia, however auxin accumulation patterns in flowers are not well understood (Krizek, 2011). According to Hartmann *et al.* (2002) auxins such as Indole-3-acetic acid (IAA) ($C_{10}H_9O_2$), 1-Naphthaleneacetic acid (NAA) ($C_{12}H_{10}O_2$) and Indole-3-butyric acid (IBA) ($C_{12}H_{13}O_2$) are involved in various growth function in plants such as stem and root growth, bud inhibition and dormancy conditions. Some of these auxins have been proved successful in root initiation, however few studies have engaged in their function of flowering in Proteaceae species. Additionally, it has been observed that rooted cuttings often show flower initiation after rooting. The aim of this study was therefore to document the influence of rooting auxins on flower and chlorophyll content of *Leucadendron elimense* E.Phillips subsp. *elimense*.

4.2.1 *Leucadendron* species as cut flowers and flowering potted plants

The Proteaceae family has 1400 species of which 330 are found in the Western Cape in South Africa with around 60 species of *Leucadendron* cone bushes (Brown, 1999; Leonhardt & Criley, 1999; Paterson-Jones, 2000; Tansley & Brown, 2000; Walker, 2018; Baessler, 2019). The name *Leucadendron* comes from the silvertree (*Leucadendron argenteum*) as it is descriptive of the species the word '*Leuca'* means white and '*dendron'* means tree (Carolus, 2008; Nurrish, 2010). *Leucadendron* species are dioecious, the female flowers produce a woody seed head, known as a cone with spirally arranged floral bracts (Lamont *et al.,* 1985; Leonhardt & Criley, 1999; Rhode, 2004; Ben-Jaacov & Silber, 2006). The male flowers only last two weeks and are inconspicuous, the plants flower from June to September and they produce the first flowers after three to four years (Leonhardt & Criley, 1999; Rhode, 2004). In general, male plants are more branched, slightly larger and have smaller leaves and a flower head (Ben-Jaacov & Silber, 2006). *Leucadendrons* are adapted to the fynbos biome and all but three species can be found in the Cape Floral Region (Ben-Jaacov & Silber, 2006).

Plants in the genus *Leucadendron* have long-lasting stems, colourful foliage and flowers, which makes them useful for the cut flower industry, the interest of *Leucadendron* spp. as cut flowers has already been there since the middle of the 18th century (Leonhardt & Criley, 1999; Reinten *et al*., 2011; Rhode, 2004). They can also be used in the landscaping industry, as potted plants or as dried products (Leonhardt

& Criley, 1999; Rhode, 2004). They have colourful foliage, for example green, red, orange, gold, silver, bright yellow or soft yellow (Rhode, 2004; Ben-Jaacov & Silber, 2006). Female plants are in higher demand for their long vase life and because they can be used in the dry flower industry, as well as for flower bouquets as a filler (Rhode, 2004; Ben-Jaacov & Silber, 2006). The male plants are desirable for their flowers, because they are white-silver, red or yellow, but only have a short market season of two to three weeks (Rhode, 2004; Ben-Jaacov & Silber, 2006). Annually 100 million cut stems are produced around the world, of those, half are *Leucadendron* spp. (Ben-Jaacov & Silber, 2006). *Leucadendrons* are popular on the Dutch market, because they are the easiest to adapt and can be propagated easily and grow vigorously (Rhode, 2004). There are 91 different species and subspecies of *Leudadendron*, but only 22 are used commercially (Rhode, 2004). The cultivars mainly used are late winter to early spring flowering (Rhode, 2004). *Leucadendron* 'Safari Sunset' comes from New Zealand, the most popular hybrid of which 90% are produced in Israel and has been on the market for over forty years (Ben-Jaacov & Silber, 2006). As more species and cultivars are in demand by a commercial market improved cultivation techniques are lacking for some.

Figure 4.1 Variation and versatility of *Leucadendron* foliage colours in *L. salignum*, *L. salignum* 'Silvan Red' and *L. argenteum.*
(Source: Waters, 2017 (left), van Riet, 2019 (middle) and Notten & van der Walt, 2008, (right).

4.2.2 Flowering and commercial importance of *L. elimense* subsp. *elimense*

L. elimense subsp. *elimense* is an endemic species to Elim on the Agulhas Plain at the most southern tip of Africa and is part of the Cape Floristic Region. This species is a single-stemmed, sparsely branched shrub, up to 1.5 m high. The leaves are elliptical: on the male plant, 13-49 x 5-19 mm and on the female plant, 14-57 x 7-21

mm; hairless, closely spaced with a narrow, firm and flexible margins. The involucral leaves (a rim of leaves surrounding the flower head), are yellow, crowded, and overlap the base of the flower head (Mustard, *et al.,* 1997; Carolus, 2008).

The male flowers are yellow, fluffy, 19 mm long and up to 46 mm across, depressed, and globular shaped while the female flowers are 20 mm wide and closely packed to the upper surface, with the sides closely overlapped by recurved involucral bracts and leaves. Both sexes have an unattractive pungent smell (Carolus, 2008).

The female flowers are responsible to develop into globular shaped cones which are 35 mm in diameter with ovate shaped bracts with some hairs (Carolus, 2008). The flowers are possibly insect pollinated as several insects are located in the flower heads while the flowers secrete nectar and a pungent odour from both sex flowers.

Several *Leucadendron* species have become part of plant breeding programs to enhance their importance as commercial cut flowers. In Western Australia, university research support has been provided for *Leucadendron* breeding projects to manage the commercialisation of new varieties intellectual property and their distributions where more than 500 interspecific cross combinations involving 140 genotypes of 27 species were evaluated for Australian conditions (Sedgley *et al.,* 2006). By 2005, this breeding protocol has delivered 200 potential new varieties with 8 released to the growing industry. Due to breeding cost the cooperation of international participants were sourced to enhance and maintain the breeding program (Sedgley *et al.,* 2006). In South Africa, the male flowers are preferred as export cut flower while female cones are more in demand. Considering the economic potential of *L. elimense* subsp. *elimense* further studies are necessary to evaluate enhanced flowering potential of this species.

4.3 Materials and methods

4.3.1 Greenhouse Experiment and environment

The experiment was conducted from the 23 May until 24 September 2019 in the propagation greenhouse on the Cape Peninsula University of Technology, Bellville campus. This part of the greenhouse is a semi-controlled environment. The sides of the greenhouse are made of shade netting to allow for natural air flow and moisture penetrating the building, however the roof prevented rain from above. Temperatures ranged between 15 and 26 °C while the relative humidity (RH) ranged between 90 to 96% as reported by Ben-Jaacov and Silber (2006); Brown and Duncan (2006), and Malan (2012). During the rooting period plant bags were placed on hot beds with a temperature of 22 °C and received intermitted mist irrigation alternating every 40 minutes for 21 seconds (Eliovson, 1965; McLennan, 1993; Brown & Duncan, 2006;

Malan, 2012; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a; ACS Distance Education, 2019).

The amount of water received per interval of irrigation measured 500 ml during the rooting stage. Once the cuttings had rooted, they were moved to a hardening off section where they received a mist spray every 50 minutes for 18 seconds, the amount of water received per interval of irrigation measured 150 ml.

4.3.2 Plant material collection and preparation

The plant material of *L. elimense* subsp. *elimense* was collected with permission on the land of the Elim church a German Mission Station in the Western Cape Province of South Africa (Schoeman & Visagie, 2014). To ensure sustainable harvesting, the plant material was collected from randomly selected, different plants with cuttings taken from 50 males and 50 female plants. The plant material was stored in moist newspaper overnight to ensure they do not dry out. The following morning the cuttings were taken to the Cape Peninsula University of Technology Bellville campus, South Africa at GPS co-ordinates - 33º 55'45.53S, 18º 38' 31. 16E. The terminal cuttings were cut into uniform lengths of 15 cm for female plants and 10 cm for male plants. The bottom 1/3 of the leaves were removed and the treatment was administered. These methodologies were recommended for collection of cuttings during the early hours in the morning to avoid heat stress, should be of terminal or sub-terminal with a length of 12 cm with a diameter of 8 mm and the lower half of the leaves removed (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). The cuttings were taken just below the node with a straight cut. The cuttings were then sprayed with Captab at 4 g/l. Two hundred terminal cuttings were then planted into perforated, transparent, bags 125 x 190 mm, obtained from Packit 354 Voortrekker Road, Maitland, Cape Town, Western Cape, with a mix of two parts coarse river sand (2 mm diameter), one-part coco peat and one-part perlite (2:1:1) (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). The rooting medium should preferably be a well-draining, acidic medium which has not been fertilised (Reinten, 2014). The cuttings should be one third deep in the medium and have good aeration (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018). The plants were then placed on the hot beds in the greenhouse and watered thoroughly.

4.3.3 Experimental treatments

The treatments consisted of three different rooting auxins, namely IAA, NAA and IBA at concentrations of 2000 ppm, 4000 ppm and 6000 ppm. The treatments were laid out in a randomised block design with ten males and ten female plants to allow for 10 repetitions for each treatment in male and female plants. The auxins were ordered from Sigma Aldrich, Unit 4 Aviation Park, 17 Pomona Road, Kempton Park. Treatments were done with cuttings dipped in the liquid for ten seconds at a depth of one to two centimetres (McLennan, 1993). Rooting auxins IAA, NAA and IBA, at different strengths were prepared by using 0.5 g in 250 ml of water, 1 g in 250 ml of water and 1.5 g in 250 ml of water to make the concentrations. Repetitions were labelled accordingly:

IAA2F = Indole Acetic Acid at 2000 ppm on cuttings of female plants
IAA2M = Indole Acetic Acid at 2000 ppm on cuttings of male plants
IAA4F = Indole Acetic Acid at 4000 ppm on cuttings of female plants
IAA4M = Indole Acetic Acid at 4000 ppm on cuttings of male plants
IAA6F = Indole Acetic Acid at 6000 ppm on cuttings of female plants
IAA6M =Indole Acetic Acid at 6000 ppm on cuttings of male plants
NAA2F = 1-Naphthaleneacetic acid at 2000 ppm on cuttings of female plants
NAA2M = 1-Naphthaleneacetic acid at 2000 ppm on cuttings of male plants
NAA4F= 1-Naphthaleneacetic acid at 4000 ppm on cuttings of female plants
NAA4M = 1-Naphthaleneacetic acid at 4000 ppm on cuttings of male plants
NAA6F = 1-Naphthaleneacetic acid at 6000 ppm on cuttings of female plants
NAA6M = 1-Naphthaleneacetic acid at 6000 ppm on cuttings of male plants
IBA2F = Indole-3-butyric acid at 2000 ppm on cuttings of female plants
IBA2M = Indole-3-butyric acid at 2000 ppm on cuttings of male plants
IBA4F = Indole-3-butyric acid at 4000 ppm on cuttings of female plants
IBA4M = Indole-3-butyric acid at 4000 ppm on cuttings of male plants
IBA6F = Indole-3-butyric acid at 6000 ppm on cuttings of female plants
IBA6M = Indole-3-butyric acid at 6000 ppm on cuttings of male plants
CF = Control no auxin treatment on cuttings of female plants
CM = Control no auxin treatment on cuttings of male plants

4.3.4 Care during rooting period of cuttings

Cuttings were continuously monitored for stress of overwatering and treated weekly with a preventative fungicide spray (Eliovson, 1965; McLennan, 1993; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018;

ACS Distance Education, 2019). A ratio 1:1 of a mixture of Captab (4 g/l) and Kelpak (5 ml/l) was sprayed on the cuttings at 12 pm. Overwatering, signs of blackening on leaves and stems and rotting were monitored during the rooting period and yellow and dead leaves were removed from cuttings.

4.3.5 Care during hardening off and growing period

On 20 August 2019 (Week 13) the cuttings were moved to the hardening off section, where they received 150 ml of water via mist sprayers every 50 minutes for 18 seconds. The plants were sprayed weekly with a mixture of Captab (4 g/l) and Kelpak (5 ml/l).

4.3.6 Determination of plant growth

On the 24 September 2019 (Week 18) the cuttings were removed from the growing medium; the growing media was carefully rinsed off the roots and measurements were done.

4.3.6.1 Wet weight

To determine the wet weight of the cuttings, each cutting was weighed before planting, and after the growing period with a standard laboratory balance, to determine if the cuttings have increased in weight.

4.3.6.2 Flowering percentage

To determine the number of flowering cuttings, the number of flowers and flower buds were counted and recorded of each cutting.

4.3.6.3 Chlorophyll content

The amount of chlorophyll in the leaves was measured with the CCM-300 chlorophyll meter.

4.3.7 Statistical analysis

The data was analysed by using two-way analysis of variance (ANOVA), with the computing software program STATISTICA 13. The occurrence of statistical difference was determined by using the Fisher Least Significance Difference (L.S.D.) at values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ levels of significance (Steel & Torrie, 1980).

4.4 Results

The experiment was conducted to determine which rooting auxin at what concentration would give the best results. Results of the rooting auxins showed statistically significant variance at P≤ 0.001 in all variables. There was no statistical significance between the male and female plants except in the wet weight.

4.4.1 Wet weight before planting

Effects of auxin treatments

The female cutting treated with the auxin NAA at 6000 ppm showed significant difference (P≤ 0.001) compared to the control before planting. The means varied between 14.83 g and 4.01 g. By the male cuttings NAA at 4000 ppm showed superior results for the wet weight before planting with 5.84 g. The auxin with the best result before planting was NAA at 6000 ppm in the female cuttings with a weight of 14.83 g (Table 4.1).

Comparisons between the female and male gender cuttings

There was significant difference (P≤ 0.001) observed with the genders before planting, because the female cuttings weighed more than the males (Table 4.1).

Interactions between auxin and gender

There was significant difference (P≤ 0.001) observed with the auxin and gender before planting, the female cuttings weighed more than the male cuttings (Table 4.1 and Figure 4.1).

Effects of auxin treatments

The female cutting treated with the auxin IAA at 4000 ppm showed significant difference (P≤ 0.001) compared to the control after harvesting. The means after harvesting varied between 23.58 g and 3.82 g. By the male cuttings NAA at 4000 ppm had the best results for the wet weight after harvesting with 10.09 g. The auxin with the best result was IAA at 4000 ppm in the female cutting with a wet weight of 23.58 g (Table 4.1 and Figure 4.1).

Comparisons between the female and male gender cuttings

There was significant difference (P≤ 0.001) observed with the gender compared to the control, the female cuttings weighed more than the males after harvesting (Table 4.1 and Figure 4.1).

Interactions between auxin and gender

The effects of auxin treatments in conjunction with gender on the wet weight of *L. elimense* subsp. *elimense* cuttings were highly significant at P ≤0.01 compared to the control (CF/CM). The auxin with the best result was IAA at 4000 ppm in the female cutting with a wet weight of 23.58 g (Table 4.1). The combination of the 4000 ppm IAA and the female cuttings proved to be more successful in wet weight than the control and the male cuttings in the experiment.

Table 4.1 Mean squares from the analysis of variance for the effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the wet weight of female and male cuttings of *Leucadendron elimense* subsp. *elimense*.

Treatment			Wet weight before (g)		Wet weight after (g)	
Code	**Auxin**	**Gender**	**Mean**	**Standard Error + Mean Group**	**Mean**	**Standard Error + Mean Group**
IAA2F	IAA 2000 ppm	F	5.53	±0.38gh	8.84	±0.94cdef
IAA2M	IAA 2000 ppm	M	4.28	±0.23gh	6.83	±0.77def
IAA4F	IAA 4000 ppm	F	11.38	±0.94bc	23.58	±6.71a
IAA4M	IAA 4000 ppm	M	5.02	±0.49gh	8.04	±0.81cdef
IAA6F	IAA 6000 ppm	F	10.34	±0.62bc	20.74	±6.94ab
IAA6M	IAA 6000 ppm	M	4.01	±0.27h	3.82	±0.47f
NAA2F	NAA 2000 ppm	F	10.72	±0.55bc	12.33	±2.34cde
NAA2M	NAA 2000 ppm	M	5.27	±0.44gh	8.47	±1.80cdef
NAA4F	NAA 4000 ppm	F	11.83	±1.41b	14.64	±3.04bc
NAA4M	NAA 4000 ppm	M	5.84	±0.51fg	10.09	±1.71cdef
NAA6F	NAA 6000 ppm	F	14.83	±0.72a	6.67	±0.59def
NAA6M	NAA 6000 ppm	M	5.78	±0.46g	5.58	±1.17def
IBA2F	IBA 2000 ppm	F	10.10	±0.89c	12.88	±2.21cd
IBA2M	IBA 2000 ppm	M	5.39	±0.29gh	7.59	±1.11cdef
IBA4F	IBA 4000 ppm	F	8.37	±0.54de	7.93	±1.76cdef
IBA4M	IBA 4000 ppm	M	4.89	±0.55gh	6.25	±0.83def
IBA6F	IBA 6000 ppm	F	7.52	±0.35ef	5.84	±0.98def
IBA6M	IBA 6000 ppm	M	4.15	±0.39gh	5.25	±0.78ef
CF	Control	F	10.00	±0.69cd	7.52	±0.62cdef
CM	Control	M	4.46	±0.33gh	4.00	±0.45f
Two-way ANOVA F-Statistics						
Auxin			12,281***		3.6044***	
Gender			351,316***		23.0439***	
Auxin* Gender			5,935***		2.6583**	

Mean values ± Standard Error are shown in columns. The mean values followed by different letters are significantly different at P ≤0.01 (**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. IAAF=Indole Acetic Acid female cutting, IAAM=Indole Acetic Acid male cutting, NAAF=1-Naphthaleneacetic acid female cutting, NAAM=1-Naphthaleneacetic acid male cutting, IBAF=Indole butyric acid female cutting, IBAM=Indole butyric acid male cutting, CF= control female cutting, CM=control male cutting

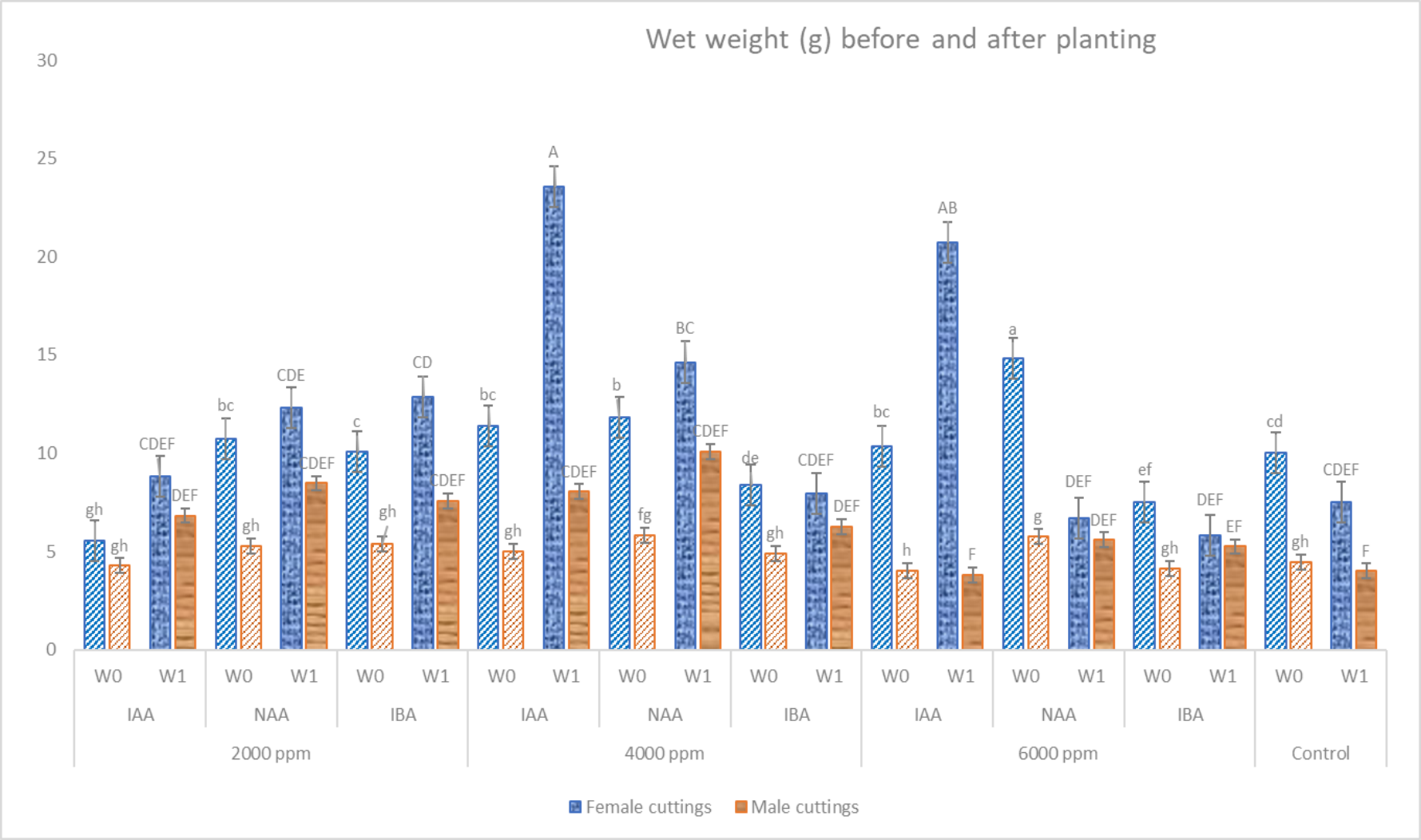

Figure 4.2 Different letters (small letters for W0; capital letters for W1) indicate significantly different mean values at P ≤0.01 (**), P ≤0.001 (***) as calculated by Fisher's least significant difference. IAA=Indole acetic acid; NAA=1-Naphthaleneacetic acid; IBA= Indole butyric acid, W0 = weight before treatment; W1 = weight after treatment.

4.4.2 Flowering percentage

Effects of auxin treatments

The female cutting treated with the auxin IAA at 4000 ppm showed significant difference (P≤ 0.001) compared to the control. The means varied between 70% and 0%. By the male cuttings NAA at 2000 ppm had the highest results for the flowering percentage at 40%. The auxin with the best result was IAA at 4000 ppm in the female plant with a flowering percentage of 70% (Table 4.2).

Comparisons between the female and male gender cuttings

There was no significant difference between the flowering of male and female plants.

Interactions between auxin and gender

There was significant difference (P≤ 0.001) observed with the auxin and gender compared to the control, the male cuttings flowered more than the females (Table 4.2).

Figure 4.3 (left) Flowering female (F) plant (right) flowering male (M) plant

Table 4.2 Mean squares from the analysis of variance for the effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the flowering percentage of female and male cuttings of *Leucadendron elimense* subsp. *elimense*.

Treatment			Flowering Percentage	
Code	**Auxin**	**Gender**	**Mean**	**Standard Error + Mean Group**
IAA2F	IAA 2000 ppm	F	45.00	±15.72ab
IAA2M	IAA 2000 ppm	M	0.00	±0d
IAA4F	IAA 4000 ppm	F	70.00	±15.28a
IAA4M	IAA 4000 ppm	M	20.00	±13.33bcd
IAA6F	IAA 6000 ppm	F	40.00	±16.33b
IAA6M	IAA 6000 ppm	M	0.00	±0d
NAA2F	NAA 2000 ppm	F	20.00	±13.33bcd
NAA2M	NAA 2000 ppm	M	40.00	±6.67b
NAA4F	NAA 4000 ppm	F	10.00	±6.67cd
NAA4M	NAA 4000 ppm	M	30.00	±15.28bc
NAA6F	NAA 6000 ppm	F	0.00	±0d
NAA6M	NAA 6000 ppm	M	10.00	±10cd
IBA2F	IBA 2000 ppm	F	10.00	±6.67cd
IBA2M	IBA 2000 ppm	M	10.00	±6.67cd
IBA4F	IBA 4000 ppm	F	0.00	±0d
IBA4M	IBA 4000 ppm	M	5.00	±5cd
IBA6F	IBA 6000 ppm	F	0.00	±0d
IBA6M	IBA 6000 ppm	M	10.00	±10cd
CF	Control	F	0.00	±0d
CM	Control	M	20.00	±8.16cd
Two-way ANOVA - F-Statistics				
Auxin			3.93231***	
Gender			1.38462ns	
Auxin*Gender			4.49231***	

Mean values ± Standard Error are shown in columns. The mean values followed by different letters are significantly different at P ≤0.01 (**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. IAAF=Indole acetic acid female cutting, IAAM=Indole acetic acid male cutting, NAAF=1-Naphthaleneacetic acid female cutting, NAAM=1-Naphthaleneacetic acid male cutting, IBAF=Indole butyric acid female cutting, IBAM=Indole butyric acid male cutting, CF= control female cutting, CM=control male cutting.

4.4.3 Chlorophyll content

Effects of auxin treatments

The auxin IBA at 2000 ppm showed significant difference (P≤ 0.001) to the control. The means varied between 378.6 mg/m² and 124.9 mg/m². The auxin with the best result was IBA at 2000 ppm in the female cutting with a chlorophyll amount of 378.6 mg/m² (Table 4.3). Whereas IAA 2000 ppm showed best results in the male cuttings.

Comparisons between the female and male gender cuttings

There was no significant difference between the amount of chlorophyll of female and male cuttings (Table 4.3, Figure 4.3).

Interactions between auxin and gender

The effects of auxin treatments in conjunction with gender on callusing of *L. elimense* subsp. *elimense* cuttings were highly significant at P ≤0.01 compared to the control (CF/CM). The combination of the 2000 ppm IAA and the female cuttings proved to be more successful in chlorophyll than the control and the male cuttings in the experiment.

Table 4.3 Mean squares from the analysis of variance for the effect of auxin, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the chlorophyll amount of female and male cuttings of *Leucadendron elimense* subsp. *elimense*.

Treatment			**Chlorophyll (mg/m²)**	
Code	**Auxin**	**Gender**	**Mean**	**Standard Error + Mean Group**
IAA2F	IAA 2000 ppm	F	343.70	±28.05ab
IAA2M	IAA 2000 ppm	M	318.30	±21.15abc
IAA4F	IAA 4000 ppm	F	291.40	±42.71abcd
IAA4M	IAA 4000 ppm	M	309.50	±21.46abc
IAA6F	IAA 6000 ppm	F	334.60	±40.13abc
IAA6M	IAA 6000 ppm	M	156.30	±45.87fg
NAA2F	NAA 2000 ppm	F	286.50	±37.96abcd
NAA2M	NAA 2000 ppm	M	248.80	±44.38bcdef
NAA4F	NAA 4000 ppm	F	337.90	±42.49abc
NAA4M	NAA 4000 ppm	M	274.30	±36.21bcde
NAA6F	NAA 6000 ppm	F	177.30	±38.65efg
NAA6M	NAA 6000 ppm	M	182.90	±44.71efg
IBA2F	IBA 2000 ppm	F	378.60	±16.38a
IBA2M	IBA 2000 ppm	M	262.40	±38.20bcde
IBA4F	IBA 4000 ppm	F	242.90	±29.08cdef
IBA4M	IBA 4000 ppm	M	313.90	±22.28abc
IBA6F	IBA 6000 ppm	F	124.90	±33.13g
IBA6M	IBA 6000 ppm	M	204.70	±27.65defg
CF	Control	F	285.90	±34.12abcd
CM	Control	M	285.10	±36.57abcd
Two-way ANOVA F-Statistics				
Auxin			5.136***	
Gender			2.481ns	
Auxin*Gender			2.567**	

Mean values ± Standard Error are shown in columns. The mean values followed by different letters are significantly different at P ≤0.01 (**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. IAAF=Indole acetic acid female cutting, IAAM=Indole acetic acid male cutting, NAAF=1-Naphthaleneacetic acid female cutting, NAAM=1-Naphthaleneacetic acid male cutting, IBAF=Indole butyric acid female cutting, IBAM=Indole butyric acid male cutting, CF= control female cutting, CM=control male cutting.

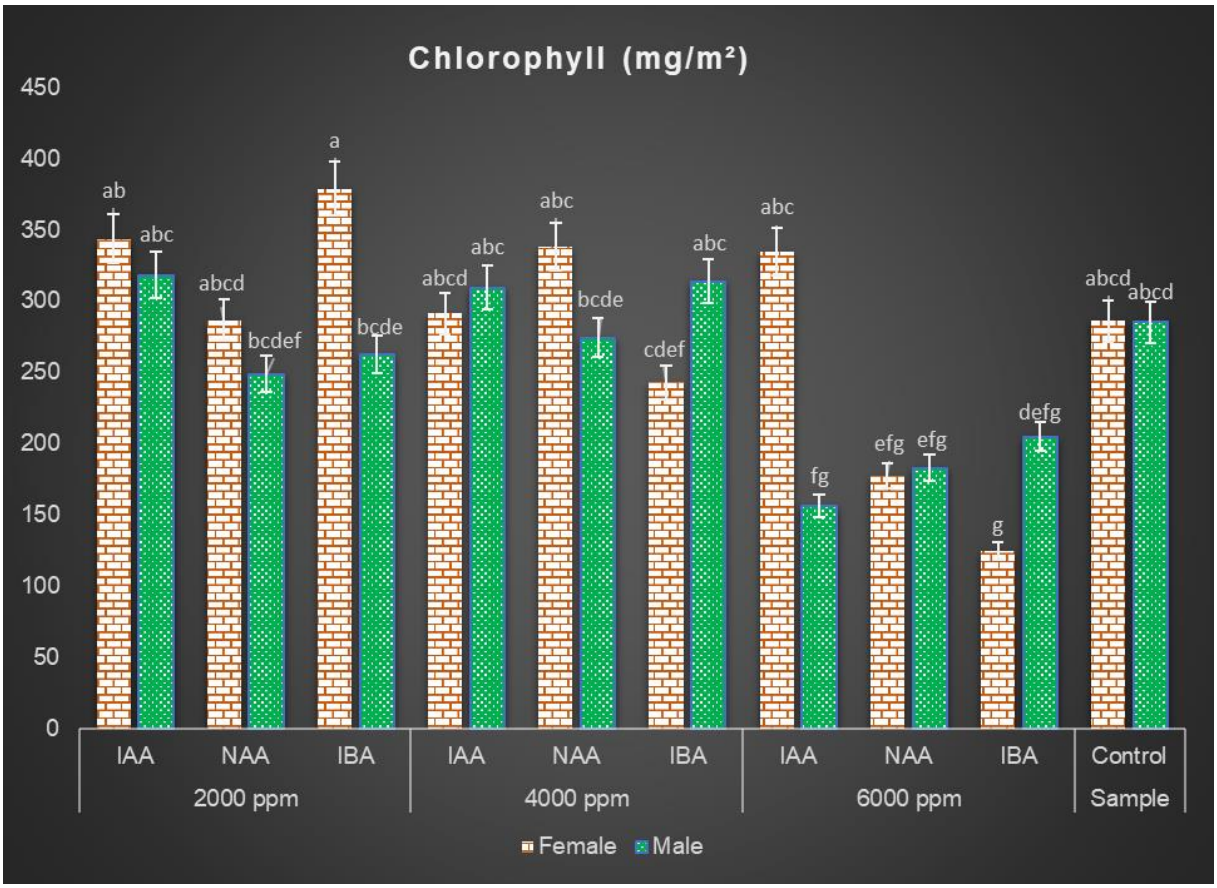

Figure 4.4 Different letters indicate significantly different mean values at P ≤0.01 (**), P ≤0.001 (***) as calculated by Fisher's least significant difference. IAA=Indole acetic acid; NAA=1-Naphthaleneacetic acid; IBA= Indole butyric acid

4.5 Discussion

Wet weight biomass increase

The wet weight (before) of *Leucadendron elimense* subsp. *elimense* showed significant results in the NAA 6000 ppm in the female plants at the beginning of the experiment. These results were followed up with a significance in the wet weight (after) in female plants treated with IAA at 4000 ppm. Wet weight increased over the experimental period with a lower concentration auxin treatment (IAAF 4000). The results concur with studies by Laubscher & Ndakidemi (2008a) who reported similar significance, while Hartmann *et al*. (2002) also documented the concentration of IAA. As the results were consistently on the female cuttings and significant in the auxin, gender and auxin vs gender measurements, these results are in agreement that possibly carbohydrate and starch accumulation, in a study with *Solanum,* resulted in an increase in weight while supported with auxins present (Yuan *et al.,* 2018).

Flowering response

From previous literature and several studies conducted on using IBA as a rooting auxin, but there are disagreements on the strength that should be used. There is also some evidence that IAA at 4000 ppm can be used successfully as a rooting auxin on *Leucadendron* sp. and species in the Proteaceae family. The cuttings for the female plants flowered most using the auxin IAA at 4000 ppm, showing that it is a possibility for the commercial cut flower market. Krizek (2011) and Cheng and Zhao (2007) reported that auxins play critical roles in several aspects of flower development, including floral meristem initiation and floral organ initiation, growth, and patterning.

Leopold and Thimann (1949) analyzed the effect of auxin on the initiation of flowering in barley after 3 weeks where NAA or IAA promoted flowering of barley. Applications of stronger auxin concentration inhibited flowering instead with complete inhibition of flowering especially in NAA. Earlier, Salisbury (1955) reported that in experimental work on *Xanthium* sp. the time of auxin application indicate that auxin inhibit flowering if applied before translocation of the flowering stimulus as well as controlled by the photoperiodic induction. Leopold and Thimann (1949) also reported that the promotion of flower initiation by auxin bud formation for vegetative buds was not promoted in the same way as for flowering buds as seasonal variations also increased flowering. Although this study did not trial seasonal changes, the time of auxin application could possibly have promoted or inhibited flower response. While auxins applied after the flowering stimulus from the leaf is complete, flower bud development is promoted. This study is also in agreement with Leopold and Thimann (1949) who observed a correlation between the number of flower primordia and the weight of the plant. This similarity between flowering and growth possibly suggests that auxin affected flower initiation almost parallel to the growth response of the plants. Both the growth and flowering were promoted by relatively low concentrations of auxin and thus possibly also inhibited by higher concentrations. This evidence is positive in promoting future production and cultivation of *Leucadendron elimense* subsp. *elimense.*

Chlorophyll production

The chlorophyll showed significance in IBA 2000 treatment on female plants. Laubscher and Ndakidemi (2008) reported similar results with IBA at 1000 ppm in *L. laxum*. While these results are aligned with this study Laubscher and Ndakidemi (2008b) documented earlier that IBA at concentrations of 4000 ppm and higher supressed growth. In contrast Rodríguez-Pérez *et al.* (2002) found that IBA at 4000 ppm were more successful for Protea Hybrid ‘Susara’. According to Pérez-Francés *et al*. (2001) *L. discolor* and *L.* ’Safari Sunset’ were more successful using 4000 ppm of

IBA auxin. Worrall (1976) reported an initial positive response with 4000 ppm of IBA which later proved to have a toxicity response. This study therefor recommended IBA at 2000 ppm for chlorophyll production. The study concurs with results from Yuan *et al.* (2108) that auxin played a significant role in chlorophyll accumulation in a study on *Solanum* fruit development.

4.6 Conclusion and recommendations

Auxins prove successful in rooting *Leucadendron* species, however this study on *L. elimense* subsp. *elimense* showed that IAA was possibly responsible for flower bud development. There was also a strong correlation in auxin response between the weight measured and increase in flower bud formation. The use of auxin could support early flowering of *L. elimense* subsp. *elimense* and therefore have potential for the cut flower industry as much of its potential remain unexplored mainly due to the unavailability of saleable plants. In summary the study demonstrates that IAA plays a significant role in flowering initiation and chlorophyll accumulation of *L. elimense* subsp. *elimense.* These results also indicate a regulation in biomass production, possibly carbohydrate accumulation. Further studies could focus on examining auxin application on established *L. elimense* subsp. *elimense* and other Proteaceae species in determining flowering inducing for commercial purposes.

4.7 Acknowledgements

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CHAPTER FIVE:

A PROPAGATION PROTOCOL FOR *LEUCADENDRON ELIMENSE* E.PHILLIPS SUBSP. *ELIMENSE* FOR CONSERVATION AND CUT FLOWER PRODUCTION

A PROPAGATION PROTOCOL FOR *LEUCADENDRON ELIMENSE* E.PHILLIPS SUBSP. *ELIMENSE* FOR CONSERVATION AND CUT FLOWER PRODUCTION

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5.1 Abstract

L. elimense subsp. *elimense* is an endemic species to the Agulhas Plain, Cape Floral Kingdom biodiversity. The species is endangered according to the Red List due to habitat loss. It also has the potential for the cut flower industry with several cultivars developed. Separate male and female cuttings were prepared using IAA, IBA and NAA rooting auxins. Callusing of cuttings started from weeks 3–4 and rooting from weeks 5–6. Female cuttings were more successful in IAA 4000 ppm treatment compared to NAA 4000 ppm for male cuttings. This study was successful in presenting a vegetative protocol of *L. elimense* subsp. *elimense* to advance commercial importance of the species as a cut flower.

Keywords: Cape Floral Kingdom, cut flowers, Red data list, rooting auxin

5.2 Introduction

Vegetative propagation techniques of many South African endemic and red listed species can increase their awareness, support their conservation and increase their commercial potential (Laubscher *et al*., 2009). *Leucadendron elimense* E.Phillips subsp. *elimense* does not propagate vegetatively (Mustart *et al*., 1997). Although Carolus (2008) reported otherwise, successful vegetative propagation techniques can enhance dwindling numbers of this species (Laubscher *et al*., 2009).

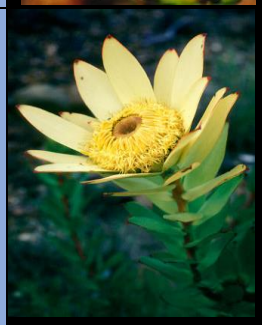
Leucadendrons are endemic to the Cape Floral Region (CFR), which is habitat to 8600 species of which 5800 are endemic and 1700 threatened in their natural habitat where more than two-thirds of the species grow on nutrient-poor mountainous sandstone soils (Hall *et al*., 1980; Winter & Botha, 1994; Cowling & Richardson, 1995; Low & Rebelo, 1996; Goldblatt, 1997; Pauw & Johnson, 1999; Tansley & Brown, 2000; Rouget *et al*., 2003a; Rouget *et al*., 2003b; Younge & Fowkes, 2003;

Rhode, 2004; Born *et al*., 2007; Manning, 2007; Reinten *et al*., 2011; Manning & Goldblatt, 2012; King, 2017). The region also is habitat to 65% of the southern African threatened and rare species (Hall *et al*., 1980). One such species, *L. elimense* subsp. *elimense* is endemic to the Agulhas Plain where it contributes largely with many others as ornamentals and cut flowers for the flower export industry (Brits *et al.*, 1983). Most flowers are continued to be harvested from the wild which contributes to their threatened status. Vegetative propagation techniques using auxin applications, optimum rooting media and environments could advance the replanting of threatened species in the wild (Laubscher *et al*., 2009). This study therefore aimed to document a successful vegetative protocol for *L. elimense* subsp. *elimense* to advance the commercialisation of this important commercial cut flower species.

5.2.1 Overview of *Leucadendron elimense* subspecies

There are four distinct subspecies: *L. elimense*; *L. elimense* E.Phillips subsp. *elimense, L. elimense* E.Phillips subsp. *nova*, *L. elimense* E.Phillips subsp. *salteri* I.Williams and *L. elimense* E.Phillips subsp. *vyeboomense* I.Williams which are very similar in growth habit but can be differentiated by the shape and length of the leaves (Table 5.1).

Table 5.1: The ecology and phylogeny of four *Leucadendron elimense* subspecies.

	L. elimense E.Phillips* subsp. *elimense	***L. elimense E.Phillips* subsp. *nova***	***L. elimense E.Phillips* subsp. *salteri I.Williams***	***L. elimense E.Phillips* subsp. *vyeboomense I.Williams***
Common name	**Elim conebush**	**Nova conebush**	**Caledon conebush**	**Vyeboom conebush**
Female				
Male				
Red list status	Endangered	Critically Rare	Critically Rare	Critically Rare
Distribution	Elim Flats from Gansbaai to Bredasdorp	Mountains north west of Caledon. Discovered in June 2002, possibly a new sub species of *Leucadendron elimense*	Bot River Valley, Hemel en Aarde Valley and Shaws Pass	Northern end of Viljoen's Pass and eastwards to Theewaterskloof Dam, now extinct at southerly extent
Habitat	Shallow soil with ferricrete over clay, 10-170 m	Shales on south slopes, 300 m	Level gravel soils over clay, 30-330 m	Level sandy to loamy soils, 330-400 m
Growth habit	Sparsely branched shrub up to 1.5 m tall	A shrub up to 2 m tall	Sparsely branched shrub up to 1.5 m tall	A shrub up to 1.5 m tall
Sexual system	Flowers of one sex on separate plants	Flowers of one sex on separate plants	Flowers of one sex on separate plants	Flowers of one sex on separate plants
Flowers	July – September	September – October	August - September	September – October
Pollinator	Insects	Insects	Insects	Insects
Fruit	Released 2 months after flowering	Dropped to the ground	Released 2 months after flowering	Dropped to the ground
Seed dispersal	Dropped to the ground	Wind, but possibly rodents, research needed	Dropped to the ground	Dropped to the ground
Seed storage	In ground litter, perhaps hoarded by rodents	In ground litter, perhaps hoarded by rodents	In ground litter, perhaps hoarded by rodents	In ground litter, perhaps hoarded by rodents
Fire survival	Killed, only seeds survive	Killed, only seeds survive	Killed, only seeds survive	Killed, only seeds survive

(Adapted from Crown Conebushes – Leucadendrons https://www.proteaatlas.org.za/conebu6.htm 2020).

5.2.2 *L. elimense* E.Phillips subsp. *elimense* endemic to the Agulhas Plain

L. elimense subsp. *elimense* is endemic to the Elim area at the most southern tip of Africa in the Cape Floristic Region (Figure 5.1). The species is a single-stemmed, sparsely branched shrub, up to 1.5 m high. The leaves are red, elliptical: on the male plant, 13-49 x 5-19 mm and on the female plant, 14-57 x 7-21 mm; hairless, closely spaced with narrow, firm and flexible margins. The involucral leaves (a rim of leaves surrounding the flower head), are yellow, crowded, and overlap the base of the flower head (Mustard, *et al*., 1997; Carolus, 2008).

The male flowers are yellow, fluffy, 19 mm long and up to 46 mm across, depressed, and globular shaped while the female flowers are 20 mm wide and closely packed to the upper surface, with the sides closely overlapped by recurved involucral bracts and leaves. Both sexes have an unattractive pungent smell (Carolus, 2008).

The female flowers are responsible to develop into globular shaped cones which are 35 mm in diameter with ovate shaped bracts with some hairs (Carolus, 2008). The flowers are possibly insect pollinated as several insects are located in the flower heads while the flowers secrete nectar and a pungent odour from both sex flowers.

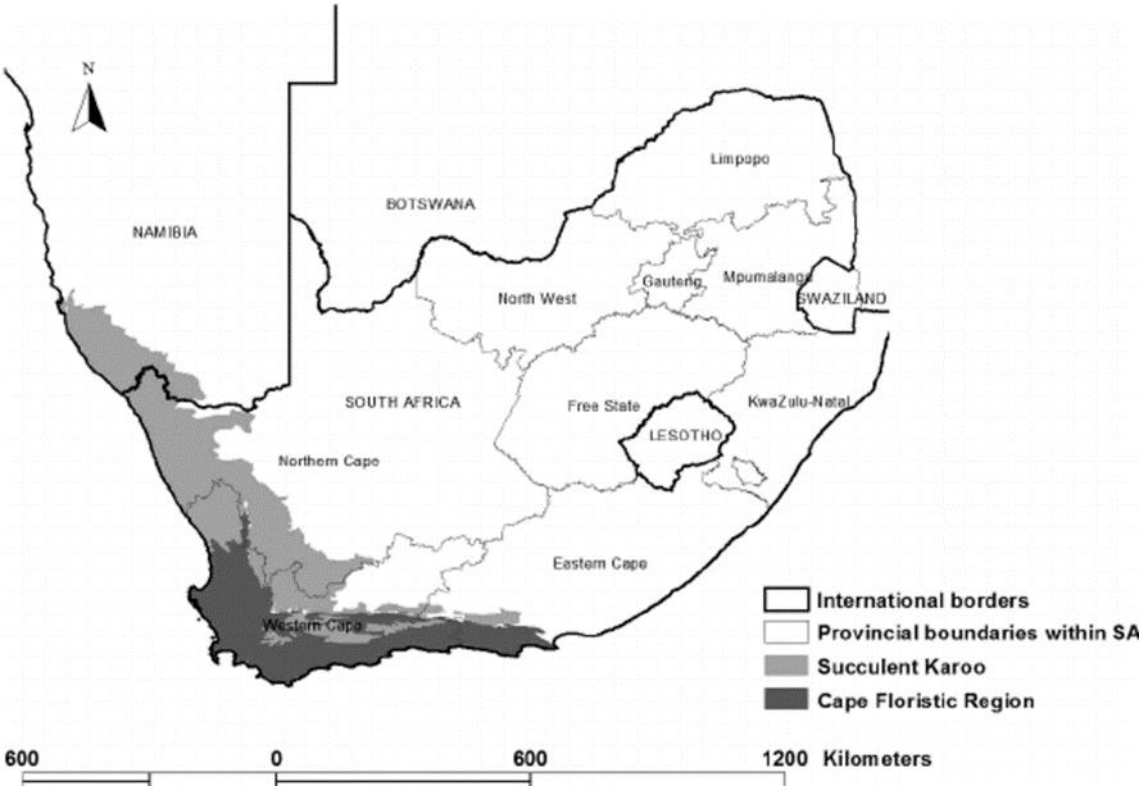


Figure 5.1 Cape Floristic Region (Source: Maze, 2005)

5.2.3 Conservation status of *L. elimense* subsp. *elimense*

L. elimense subspecies are listed as problematic in the The Red List of Threatened South African plants with three subspecies *L. elimense* E.Phillips subsp. *nova, L. elimense* E.Phillips subsp. *salteri* I.Williams and *L. elimense* E.Phillips subsp. *vyeboomense* I.Williams are listed as critically rare (Raimondo *et al.*, 2009). For several years since 1980, *L. elimense* E.Phillips subsp. *elimense* has been classified as "Endangered" in the Red List data for threatened species in South Africa with eight populations being in four areas and population sizes ranging between twelve to a couple of hundred plants in heavily threatened coastal lowland habitats (Hall *et al*., 1980; Hall & Veldhuis, 1985; Rebelo, 2001; Carolus, 2008). Before 1999, 10 000 plants were counted, shortly after the numbers dropped to 7 000 plants in eight populations (Brown, 1999). The species remained at risk with becoming extinct with only 2000 plants remaining in their natural habitat (Mustard *et al*., 1997; Protea Atlas, 2008; SANBI, 2010). In 1996 the status improved to vulnerable, where the risk of extinction is slightly lower, however the species was placed back in the "Endangered" category in 2009 (Hilton-Taylor, 1996; Raimondo *et al*., 2009; SANBI, 2010). An endangered category indicates that the species is facing high risks of extinction according to IUCN criteria.

The Protea Atlas (2008) project recorded that for every live plant found nine were dead, while many looked unhealthy and yellow and some reported as many live plants as dead ones, while others reported more dead than alive ones. The reasons for declining numbers are alien invasive species such as *Acacia* spp., *Myrtus* spp. and *Pinus* species. While only 15% remain alien free, 63% has sparse alien vegetation and 20% has abundant amounts and 2% are smothered in dense alien vegetation (Hall & Veldhuis, 1985; Protea Atlas, 2008; Protea Atlas Project, 2019). Other reasons are grazing and trampling by cattle, too frequent fires, ploughing, quarrying and flower picking (Hall & Veldhuis, 1985). Due to extensive natural habitat destruction remaining plants are mainly seen growing along gravel roads in shallow ferricrete Elim asteraceae soils (Mustard *et al*., 1997; Carolus, 2008). The urgency status for conservation of this species is of high priority as it only grows in the "Elim flats" where it is up to the Elim community and the surrounding farmers to protect the species from extinction (Hall & Veldhuis, 1985; Protea Atlas Project, 2019).

5.2.4 Commercial importance of *L. elimense* subsp. *elimense*

Several *Leucadendron* species have become part of plant breeding programs to enhance their importance as commercial cut flowers. In Western Australia, university

research support has been provided for *Leucadendron* breeding projects to manage the commercialisation of new varieties intellectual property and their distributions where more than 500 interspecific cross combinations involving 140 genotypes of 27 species were evaluated for Australian conditions (Sedgley *et al*., 2006). By 2005, this breeding protocol has delivered 200 potential new varieties with 8 released to the growing industry. Due to breeding cost the cooperation of international participants were sourced to enhance and maintain the breeding program (Sedgley *et al*., 2006).

5.2.5 Propagation of *Leucadendron* from seed

Leucadendron species are mainly propagated from seed as the most successful method of propagation of the genus in South Africa (Ben-Jaacov & Silber, 2006). *L. elimense* subsp. *elimense* falls under the group crown conebushes which release their fruit after five months of ripening (Rebelo, 2001). The genus has a natural mechanism, Alatosperma which refers to the flattened seeds which remain within the cone for one year or more to allow after-ripening of the embryo and the spontaneously release from the cones when germination conditions becomes more favourable (Brits, 1986; Carolus, 2008; Reinten, 2014). Seeds are hand collected from the previous year's cones and are stored in a warm, dry place to be sown in autumn in the winter rainfall area where fluctuating temperatures between day and night activate seed germination, with night temperatures dropping between 5 to 8°C and day temperatures are between 15 to 20°C (Eliovson, 1965; McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Carolus, 2008; Malan, 2012; Reinten, 2014). Dormancy of seed can be overcome when seeds are soaked in sulphuric acid for five to ten minutes and then rinsed in clean water (McLennan, 1993; Malan, 2012). Another way to break dormancy is by mechanically scarifying the seed with sandpaper, to increase the oxygen supply to the embryo (Brown & Duncan, 2006; Malan, 2012). The seeds can also be treated with smoke or with a smoke derivative and scarified to break dormancy (Brown & Duncan, 2006; Malan, 2012). A fourth option to activate the embryo is to soak the seed in 1% hydrogen peroxide with smoke primer paper for 24 hours (Brits, 1986; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012). Older seeds lose their viability and can be soaked in a solution containing gibberellin (Brown & Duncan, 2006). The seeds should be treated with a fungicide to prevent post-emergence infection (Brown & Duncan, 2006). It is also recommendable to use 500 ml plastic seedling bags as they provide improved results compared to using seedling trays (Oertel & Oertel, 2018b). A recommended media of two parts of coarse river sand, two parts peat and one part of vermiculite or perlite with a pH of 5.5 allow successful germination in providing adequate drainage and aeration to prevent rotting of seed in a too wet media (Eliovson, 1965;

McLennan, 1993; Carolus, 2008; Oertel & Oertel, 2018b). Initially the media should be drenched with a commercial fungicide (McLennan, 1993). The seedling bags should be placed under mist sprayers in a warm environment with sufficient ventilation (McLennan, 1993). Germination will commence after one to three months when the roots should be strengthened with the application of kelp seaweed, liquid fertiliser (McLennan, 1993; Brown & Duncan, 2006; Carolus, 2008; King, 2018). After two to six true leaves have formed the seedlings are ready to be pricked out into individual bags, where they are kept for one year and grown to a height of 15 to 30 cm before being transplanted into the open ground usually with the following rainy season (Eliovson; 1965; Carolus, 2008).

5.2.6 Propagation of *Leucadendron* from cuttings

Many species of the *Leucadendron* genus have been propagated successfully in raising clones for cut flower orchard plantings. In the southern hemisphere the best time for cuttings is from March to April (Eliovson; 1965; McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014). Terminal or sub-terminal cuttings should be made early in the morning to avoid heat and drought stress and should be 12 cm long and have a diameter of 8 mm (Eliovson; 1965; Ben-Jaacov *et al*., 1986; McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014; Oertel & Oertel, 2018a; ACS Distance Education; 2019). The leaves on the lower half of the cutting should be removed (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Oertel & Oertel, 2018a; Andrews, 2018).

Several studies reported that indole butyric acid (IBA) at 0.1% can be used to stimulate root growth, at a concentration of 2000 ppm for Proteaceae (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). The options would be to dip the cuttings in IBA powder at 4 g per litre dissolved in 50% ethanol for five to ten seconds (Brown & Duncan, 2006). Liquid IBA has shown best results, the cuttings should be placed in the liquid for ten seconds at a depth of one to two centimetres (McLennan, 1993). When powder IBA is used, it can burn the cutting if too much is applied (McLennan, 1993). Seradix 2 or liquid Dip and Grow can also be used as a rooting stimulant (Brown & Duncan, 2006; Malan, 2012). The best results for Proteas have been to use 4000 ppm of IBA, while 2000 ppm of IBA is better for cone bushes (Ben-Jaacov & Silber, 2006). Indole-3-acetic acid (IAA) had a positive effect on the rooting of *L. laxum* in a shaded tunnel environment (Laubscher & Ndakidemi, 2008b).

Cutting length of 10 mm should be dipped into the correct strength for ten seconds (Ben-Jaacov & Silber, 2006). The growing media can be in transparent growing bags and can either be sand, peat, polystyrene in equal amounts or two to three parts river sand with one-part peat, (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). In comparison with other growth media tested, a bark and polystyrene media was more effective in the stimulation of rooting, survival of cuttings, root length and number of roots formed for *L. laxum* (Laubscher & Ndakidemi 2008a).

The cuttings should be one third deep in the media and have good aeration and be misted (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). The air temperature should be at 26°C, while the root zone should be 18°C (McLennan, 1993; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Malan, 2012). They should be placed on hotbeds and every hour the cuttings should receive a mist spray, but only during the day, and receive a spray programme against diseases (Eliovson, 1965; Ben-Jaacov *et al*., 1986; McLennan, 1993; Brown & Duncan, 2006; Malan, 2012; Reinten, 2014; Oertel & Oertel, 2018a; Andrews, 2018; ACS Distance Education, 2019).

After six to sixteen weeks have passed the cuttings will begin to form roots and are ready to be planted out, once the roots are well-developed and appear discoloured on the sides of the bag (Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018). The plants are ready to be planted out into well-drained, acidic soil that has not been fertilised (Reinten, 2014). Research in Israel has shown that *L*. 'Safari Sunset' can be grown in three to five months by using 15 cm long cuttings, dipped in 4000 ppm of IBA and are grown in a Styrofoam-peat media under intermittent mist with 25% reduced natural light (Ben-Jaacov *et al*., 1986; Leonhardt & Criley, 1999). Cuttings will root in a period of four weeks (Leonhardt & Criley, 1999). Although many of these propagation techniques are being used successfully, it remains uncertain if this methodology could be used for *L. elimense* subsp. *elimense* as little evidence could be found to support this. It is therefore important to study the vegetative propagation of the species to increase plant numbers.

5.2.7 Auxin treatment requirements for *Leucadendron*

In the 1930's it was discovered that natural growth substances stimulated and controlled root formation, and some synthetic substances could do the same (Hammet, 1973). Shortly after root stimulating rooting substances were made available to the horticultural industry (Hammet, 1973). Today rooting auxins come in

liquid, powder and gel form, they are made of auxins, which are naturally occurring plant auxins, but are mostly commercial products produced in laboratories (Bagust, 1994; Dyer, 2019). For Proteaceae propagation IBA is used, depending on the species it is best applied at 2000 ppm or 4000 ppm (McLennan, 1993; Faruchi *et al*., 1997; Ben-Jaacov & Silber, 2006; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). In order to add value to the market with the introduction of new varieties it is important to find a propagation protocol for *L. elimense* subsp. *elimense*.

5.3 Materials and methods

Mother stock plants of *L. elimense* subsp. *elimense* were sourced on 23 May 2019 from the Elim Moravian Mission church land (Schoeman & Visagie, 2014), next to Sandberg Fynbos Reserve on the Agulhas Plain. As very few records are available cultivated plants exist, permission was obtained to collect cutting material from their natural habitat. Stock plants were healthy, to ensure rooting potential during the experiment. The plants were growing in full sun exposure position. The timing of the year was important as autumn is their active growing season and the plants are not in flower, meaning all the energy can be put into root formation. Plants are actively growing from November to May (Protea Atlas, 2008). To ensure sustainable harvesting the plant material was collected from randomly selected, different plants with cuttings taken from 50 females and 50 male plants. Cuttings were collected during the morning and wrapped in moist newspaper so that they would survive during the night and to be planted the next day. The cuttings were stored in a cool environment overnight making sure that the material was not too wet for prolonged periods to prevent fungal diseases. The following morning the cuttings were taken to the Cape Peninsula University of Technology Bellville campus, South Africa at GPS coordinates - 33º 55’45.53S, 18º 38’ 31. 16E.


Figure 5.2 *L. elimense* subsp. *elimense* mother stock growing in its natural environment (Source: Liedtke, 2019 and Laubscher, 2009).

5.3.1 Week 1 - Preparation for cuttings

Preparing rooting media

Growing media of a mixture of coarse river sand (2 mm diameter), coco peat and perlite (2:1:1) was prepared for the cuttings. The media was moistened to absorb water, but not too wet to be oversaturated. Two hundred perforated, transparent bags with a size of 125x190 mm were filled with the media for the cuttings.

Preparing rooting auxin

Rooting auxins IAA, NAA and IBA, from Sigma- Aldrich, at different rooting strengths were prepared by using 0.5 g in 250 ml of water making 2000 ppm, 1 g in 250 ml of water making 4000 ppm and 1.5 g in 250 ml of water making 6000 ppm for each of the rooting auxins. The prepared auxins were kept in dark glass containers and stored at 4-6 degrees C.

Preparing, planting and placing cuttings

Fifty female and fifty male tip cuttings of *L. elimense* subsp. *elimense* were cut into the correct length of around 15 cm. The cuttings were taken just below the node with a straight cut. The bottom 1/3 of the leaves were removed and the base of the cuttings were dipped into respective rooting auxins for 10 seconds and then placed into the perforated, transparent bags with the growing media and placed onto the hot beds. All cuttings were sprayed with Captab (4 g/l), a preventative fungicide.

Figure 5.3 Female cuttings (left) compared to male cuttings (right) prepared to 15 cm lengths (Source: Liedtke, 2019).

5.4 Results

5.4.1 Week 2-3 Acclimatising to the rooting environment

Once cuttings were planted, they were monitored to ensure that they received bottom heating at 22 °C and misting irrigation every 40 minutes for 21 seconds. The amount of water was measured to 500 ml per mist spray to ensure that cuttings did not received too much water. The rooting media was constantly checked to ascertain that not too much water was received. Towards end of week 3 callusing started showing signs on bottom ends of the stems. Cuttings received a weekly spray of Captab (4 g/l) as a preventative measure against fungi and Kelpak (5 ml/l) as a growth hormone to promote healthy cuttings.

5.4.2 Week 4-5 Start of rooting

Cuttings received a weekly spray of Captab (4 g/l) and Kelpak (5 ml/l). Rooting of female cutting of IAA at 4000 ppm took place in week 5. A few deaths of cuttings occurred, most likely due to a fungal problem, even though precautionary measures were taken.

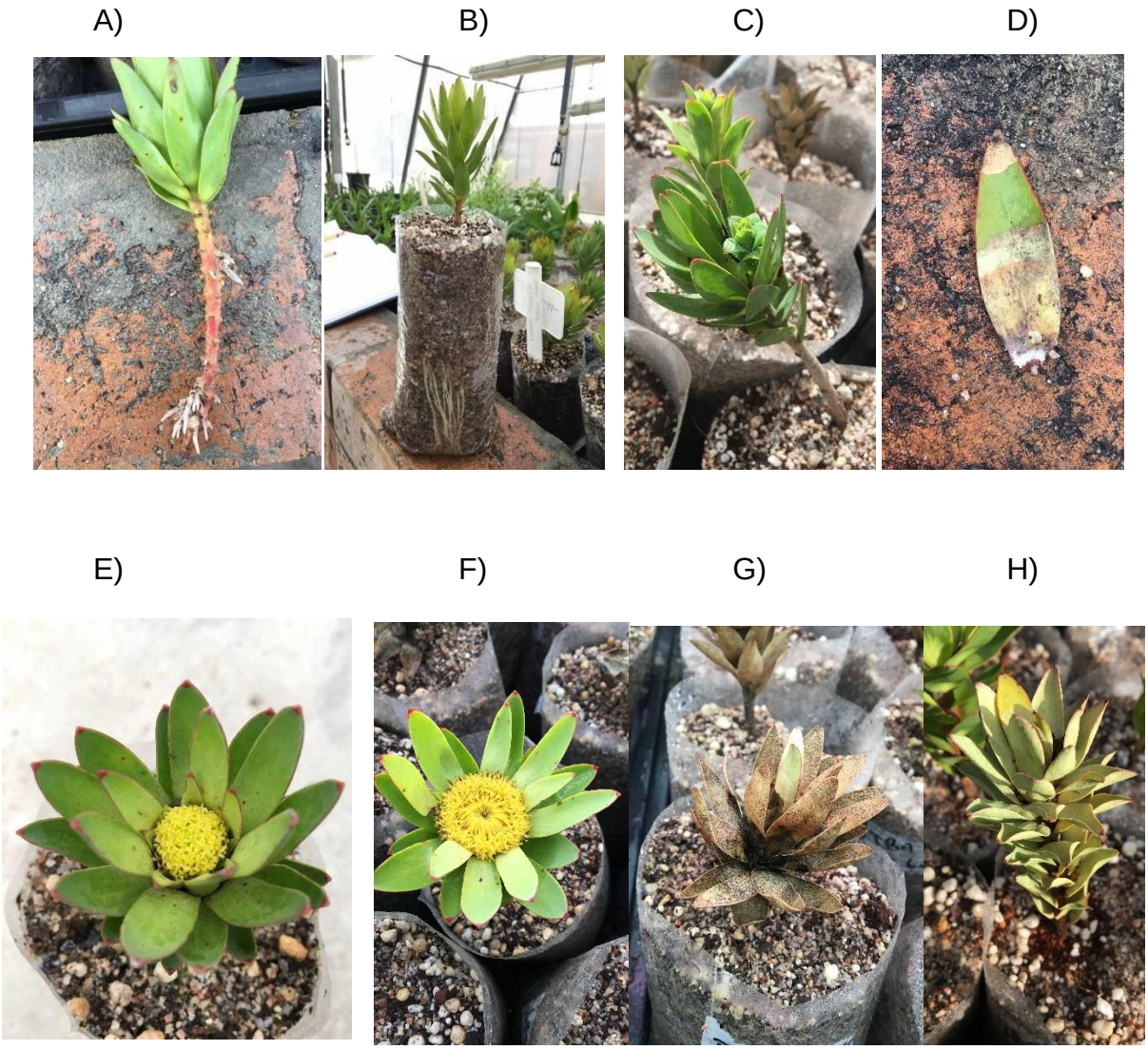


Figure 5.4 A) Rooting of IAA at 4000 ppm female cutting; B) Visible rooting at week 6; C) A new shoot visible; D) A sick leaf of one of the plants, showing signs of an infection; E) Flowering female cutting; F) Flowering male cutting; G) Death of cutting IBA 6000 ppm; H) Death of cutting of control (Source: Liedtke, 2019).

5.4.3 Week 6-9 Visible rooting

Captab (4 g/l) and Kelpak (5 ml/l) were applied weekly to promote healthy growth and to prevent fungal diseases. Visible rooting by many of the cuttings through the bag, but there were more deaths of plants, due to fungal infections of the stem and leaves.

5.4.4 Week 10-12 Prepare cuttings for hardening off

The plants were sprayed weekly with a mixture of Captab (4 g/l) and Kelpak (5 ml/l).

5.4.5 Week 13-17 Hardening off

On the 20th of August 2019, week 13, the cuttings were moved to the hardening off section, where they received 150 ml of water via mist sprayers every 50 minutes for

18 seconds. During week 14 the cuttings started producing flowers. The cuttings were sprayed weekly with a mixture of Captab (4 g/l) and Kelpak (5 ml/l).

5.4.6 Week 18 Growth measurements

On the 24th of September 2019, week 18, the cuttings were removed from the growing media and the results were measured. The percentage of callusing, rooting and flowering was measured by looking at the cuttings and seeing whether it had taken place. The root length was determined with a standard ruler by measuring the longest root. The number of roots were determined by counting the number of roots on each cutting. The wet weight was measured by using a standard balance. The amount of chlorophyll was measured with a chlorophyll meter. From the results in chapter 3 and 4 it was clear that IAA 4000 ppm had the highest success rate for the female cuttings, while NAA at 4000 ppm had the highest success rate for the male cuttings (Figure 5.5 and 5.6).

Table 5.2: A proposed vegetative production schedule for the commercial cultivation of *L. elimense* subsp. *elimense* female and male cuttings rooted in a semi-controlled greenhouse at 16 - 28 °C with bottom heating.

	Weeks	Developmental stages	Actions	Media	Watering schedule	Treatments Female cutings	Treatments Male cuttings	Growth Measurements
Materials and methods	**0**	Stock plants	Select healthy mother plants	Plants grow in natural habitat	Keep moist and cool in sealed container	Keep labelled	Keep labelled	Select non-flowering shoot tips
	1	Unrooted cuttings	Make tip cuttings and plant in perforated transparent bags 125x190 mm	river sand, coco peat and perlite (2:1:1)	Misting every 40 minutes for 21 seconds (500 ml per mist spray)	Apply IAA 4000 ppm as liquid dip	Apply NAA 4000 ppm as liquid dip	Tip cuttings measured 15 cm Low mortality rate. monitor watering
Results	**2-3**	Unrooted cuttings	Cutting bags on heated bed	river sand, coco peat and perlite (2:1:1)	Misting every 40 minutes for 21 seconds (500 ml per mist spray)	Captab (4 g/l)	Captab (4 g/l)	Callusing start. Monitor watering
	4-5	Unrooted cuttings	Cuttings bags on heated beds	river sand, coco peat and perlite (2:1:1)	Misting every 40 minutes for 21 seconds (500 ml per mist spray)	Captab (4 g/l) and Kelpak (5 ml/l)	Captab (4 g/l) and Kelpak (5 ml/l)	First rooted cutting with some deaths.
	6-9	Rooted cuttings	Cuttings on heated beds	river sand, coco peat and perlite (2:1:1)	Misting every 40 minutes for 21 seconds (500 ml per mist spray)	Captab (4 g/l) and Kelpak (5 ml/l)	Captab (4 g/l) and Kelpak (5 ml/l)	Visible rooting with further deaths.
	10-12	Rooted cuttings	Prepare cuttings for hardening off	river sand, coco peat and perlite (2:1:1)	Misting every 40 minutes for 21 seconds (500 ml per mist spray)	Captab (4 g/l) and Kelpak (5 ml/l)	Captab (4 g/l) and Kelpak (5 ml/l)	More rooting visible through bags
	13	Rooted cuttings	Remove from heated bed and move to hardening off area	river sand, coco peat and perlite (2:1:1)	Reduce watering to half	Captab (4 g/l) and Kelpak (5 ml/l)	Captab (4 g/l) and Kelpak (5 ml/l)	Hardening off taking place.
	14-18	Rooted cuttings	Measurements were taken in week 18.	river sand, coco peat and perlite (2:1:1)	Reduce watering watering to half	Captab (4 g/l) and Kelpak (5 ml/l)	Captab (4 g/l) and Kelpak (5 ml/l)	Flower buds and flowers appear Growth measurements taken

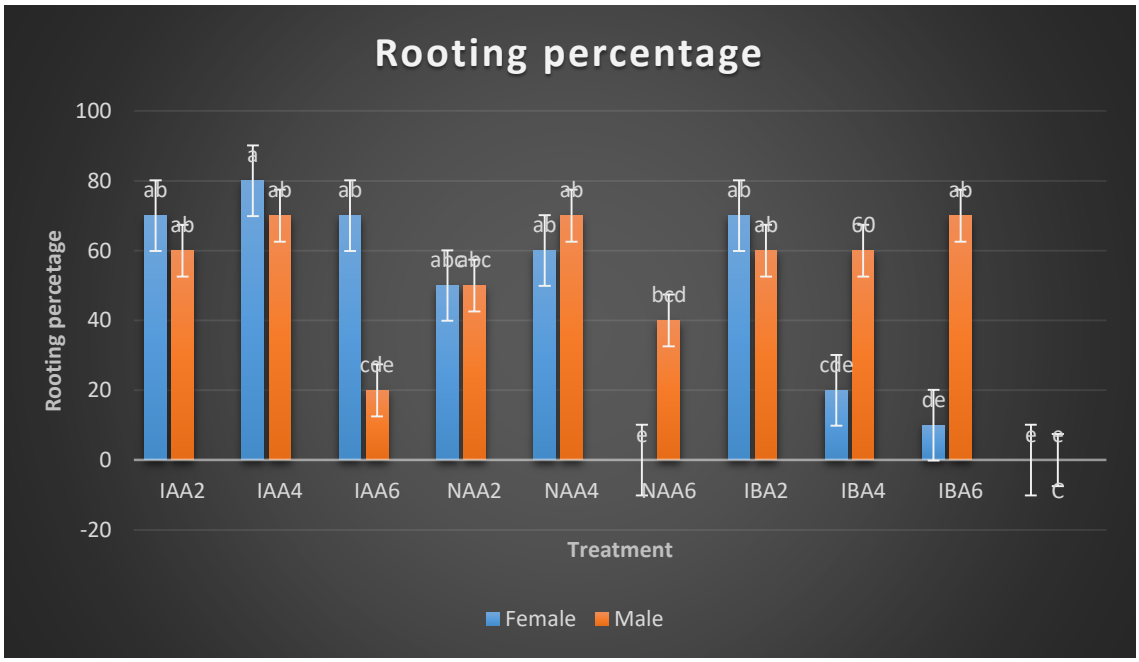


Figure 5.5 The effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the rooting success of female and male cuttings of *Leucadendron elimense* subsp. *elimense.*

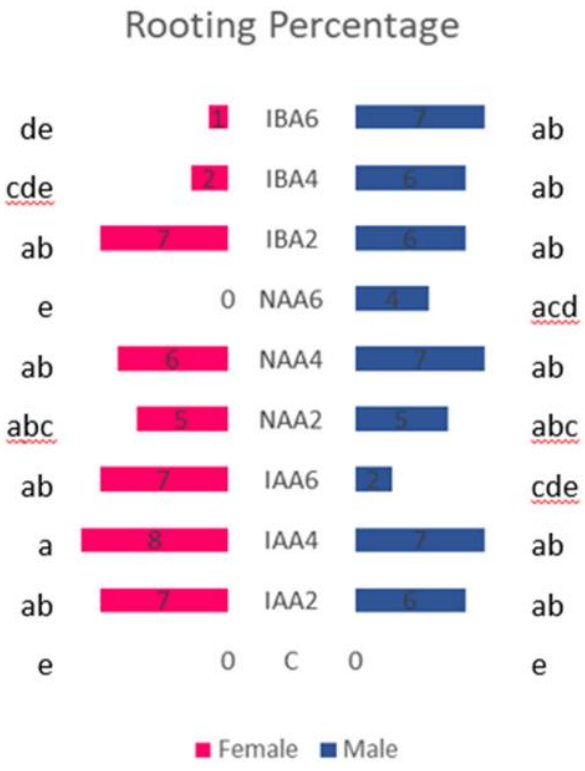


Figure 5.6 Comparison of the effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the rooting success of female and male cuttings of *Leucadendron elimense* subsp*. elimense*.

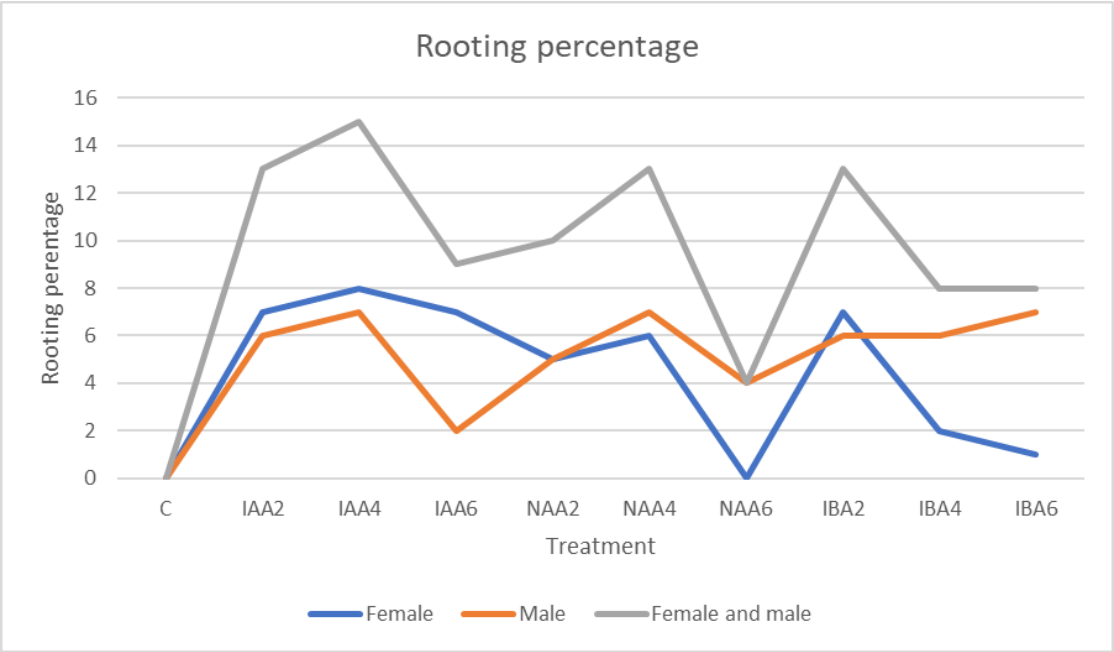


Figure 5.7 The effect of different rooting auxins, Indole acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA), on the rooting success of female and male cuttings of *Leucadendron elimense* subsp. *elimense*.

5.5 Discussion and conclusion

IAA rooting auxin

The most successful rooting of *L. elimense* subsp. *elimense* were measured with IAA at 4000 ppm in the female cuttings. These results were supported in a rooting study by Laubscher and Ndakidemi (2008a) of *L. laxum* successfully using IAA at 4000 ppm. Hartman *et al.* (2002) documented the benefits of IAA in rooting plants as was found in this study for the propagation protocol for *L. elimense* subsp. *elimense*.

Flowering and wet weight had significant difference for the female cuttings treated with IAA at 4000 ppm. This also agrees with the rooting success. Laubscher & Ndakidemi (2008a) and Hartmann *et al.* (2002) who also observed significant difference in the root length and number of roots with 4000 ppm of IAA.

NAA rooting auxin

The male gender cuttings showed a higher success rate in the NAA auxin treatments, however the results showed that NAA was not significant during the study. It is speculated that the concentration strength applied could be sufficient for root development, however over applications especially powder preparations can cause burning of the parenchyma tissue with delay in rooting and or death of the cutting

(McLennan, 1993). The male cuttings did not show consistent results, the only certain result was that NAA was the best auxin for the male cuttings, as it showed good results in two of the three categories.

IBA rooting auxin

IBA has been reported as the most popular auxin used for general cutting propagation with various degrees of success documented in various Proteaceae species. This study however found that IBA was not significant in rooting *L. elimense* subsp. *elimense* and therefore remains in contrast with several other studies which recommended that a concentration of 0.1% IBA will stimulate root growth more specifically a concentration of 2000 ppm is recommended for *Leucadendron* species (McLennan, 1993; Faruchi *et al*., 1997; Brown & Duncan, 2006; Reinten, 2014; Andrews, 2018; Oertel & Oertel, 2018a). This study concurs with Laubscher & Ndakidemi (2008b) that IBA at concentrations of 4000 ppm and higher supressed rooting, which guide the propagator to be more selective in auxin application type and quantity.

The rooting requirements for *L. elimense* subsp. *elimense* have shown that the species responded to IAA and NAA in rooting female and male cuttings. The plant can be grown successfully propagated from vegetative shoot tip cuttings. This outcome has the potential in the horticultural industry, especially as a cut flower cultivation with further research needed on flowering control.

5.6 Acknowledgements

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CHAPTER SIX:

GENERAL DISCUSSION AND RECOMMENDATIONS AND CONCLUSION

6.1 General discussion and recommendations

Chapter 2 focused on *L. elimense* subsp*. elimense* an 'Endangered' in the red data listed species' potential for the cut flower industry for both female cones and male flowers (Mustard *et al*., 1997; Protea Atlas, 2008; SANBI, 2010). This chapter found there have been limited research done on the propagation this species and therefore further studies on vegetative propagation is essential to enhance the potential of this species for both commercial and conservation purposes.

Chapter 3 found that rooting of *L. elimense* subsp. *elimense* was most successful for female cuttings using the auxin IAA at 4000 ppm. IAA at 4000 ppm showed significant difference to the control in callusing and rooting percentage, root length and number of roots. For the male cuttings NAA at 4000 ppm showed best results in these categories. It was found that the cuttings treated with 6000 ppm of the auxins did not perform well, as it may have been too strong and burnt the plants, preventing them from callusing and rooting. One can therefor suggest that IAA at 4000 ppm is used as a rooting auxin for female cuttings and NAA at 4000 ppm for male cuttings (Hartmann *et al*., 2002; Laubscher & Ndakidemi, 2008a). Further studies would be required on time of year, growing media, watering frequency and amount, relative humidity, and temperature to establish an optimal protocol for cultivation of the plant.

Chapter 4 found that there is an effect on the wet weight and flowering percentage of *L. elimense* subsp. *elimense* (Krizek, 2011; Cheng & Zhao, 2007). It was shown flower production was most successful in the female cuttings using the auxin IAA at 4000 ppm, for the male cuttings NAA at 2000 ppm was most successful. The wet weight of IAA 4000 ppm was the highest for the female cuttings, while NAA 4000 ppm had the best result for the males. Further studies would be necessary to see which time of year, growing media, watering frequency and amount, relative humidity, and temperature to establish an optimal protocol for cut flower production of the plant.

Chapter 5 found the potential for *L. elimense* subsp. *elimense* as a cut flower and therefore shows a propagation schedule for growing female and male plants of *L. elimense subsp. elimense* using IAA 4000 ppm for female plants and NAA 4000 ppm for male plants. There are many factors that should be studied further such as time of year, growing media, watering frequency and amount, relative humidity, and temperature to establish a successful propagation schedule. This study did show however that *L. elimense subsp. elimense* can be propagated vegetatively and has potential as a horticultural crop and needs to be protected from going extinct.

6.2 Conclusion

In conclusion this study has reported that several species of the Proteaceae family have gained popularity as commercial cut flowers with only some species being cultivated to save natural populations from overharvesting. One species, *L. elimense* subsp. *elimense* have reached 'Endangered' levels due to a continued habitat destruction with population numbers continue to decline and little evidence of a recovery of the natural habitat. The study reported that a demand for cut flowers from female plants are the highest while there has been no record of the vegetative propagation of the species.

This study showed that *L. elimense* subsp. *elimense* can be successfully propagated vegetatively from shoot tip cuttings using IAA 4000 ppm rooting auxin. The rooting trials on *L. elimense* subsp. *elimense* have shown that the species responded significantly to IAA and NAA in rooting female and male cuttings, respectively.

This study showed that auxin treatment IAA was possibly responsible for flower bud development in *L. elimense* subsp. *elimense.* There was also a strong correlation in auxin response between the weight measured and increase in flower bud formation. The use of auxin could support early flowering of *L. elimense* subsp. *elimense* and therefore have potential for the cut flower industry as much of its potential remain unexplored mainly due to its unavailability of saleable plants. This study demonstrated that IAA possibly played a significant role in flowering initiation and chlorophyll accumulation of *L. elimense* subsp. *elimense.* These results also indicated a regulation in biomass production, possibly carbohydrate accumulation.

This study presented an optimal protocol for the cultivation of *L. elimense* subsp. *elimense* documenting seasonality, growing and environmental conditions to propagate the species successfully for both female and male plants. Cultivating plants could contribute to the species 'Endangered' red listed status which could aid in saving the species in its natural habitat and support restoration ecology. Cultivation of the species could provide data for commercial cut flower growers and farmers to improve growing the species. This would enhance flowering of female and male plants for their cut flower potential to support the production of quality export cut flowers and flowering potted plants.

Further studies on propagation of the species could be explored in using IAA auxin concentrations for In-vitro culture purposes, cultivation during different times of the year to support clonal production. Further studies could also focus on examining

auxin application on established *L. elimense* subsp. *elimens* in improving flowering for commercial purposes.

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