AN ECO-TOURISM AND CONSERVATION PERSPECTIVE OF ENDANGERED PROTEACEAE OF THE CAPE FLORAL KINGDOM ON THE AGULHAS PLAIN

by

CHARLES PETRUS LAUBSCHER

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Supervisor: **Dr P Ndakidemi**
Co-supervisor: **Prof MS Bayat**
Co-supervisor: **Prof A Slabbert**

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DECLARATION

I, Charles Petrus Laubscher, 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 presents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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Signed	**Date**

ABSTRACT

The growing global perspective on conservation in combination with the rapid changes in the environment due to ever increasing human demands has placed more emphasis on the plight of threatened and endangered Proteaceae on the Agulhas Plain. Large parts of the Agulhas Plain contribute to the commercial cut flower export industry. The population numbers of *Leucadendron elimense* subsp. e*limense*, *L. laxum*, *L. platyspermum* and *L. stelligerum* (PROTEACEAE) have been drastically reduced as many species are illegally harvested. The continued destruction of natural habitats has made environmentalists and Protea flower producers aware of the need for developing future conservation strategies. An increase in ecotourism on the Agulhas Plain is important in view of its role in extended conservation and job creation. The Agulhas Plain is a region where conservation, ecotourism and agriculture could work together to maintain a balance of protection, enjoyment and commercial gain from the habitat. Potential developments of ecotourism on the Agulhas Plain are hindered through poor agricultural practices and a lack of conservation of the natural habitat. Landowners and cut flower producers have to stay abreast of global changes if they are to be responsible for the protection of the environment. In this respect the usage of land is linked to skills, attitudes, knowledge and an understanding of the environment. There is a lack of guidance and available information in the conservation of the Agulhas Plain while the ecotourism potential of the Cape Floral Kingdom remains undeveloped.

The objectives of the study are: a) to collect and survey scientific data on current practices of landowners, flower producers and exporters to determine the probable causes of destruction of Red Data species and their influences on ecotourism development on the Agulhas Plain. This study aims to make recommendations on the propagation and the conservation of threatened species and the ecotourism potential on the Agulhas Plain; b) to test the rooting ability of *L. laxum* using four liquid hormone concentrations of IBA or IAA and four different rooting mediums. Differences in rooting in an environmentally controlled greenhouse environment with bottom heat and a shaded tunnel is also tested. The study aims to develop new propagation techniques to increase successful and economical propagation of the species, to solve problematic and difficult propagation techniques and to relieve the threatened status of species. The study used a self-administered survey questionnaire distributed amongst growers, farmers and exporters to determine the knowledge, skills, values and attitudes in the harvesting, propagation, conservation and ecotourism development of Red Data species on the Agulhas Plain.

Findings of the study: The training of individuals and farm workers was found to be limited. The habitat is continuing to be used for grazing animals, while the occurrence of fires were seen as the second biggest threat to the endangered species. Permits are abused and the lack of authorities visiting farms is clearly evident. Although a positive attitude towards conservation and the promotion of ecotourism exists, a lack of information, guidance and facilities is evident. The survey revealed that little is known about the propagation of the many rare and threatened species of the Agulhas Plain. The need to develop propagation techniques which could increase the awareness and rehabilitation of species habitats was identified.

Four separate studies tested the rooting ability of *L. laxum* using liquid auxin preparations of 500, 1000, 2000 and 4000 ppm and a control in combination with four different growth media, namely: a) bark / polystyrene; b) peat moss / polystyrene; c) bark / river sand / polystyrene; and d) perlite / river sand. The experiments used a randomised block design with 10 cuttings per treatment. In experiment one the cuttings were rooted under temperature controlled greenhouse conditions with bottom heat using IBA auxin treatments. The bark / river sand / polystyrene medium had the highest (45%) survival rate in the 500 ppm auxin treatment. The application of 1000 ppm of IBA in the bark / river sand / polystyrene medium significantly increased the number of roots and longest roots per cutting. The bark / polystyrene medium supplied with zero IBA had the largest number of roots per cutting (6.5). The bark / polystyrene medium yielded the highest and most significant callusing formation (30%), highest percent of cuttings that rooted (28%), cuttings with a higher survival rate (57%), and cuttings with a higher number of roots (3.5) as well as the longest roots (7.35 mm). IBA supplied at 1000 ppm produced greater root numbers when compared with all other treatments. In experiment two, the cuttings were rooted under 40% shade environment with IBA auxin treatments. The IBA application in comparison with the control significantly improved rooting percentages in the a) peat / polystyrene and b) bark / river sand / polystyrene mediums. The bark / polystyrene medium was most effective in the stimulation of roots, survival of cuttings, promotion of root length and the number of roots that developed. The overall application of IBA induced more root and shoot formation. In experiment three cuttings were rooted under temperature controlled greenhouse conditions with bottom heat and were exposed to IAA auxin treatments. Compared to other mediums, bark / polystyrene had the highest significant results in root and shoot growth, as well as percentage that callused, rooted and survived. The IAA treatments at different concentrations had significant effects on rooting, callusing, shoot growth, root lengths and numbers of roots per cutting. In experiment four the cuttings were rooted in a 40% shade environment with IAA auxin treatments. Overall, the exogenous supply of IAA had a positive effect in the advancement of rooting in *L. laxum* grown in a) peat / polystyrene and b) bark / river sand / polystyrene mediums. The a) bark / polystyrene and b) peat / polystyrene mediums were significantly superior in promoting different aspects of rooting when compared with the other mediums. Evidence from this study was conclusive that the shade tunnel was an economical and successful environment in rooting *L. laxum.*

The study recommends that the destruction of Red Data species should receive greater conservation attention. The development of farm agri-tourism is important to develop ecotourism facilities, such as accommodation and educational tours on flower farms. Support is needed to help local communities in developing tourist based products that could increase employment in the region. Future research projects are necessary to investigate the propagation of other Red Data species and to increase the awareness of the plight of endangered species. The preservation of this flora is highly important for the future development of sustainable ecotourism on the Agulhas Plain.

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BIOGRAPHICAL SKETCH

Charles Petrus Laubscher was born in Ceres, South Africa, on the 30th October 1959. He attended Ceres Primary School and matriculated at Grabouw High School in 1977. He completed two years military training and became a captain in the Infantry corps in 1979. He enrolled at the Cape Technikon in 1980 and obtained the ND Horticulture in 1982, ND Parks and Recreation Management in 1985, NHD Horticulture in 1986 and MTech Horticulture in 1999. He has been employed at the Cape Peninsula University of Technology for the past 19 years and is currently a senior lecturer and head of programme for horticulture.

DEDICATION

This thesis is dedicated to my:

Parents	*Constant Laubscher,*
	21.03.22 – 01.03.94
	Alta Laubscher
	15.09.29 – 02.08.06

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
ABI	Agulhas Biodiversity Initiative
AP	Agulhas Plain
ARC	Agricultural Research Council
Asexual propagation	Vegetative reproduction of plants
C.A.P.E.	Cape Action Plan for the Environment
CFK -	Cape Floral Kingdom
CPUT	Cape Peninsula University of Technology
CREW	Custodians of Rare and Endangered Wildflowers
Cultural conditions	Conditions such as temperature, soil media, light intensity, fertilising, pest management, etc. that influence growth of plants.
DEAT	Department of Environmental Affairs and Tourism
Elim asteraceous fynbos	Vegetation associated with shallow, gravely soils derived from patches of silcretes and ferricretes which cap much of the Bokkeveld shales in the Southern Overberg.
EuroGAP	European Good Agricultural Practice
FVCT	Flower Valley Conservation Trust
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IUCN	International Union for Conservation of Nature
***L. elimense* subsp. Elimense**	*Leucadendron elimense* sub-species Elimense
L. laxum	*Leucadendron laxum*
L. platyspermum	*Leucadendron platyspermum*
L. stelligerum	*Leucadendron stelligerum*
Medium	A substrate used for growing plants
PROTEA ATLAS	Protea Atlas Project
Red Data List	The list of threatened or potentially threatened plant species
SANBI	South African National Biodiversity Institute
SAFEC	South African Flower Export Council

SAPPEX	South African Protea Producers and Exporters Association
Sexual propagation	Seed plant propagation
SPSS	Statistical Package of Social Sciences
Stratified random sampling	A method of sampling used on a population that is different or heterogeneous
ppm	Parts per million
UNDP	United Nations Development Programme
Vegetative propagation	Non-sexual propagation
WCNCB	Western Cape Nature Conservation Board;

CHAPTER ONE

LITERATURE REVIEW AND INTRODUCTION

Review

Conservation and propagation of endangered Proteaceae on the Agulhas Plain: Sustainable ecotourism development

Charles P. Laubscher[1], Patrick A. Ndakidemi[2]*, Mohammed S. Bayat[1] and André Slabbert[1]

[1]Faculty of Business, Cape Peninsula University of Technology, PO Box 652, Cape Town, 8000, South Africa.
[2]Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 652, Cape Town, 8000, South Africa.

1.1 Abstract

Large parts of the Agulhas Plain contribute to the commercial cut flower export industry. However, this industry is causing the destruction of many Proteaceae species both endemic and threatened. The Agulhas Plain is a large region (34°50′00″S 20°00′09.15″E / 34.833333°S 20.0025417°E) where threatened Red Data species are not sufficiently valued as many landowners and local communities lack vigilance in the preservation of these species. Flowers are harvested from their natural habitat and trading permits are misused while the lack of authorities inspecting farms is clearly evident. Environmental threats include wine farming, alien invasive plants and wild fires. The occurrence of fires has become one of the biggest threats to the area. Involvement of stakeholders such as conservation, tourism and government departments are important for conservation to succeed. Education and training of farm workers remains undeveloped. Propagation techniques are limited as no Red Data species are propagated to increase an awareness of these species. There is a lack of guidance and available information in the conservation of the Agulhas Plain while the ecotourism potential of the Cape Floral Kingdom (CFK) remains underdeveloped. Current practices which cause the destruction of red data species and the ecotourism potential on the Agulhas Plain are assessed in this review.

Keywords: auxins; rooting mediums; cut flowers; flowering potted plants; conservation; propagation; Red Data species; sustainable tourism; threatened species

Abbreviations: ABI = Agulhas Biodiversity Initiative; C.A.P.E. = Cape Action Plan for the Environment; CFK = Cape Floral Kingdom; EuroGAP = European Good Agricultural Practice; FVCT = Flower Valley Conservation Trust; IAA = Indole-3-acetic acid; IBA= Indole-3-butyric acid; IUCN = International Union for Conservation of Nature; SAFEC = South African Flower Export Council; ppm = parts per million; UNDP = United Nations Development Programme; WCNCB = Western Cape Nature Conservation Board

1.2 INTRODUCTION

In the Western Cape, the Cape Floral Kingdom (CFK) consists of 330 species of Proteaceae of which some are near extinction (Rebelo, 1995). The United Nations Development Programme (UNDP) views the Agulhas Plain as an area which has the highest priority for conservation and the largest number of lowland threatened species in South Africa (UNDP, 2003). The area is also known for important vegetation types such as Elim Asteraceous Fynbos, limestone proteoid, restioid fynbos and neutral sand proteoid vegetation types, all of which are highly threatened in the wild (UNDP, 2003). Elim Asteraceous Fynbos contains many endemic Proteaceae species such as *Leucadendron elimense*, *L. laxum*, *L. stelligerum* (Mustard et al., 1997). These plant species are classified as endangered plants on the Red Data List of South African Plants (Hilton-Taylor, 1996). The Protea Atlas (2008) classifies *L. elimense* and *L. platyspermum* as vulnerable species. *L. stelligerum* is found in only three populations, totalling 1000 plants in the Voëlvlei area (Rebelo, 1995). According to Mustard et al., (1997) only 5 000 *L. laxum* plants remain in their natural habitat.

The International Union for Conservation of Nature (IUCN) Red Data list of threatened species recorded more than 11 000 plant species which are threatened with extinction in South Africa (Jeffery, 2003). Leucadendrons are seriously threatened by expanding farm land, uncontrolled veld fires, alien invasive plants and improper cut flower harvesting in the natural habitat (Mustard et al., 1997; Robyn and Littlejohn, 2002). Some species are naturally rare because of reproductive reasons (Paterson-Jones, 2000). Many plant populations will disappear if the destruction of these sensitive habitats continues. This would result in a loss of biodiversity and endemic species (Jamieson, 2001; Robyn and Littlejohn, 2002; Cooper, 2003) and ultimately may affect ecotourism activities in the Western Cape Region.

Agriculture and the keeping of livestock have been identified as the largest cause of habitat destruction (Lombard et al., 1997). On the Agulhas Plain, 22.5 % of the Asteraceous Fynbos and

Renosterveld fertile soils are already covered in cereals, vineyards, pastures and cultivated flowers (UNDP, 2003). Studies on the vegetative propagation of some of the threatened Proteaceae species are lacking (Hartmann et al., 2002; Wu et al., 2007a). Their inception could increase awareness and facilitate rehabilitation of these species in their natural habitats. Future studies should also aim at providing information on the impact of current agricultural practices on sensitive habitats such as those involving Proteaceae and hence alleviating the threat on the biodiversity of these species on the Agulhas Plain.

1.3 PROMOTING ECOTOURISM THROUGH PROPER MAINTENANCE OF ENDANGERED PLANT SPECIES

The Agulhas Plain has the potential to become a major tourist destination with tourists visiting these sites for its beautiful scenery and large diversity of plant species (Cowling, 1993). In the Western Cape, most of these plant species form part of the Cape Floral Kingdom (CFK) which is documented as a world heritage site under exceptional habitat threat (Cowling and Richardson, 1995). Tourist visits to the Western Cape Province are expected to have grown with already 51% of international tourists visiting the area (ABI, 2008). The proximity of the Agulhas Plain region to both the Garden Route and the Cape Peninsula also presents positive ecotourism potential (ABI, 2008). The region has been recognised as having the most distinctive, most diverse and the highest density of endemic flora in the world (Coetzee and Littlejohn, 1994), yet the ecotourism potential in this area remains undeveloped (Cowling and Richardson, 1995), and many parts of the land are threatened with the loss of endemic species which form part of tourist attractions.

Therefore, landowners should realise the value of conserving the remarkable flora of the Agulhas Plain for future generations and the possible income benefit derived from ecotourism. This is supported by the fact that many farms on the AP are not sustainable for a variety of crops or livestock and would generate far more wealth in the development of ecotourism activities (Cowling, 1993; Paterson-Jones, 2000; Robyn and Littlejohn, 2002).

The Agulhas Plain is a region where conservation, ecotourism and agriculture could work together to maintain a balance of protection, enjoyment and commercial gain of the habitat. An increase in ecotourism on the Agulhas Plain is furthermore important in view of the role of extended conservation and job creation. More studies are needed to determine the effect that landowners, habitat destruction and the loss of red data species have on ecotourism development on the Agulhas Plain.

1.4 CONSERVING ENDANGERED SPECIES THROUGH CORRECT CUT FLOWER PRACTICES

The demand for new and interesting flowering plants around the world (Barzilay et al., 1992) has resulted in a strong interest in the diverse biodiversity of South African flora (Milandri et al., 2008). Most Proteaceae such as Leucadendrons are considered speciality flowers due to their unique and naturally bright colours. The export flowers are favoured for their bright yellow colour and attractive cones (Dodd and Bell, 1993; Coetzee and Littlejohn, 1994; Moody, 1995; Paterson-Jones, 2000; Berney, 2000; Jamieson, 2001). Export quantities for Leucadendron flowers increase by 27% in the European winter period (Rabie, 2002, Rabie, 2003). Some species that are exported include *L. laxum, L. stelligerum* and *L. platyspermum* (Rabie, 2002). These popular flowers continue to be harvested for commercial gains (Robyn and Littlejohn, 2002) and sometimes through incorrect cut flower practices such as over-harvesting and incorrect pruning methods. A total of 80% of Leucadendrons are harvested from low income farm land (Dodd and Bell, 1993; Mustard et al., 1997; McVeigh, 1998; Paterson-Jones, 2000; Robyn and Littlejohn, 2002; ABI, 2008). Over-harvesting of one species such as *L. laxum* from 436 hectares of habitat has caused this species to be listed as endangered in the CFK (Hilton-Taylor, 1996; Mustard et al*.,* 1997; Robyn and Littlejohn, 2002). Over-harvesting threatens many jobs of low-income people as natural habitats are depleted from a reserve of seed required for the next generation of species (Coetzee and Littlejohn, 1994). Because of the demand for *L. laxum*, more farmers should be encouraged to use sustainable cultivation and harvesting techniques, as field harvesting continues to destroy the habitats of many species.

It is therefore of great importance to investigate the harvesting practices of cut flower producers to determine at what level Red Data species are destroyed and which measures could be proposed for future conservation of the habitat.

1.5 ENVIRONMENTAL THREATS TO THE CONSERVATION AND PROPAGATION OF ENDANGERED PROTEACEAE

The habitats of many indigenous species such as those involving Proteaceae have been replaced by commercial vineyards. The Agulhas Plain produces high quality wines as the environmental conditions are conducive for vine growing (Hughes, 2002). These farming practices could pose further threats to the environment in which several members of Proteaceae family could struggle to survive. The impact of these new wine farms on the Agulhas Plain species remains unknown and the effect of further expansion of this farming practice remain uncertain and needs to be validated.

The Agulhas Plain consists of small pockets of broken up land where small scale dairy farming is practiced (Mustard et al*.,* 1997). Close to half of the cattle farms on the Agulhas Plain rely on natural habitat grazing, with cattle often grazing along wetlands (ABI, 2008). Many of these areas contain sensitive, endemic plant species, such as *L. laxum*. This type of grazing could further add pressure on the natural habitat of threatened species which could result in their extinction. The need for appropriate conservation measures by farmers is important to protect these sensitive habitats.

The spread of alien invasive species have been recorded as the second biggest threat to biodiversity on the Agulhas Plain, with more than 40% of the area being infested and 14.7% of the natural habitat being already lost to alien species (Turpie, 2004; ABI, 2008) such as Mediterranean Pine and Australian Acacia plant species (Mustard et al., 1997). These species have changed the soil pH and nutrient levels (ABI, 2008). Soil types have also become affected by layers of pine needles and leaf mulch, which prevent the germination of natural vegetation species (UNDP, 2003). The removal of alien species has always been problematic and costly (Coetzee and Littlejohn, 1994; De Villiers, 2002). For sustainable conservation of endangered plant species, measures to eradicate alien invasive species are important in reclaiming natural habitats.

The Agulhas Plain is largely affected by seasonal wild veld fires during the dry summer months (Seydack et al., 2007). Unnecessary and irresponsible burning of the natural habitat is a further threat which reduces the biodiversity. Poor fire management control systems (ABI, 2008) along with irresponsible burning in the habitat are dually responsible for the loss of many plant populations. Burnt landscapes can take years to recover and result in a loss of revenue from tourist’s visits to the area. This occurrence was seen during the 2006 fires on the Agulhas Plain which destroyed ecotourism establishments and in excess of 40 thousand hectares of land. The result of this fire left many local people without employment for two to three years.

Such occurrences of unplanned burning can have a large negative impact on post-fire regeneration and cause the further extinction of species (Cowling and Richardson, 1995; Coetzee and Littlejohn, 1994). The impact of fire on the biodiversity of species is complex. For instance, fire can reduce the seed production output of species through damage caused by the heat of the burning plant material but also assist in germination of seed (Brown and Duncan, 2006).

On the Agulhas Plain the existence of sensitive and threatened plant populations and their impeding environmental destruction has not been sufficiently publicised or brought to the attention of the landowners. The question should be asked whether threatened plant populations should not be made more visible by being marked by conservation authorities in their habitat, so service providers can be made aware of their locations? This suggestion would require concerted effort and co-operation between conservation authorities and municipal / provincial administration. The marking of threatened populations can also benefit tourists visiting the area. Ecotourism could provide nature lovers with the challenge to follow marked red data species trails to explore discover and enjoy these remarkable plant species in their natural habitat. To date there have been few studies on the causes of destruction of Red Data species on the Agulhas Plain. More studies targeting these aspects are recommended.

1.6 STRATEGIC PROPAGATION OF THREATENED RED DATA SPECIES

Plant species in nature will continue to survive provided conservation and horticultural efforts are made to maintain them (Jeffery, 2003). Informal interviews with farmers indicated that a lack of propagation techniques for threatened species exists. Many farmers practice basic propagation skills and some have developed and practise their own methods on an ad hoc basis. Many commercial growers are often reluctant to share techniques due to commercial competitiveness as their propagation information is not shared with other growers who may require the information (Laubscher, 1999).

On the Agulhas Plain, propagation of Red Data Species is mainly done by seeds. In this method, seed propagation can be natural whereby plants regenerate themselves in the natural habitat (Hartmann et al., 2002). Alternatively, seeds can also be used by flower producers to establish cut flower fields in their natural habitat (Robyn and Littlejohn, 2002). Unfortunately many seeds sown that have been introduced from other areas to an existing habitat have had a major impact on that environment. These foreign plant species, with new genes can change the biodiversity of an existing habitat irreversibly (Littlejohn, 2002) through genetic changes within species of that habitat.

Vegetative propagation techniques on the other hand would ensure faster rooting of higher quality plant products (Robyn and Littlejohn, 2002). Such plants also flower a year earlier than seed grown plants (Reinten et al., 2002) and have the potential to maintain the unique characteristics of the species (Brown and Duncan, 2006) and flower at predictable times (Reinten et al., 2002). An increase in the propagation of threatened species through vegetative means could benefit ecotourism where new plants could be replanted in the natural habitat where species are threatened, especially after fires (Robyn and Littlejohn, 2002). In addition, plants can also be grown for commercial plantations or for selling to other growers and farmers. The demand for Proteaceae such as L. *laxum* has increased to more than 23673 flower stems per annum (Middlemann, 2004). This growing market has created a further need for the improvement of propagation and cultivation techniques. Advanced techniques could also increase royalties and the sale of patented cultivars for future export purposes (Robyn and Littlejohn, 2002). Literature on propagation techniques of many species remains limited (Laubscher, 1999). Studies on the vegetative propagation of Red Data species are important to identify and expand the vegetative propagation possibilities of species such as *L. laxum* which has not been previously documented.

1.7 AUXIN TREATMENTS IN VEGETATIVE PROPAGATION OF DIFFICULT TO ROOT RED DATA SPECIES

Proteacea species are generally propagated vegetatively from 150 mm stem cuttings (SAFEC, 2002; Reinten et al., 2002). Cuttings should be taken during dry weather from semi-hardwood stems after shoot elongation (Aug-Nov), rinsed with a fungicide before planting and sprayed weekly with a fungicide (Ofori et al., 1996; Newton et al., 1992; Reinten et al., 2002; SAFEC, 2002). Limited studies have been found which document the influence of different concentrations of auxin on rooting Proteaceae (Perez-Frances et al., 1995). Most members of this family are categorised as difficult to root plants and may require special auxin treatments and special environmental conditions to facilitate their propagation (Hartmann et al., 2002; Wu et al., 2007a). Auxins have proved to stimulate root and shoot growth in some difficult to root plant species (Baraldi et al., 1993; Wu and du Toit, 2004; Wu et al., 2007a, b).

Auxins play an important role in speeding up the percentage of uniform rooting and in increasing rooting percentages of cuttings (Dodd and Bell 1993; Hartmann et al., 2002; Fogaça and Fett-Neto, 2005). It is uncertain what specific concentrations of Indole-3-butyric acid (IBA) or Indole-3-acetic acid (IAA) would be successful in rooting some Proteaceae species. An increase in root numbers using IBA has been reported in plants such as Olive, *Dorycnium* spp, *Shorea leprosula*, *Leucospermum patersonii, Leucospermum patersonii* and *Protea obtusifolia* (Wiesman and Lavee, 1995; Rodríguez Pérez, 1992; Aminah et al., 1995; Alegre et al., 1998). The auxin accumulation in shoots has been successful in vegetative propagation, however IBA applications could also inhibit rooting, or act together with other compounds to control plant functions such as root formation (Jones and Hatfield, 1976; Zimmerman, 1984; Volper et al., 1995; Hartmann et al., 2002; Reinten et al., 2002; Rout, 2006). Higher levels of auxin than those found in plant cells could result in cell death (Hartmann and Kester, 1983; Hartmann et al., 2002). IBA is one form of auxin that is effective in the rooting of a large number of plant species (Hartmann et al., 2002). In some woody species aryl esters and aryl amid of IBA are similar or more effective than acid formulations in root initiation (Hartmann et al., 2002).

Auxins such as IAA, supplied exogenously to cuttings, have been shown to promote rooting through meristematic cell division in many species (Liu et al., 1996; Liu et al., 1998; De Klerk et al., 1997; Robyn and Littlejohn, 2002; Hartmann et al., 2002; Rout, 2006). Natural occurring IAA in plants may not be adequate to enhance auxin functions (Hartmann et al., 2002). Applications of IAA may induce economically viable results in Proteaceae by increasing rooting percentages. The aryl esters of IAA are similar or more effective than acid formulations in root initiation. Several studies have reported correlations between higher IAA concentrations in plant tissues and rooting in different plant species (Hartmann et al., 2002). From the above background, it is clear that the need exists to test auxin application in propagating Red Data species by establishing the optimum level(s) of IAA necessary to stimulate root development. Information from such studies is likely to provide suitable auxin applications for individual plant species which are necessary to stimulate rooting. These methods are necessary to help farmers and growers to successfully propagate and cultivate Red Data species such as *L. laxum*.

1.8 INFLUENCE OF ROOTING MEDIUMS IN VEGETATIVE PROPAGATION OF DIFFICULT TO ROOT RED DATA SPECIES

A number of studies have shown that selecting the correct rooting medium for different plant species has a profound influence on the rooting of cuttings (Leakey et al., 1990; Ofori et al., 1996; Hartmann et al., 1983). Rooting mediums for Proteaceae should be light with good drainage and also retain sufficient moisture (SAFEC, 2002). Combinations of rooting components such as bark, peat, polystyrene and river sand should be tested to ensure faster, better quality root formation (Brown and Duncan 2006). Success in bark / polystyrene mediums has been reported in various Proteaceae species (Brown and Duncan, 2006; Reinten et al., 2002). Milled pine bark is a medium widely used and must be of good quality (Owings, 1996). However, past studies have shown that pine bark is very rich in phenolic compounds, alkaloids and cyanogenic glycosides (Machrafi and Prevost, 2006) which can also have an inhibitory effect on the growth of plants (Still et al., 1976; Rice, 1984). Good quality shredded milled pine bark is recommended for pH control (Owings, 1996) or can be substituted with a coarse grade peat moss to enhance the water holding capacity of the growing medium (Lamb, 1972; Hartmann et al., 2002; Reinten et al., 2002. Matkin (2008) also suggested using a medium of pine bark / peat as a good combination for rooting. An average pH of 6.5-7 is recommended for rooting Proteaceae (SAFEC, 2002).

Aeration and good water holding capacity are necessary characteristics for a rooting medium to positively favour the rooting physiology in different plant species (Grange and Loach, 1983; Reinten et al., 2002; Hartmann et al., 2002; SAFEC, 2002). Good aeration is essential for some Proteaceae species which are slower to root and the cuttings therefore require a longer period in the medium which must be of good quality. Polystyrene improves aeration, whereas washed river sand provides coarseness and drainage (Hartmann et al., 2002). A medium with a good air to water ratio such as bark and polystyrene is key to successful rooting (Leakey et al., 1990; Matkin, 2008). Adequate drainage should be provided to prevent *Phytophtora* fungal infection of roots (Reinten et al., 2002; SAFEC, 2002). Studies are needed to test the suitability of rooting mediums for individual Red Data species. Information from such studies is likely to provide improved rooting mediums which will stimulate better rooting of difficult to root Red Data species such as those found on the Agulhas Plain.

1.9 SELECTION OF IDEAL ROOTING ENVIRONMENTS

An ideal rooting environment is necessary to provide optimum conditions for successful rooting, thereby ensuring the maximum quantity and quality of rooted plants. Bottom heat (20-25 ºC) and misting are known to enhance rooting of Proteaceae cuttings (Brown and Duncan, 2006; Reinten et al., 2002). Propagation environments for many threatened Proteaceae species such as *L. laxum* have not been well established. Documentation exists on the rooting period for

Proteaceae species which varies between 8 and 12 weeks. However, some species are slower to root and would benefit from extended rooting periods in order to increase rooting percentages (Brown and Duncan, 2006; Kibbler et al., 2004). Facilities to root plants must be cost effective to make production economically viable for growers with limited greenhouse facilities (Laubscher, 1999). Atmospheric air circulation is essential in rooting Proteaceae cuttings (Reinten et al., 2002; SAFEC, 2002). No information has been found on rooting these species under shade house conditions. Cheaper built structures could prove to be more economical for farmers as the cost of construction, maintenance and electricity usage could be reduced. More efficient environmental propagation structures would in turn add value to the rapid multiplication and hence the domestication of plant species which are listed as endangered on the Agulhas Plain. Ecotourism could also benefit from guided tours of the propagation facilities on farms informing tourists how these interesting plants are grown. Most farmers have no propagation structures for rooting indigenous plant species. The need exist for the investigation of suitable structures which will guarantee success in propagation of Red Data species. Information from such studies is likely to provide guidance on cost effective and simpler propagation structures compared with expensive propagation greenhouses.

1.10 CONSERVATION STRATEGIES: INVOLVEMENT OF ALL STAKEHOLDERS

The lack of focus on priorities and the failure to reduce the ongoing degradation of the biodiversity on the Agulhas Plain remain causes for concern (Rebelo, 1997; Rouget et al., 2003). The success and development of ecotourism on the Agulhas Plain should be based on well planned conservation strategies as this region is recognized as having a biodiversity that has globally significant and irreplaceable vulnerable plant species (UNDP, 2003). Any viable and successful conservation strategies should involve all stakeholders dealing with ecotourism and biodiversity on the Agulhas Plain. Many institutions, including conservation, tourism and government departments have become directly involved in the biodiversity of the CFK (Younge and Fowkes, 2003). For instance, strategies for the Cape Action Plan for the Environment (C.A.P.E.) were formulated to inform landowners of their responsibilities in managing the biodiversity of the region (Lochner et al., 2003). These include the establishment of private and public conservancies (Cooper, 2003). Further efforts to control flower harvesting are supported by Sappex, who encourages the European Good Agricultural Practice (EuroGAP) code of practice with sustainable harvesting (Patterson, 2005). The Flower Valley Conservation Trust (FVCT) is a good example of practicing sustainable harvesting (Privet, 2002; ABI, 2008; Flower Valley, 2008). The Western Cape Nature Conservation Board (WCNCB) should play a major role in conserving the biodiversity and ecotourism on the Agulhas Plain, yet informal interviews with farmers for this study revealed a negative attitude towards the WCNCB because of poor management. The WCNCB lacks the capacity to police and control flower harvesting and to protect endangered species (ABI, 2008).

Effective conservation strategies involving all stakeholders are likely to reduce illegal harvesting and lessen the depletion of vulnerable Red Data species in the wild. Other conservation efforts include the purchasing of land for conservation, the control of alien invasive species and private and government land owners recognizing the commercial potential for ecotourism (Paterson-Jones, 2000). Exploitation of the environment could be minimized if the successful development of ecotourism potential is regulated by the conservation of the natural habitat on the Agulhas Plain by all stakeholders.

1.11 Conclusion

This review showed that threatened Proteaceae species such as *L. laxum* are continually being destroyed in the wild. The various factors responsible for the loss of important species which may attract tourists on the Agulhas Plain remain uncertain. Further studies are necessary to investigate the management practices of landowners, such as wild flower harvesting, orchard plantings and the control of fire and alien plants to conserve the endangered plant species on the Agulhas Plain. In the literature, there is little information on vegetative propagation of Red Data species. Developing improved propagation techniques of Proteaceae species such as the use of auxin applications, optimum rooting environment and mediums could help advance the growing and replacement of threatened species. Further studies should aim at developing new propagation techniques to advance the replanting of threatened species in the wild.

The objectives of the study are:

1. to collect and survey scientific data on current practices of landowners, flower producers and exporters to determine the probable causes of destruction of Red Data species and their influences on ecotourism development on the Agulhas Plain. This study aims to make recommendations on the propagation and the conservation of threatened species and the ecotourism potential on the Agulhas Plain;
2. to test the rooting ability of *L. laxum* using four liquid hormone concentrations and four different rooting mediums. The study aims to develop new propagation techniques to

increase successful and economical propagation of the species, to solve problematic and difficult propagation techniques and to relieve the threatened status of species:

- to assess the influence on callusing, root development and root length of *L. laxum* using IBA hormone and an environmentally controlled greenhouse with bottom heat:
- to assess the influence on callusing, root development and root length of *L. laxum* using IBA hormone and a shaded tunnel environment;
- to assess the influence on callusing, root development and root length of *L. laxum* using IAA hormone and an environmentally controlled greenhouse with bottom heat;
- to assess the influence on callusing, root development and root length of *L. laxum* using IAA hormone and a shaded tunnel environment.

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1.13 REFERENCES

ABI, 2008. Agulhas Biodiversity Initiative research report. http://www.flowervalley.org.za/abi.html [5 November 2008].

Alegre J, Toledo JL, Martinez A, Mora O, De Andres EF (1998). Rooting ability of *Dorycnium* spp. Sci. Hort. 76: 123-129.

Aminah H, Dick JMc.P, Leakey RRB, Grace J, Smith RI (1995). Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. For Ecol. Manag. 72: 199-206.

Baraldi R, Bertazza G, Predierii S, Bregoli A, Cohen JD (1993). Uptake and metabolism of Indole-3-butyric acid during the in vitro rooting phase in pear cultivar (*Pyrus comunis*). Acta. Hort. 329: 289-291.

Barzilay A, Ben-Jaacov J, Cohen A, Ion A, Halevy H (1992). Mini-gladiolus as a flowering pot plant. Sci. Hort. 49:117-124.

Berney J (2000). Combined research to benefit Protea growers. Aust. Hort. 98(2).

Brown N, Duncan G (2006). Manual for growing Proteaceae in greenhouses Joseph Knight in 1809 www.protea.worldonline.co.za/growknight.htm : 14.

Coetzee JH, Littlejohn G (1994). Indigenous South African Flower Industry. Hortagrow (8), August 1994.

Cooper KH (2003). A future for SA environmental conservation. Park and Grounds. May 2003. 50-52.

Cowling R (1993). Ecotourism - What is it and what can it mean for conservation. Veld and Flora, 79(1): 3-5.

Cowling R, Richardson D (1995). Fynbos. South Africa's Unique Floral Kingdom. Fernwood Press, Cape Town.

De Klerk G, Ter Brugge J, Marinova S. (1997). Effectiveness of IAA, IBA and NAA during adventitious roots, new concepts, new possibilities. In Vitro Cell. Dev. Biol. 35:188-189.

De Villiers T (2002). First Words: A farmer's viewpoint. Veld and Flora, 88(2): 42.

Dodd J, Bell DT (1993). Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. Aust. J. of Bot. 18:281-293.

Flower Valley (2008). Annual Report.
http://www.givengain.com/cause_data/images/1866/FlowerValleyReport2008.pdf
[9 February 2009].

Fogaça CM, Fett-Neto AG (2005). Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrance. Plant Growth Regul. 45: 1-10.

Grange RI, Loach K (1983). The water economy of un-rooted leafy cuttings. J. of Hort. Sci. 58 (10): 1-17.

Hartmann HT, Kester DE. (1983). Plant propagation; principals and Practices. Prentice-Hall International, London.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant Propagation Principles and Practices. 7th Edition. Prentice Hall. New Jersey :367-374.

Hilton-Taylor C (1996). Red Data List of Southern African Plants. Strelitzia 4. National Botanical Institute, Pretoria.

Hughes C (2002). The potential impact of viticulture expansion on biodiversity in the Cape Floristic Region. MSc Thesis, University of Cape Town, Cape Town.

Jamieson HG (2001). *Leucadendron laxum* I. Williams. Kirstenbosch Botanic Garden, Cape Town, South Africa.

Jeffery R (2003). The challenges for the defence force. Equus, 9(1): 8-9, March.

Jones OP, Hatfield SGS (1976). Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds. J. Hort. Sci. 51:495-499.

Kibbler H, Johnston ME, Williams RR (2004). Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell: 2 Seasonal influences of temperature, rainfall, flowering and auxins on the the stock plant. Sci. Hort. 102: 343-358.

Lamb, JGD (1972). The use of Peat in Propagating Hardy Nursery Stock at Kinsealy. Acta Hort. 26: 125-128.

Laubscher CP (1999). Rooting techniques for selected tree species. M Thesis. Unpublished. Cape Technikon, Cape Town.

Leakey RRB, Mesén JF, Tchoundjeu Z, Longman KA, Dick, JMcP, Newton A, Martin A, Grace J, Monro RC, Muthoka PN (1990). Low technology techniques for the vegetative propagation of tropical trees. Commonwealth For. Rev. 69: 247-257.

Littlejohn G (2002). Legal Issues, Planning and Planting. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Agricultural Research Council, Elsenburg, South Africa pp: 82-90.

Liu Z-H, Hsiao I-C, Pan Y-W (1996). Effect of naphthaleneacetic acid on endogenous indole-3-acetic acid, peroxidase and auxin oxidase in hypocotyls cuttings of soybean during root formation. Bot. Bull. of Aca. Sin. 37:247-257.

Liu Z-H, Wang W-C, Yen Y-S (1998). Effect of hormone treatment on root formation and endogenous indole-3-acetic acid and polyamine levels of *Glycine max* cultivated *in vitro*. Bot. Bull. of Aca. Sin. 39:113-118.

Lochner P, Weaver A, Gerderblom C, Peart R, Sandwith T, Fowkes S (2003). Aligning the diverse: The development of a biodiversity conservation strategy for the Cape Floristic Region. Biol. Con. 112: 29-43.

Lombard, A., Cowling, R.M. Pressey, R.L. & Mustard, P.J. 1997. Reserve design on the Agulhas Plain, South Africa: a flexible tool for conservation in a species-rich and fragmented landscape. Cons. Biol. 11: 1101-1116.

Machrafi Y, Prevost D (2006). Toxicity of phenolic compounds extracted from bark residues of different ages. J. Chem. Ecol. 32:2595-2615.

Matkin OA (2008). Perlite Vs. Polystyrene In Potting Mixes. The Schundler Company 150 Edison, NJ 08817 http://www.schundler.com/polystyrene.htm. [15 September 2008].

McVeigh S (1998). Fabulous fynbos a favourite for the small-scale farmer. Farmers Weekly, 4: 30-32.

Middlemann M (2004). Fynbos industry statistics - Statistics appendix. Sappex News, 120.

Milandri S, Laubscher CP, Ndakidemi P (2008). Hydroponic culture of *Gladiolus tristis*: Application of paclobutrazol for flowering and height control. Afr. J. Biotechnol. 7(3): 239-243.

Moody H (1995). Versatile Leucadendrons and Leucospermums. Aust. Hort. 5:111-112.

Mustard P, Cowling R, Albertyn J (1997). Southern Overberg. South African Wild Flower Guide 8. National Botanical Institute. National Book Printers. South Africa.

Newton AS, Mesén JF, Dick JMcP, Leakey RRB (1992). Low-technology propagation of tropical trees: rooting physiology and its practical implications. In: Mass production Technology for genetically Improved Fast Growing Forest Tree Species, Vol 2 AFOCEL, Nangis, pp: 417-324.

Ofori DA, Newton AC, Leakey RRB, Grace J (1996). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentrations, leaf areas and rooting medium. Forest Ecol. & Manage. 84: 39-48.

Owings AD (1996). Variations in pH from different bark sources. Combined Proceedings International Plant Propagators' Society, 46:699.

Paterson-Jones C (2000). The Protea family of Southern Africa. Struik Publishers. Cape Town.

Patterson T (2005). Sappex develops a code of practice. Talking Flowers Africa. J. for Floric. SA. July 2005.

Perez-Frances JF, Exposito A, Rodriguez JA (1995). Effect of different factors on in vitro multiplication of *Leucadendron* 'Safari Sunset' (Proteaceae). Acta Hort. 387**:** 115-120.

Privet S (2002). Putting fynbos to work on the Agulhas Plain. Veld & Flora, 88(2): 50-51.

Protea Atlas (2008). Current Rarity Status of the Proteaceae 1990. http://protea.worldonline.co.za/redstatus2.htm [30 October 2008].

Rabie M (2002). Export statistics for season June 2001 to July 2002. (sappex@hermanus.co.za). [15 June 2004].

Rabie M (2003). Research/ Navorsing – "Think Tank / Dinkskrum". (sappex@hermanus.co.za). [15 June 2004].

Rebelo AG (1995). *Proteas.* A Field Guide to the Proteas of Southern Africa. Cape Town: Fernwood Press.

Rebelo AG (1997). Conservation. In Cowling, R.M. Richardson, D.M. & Pierce, S.M. (eds). Vegetation of Southern Africa. Cambridge: Cambridge University Press: 571-590.

Reinten E, Gertze S, Arends L (2002). Propagation methods. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Elsenburg, South Africa, pp: 50-70.

Rice EL (1984). Allelopathy. Academic Press, Orlando, pp272-274.

Robyn A, Littlejohn G (2002). Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production, pp: 22-48.

Rodríguez Pérez JA (1992). Propagation by leaf bud cuttings of *Leucadendron* 'Safari Sunset', *Leucospermum cordifolium*, *Leucospermum patersonii* and *Protea obtusifolia*. Acta. Hort. 316: 35-46.

Rouget M, Richardson DM, Cowling RM, Lloyd JW, Lombard AT (2003). Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region. Biol Cons. 112: 63-95.

Rout GR (2006). Effect of auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. Plant Growth Regul. 48:111-117

SAFEC (2002). South African Flower Export Council: Preparing orchards for planting of PROTEACEAE. Certificate course in flower export readiness. Unpublished booklet. South Africa.

Seydack AHW, Bekker SJ, Marshall AH (2007). Shrubland fire regime scenarios in the Swartberg Mountain Range, South Africa: implications for fire management. Int. J. Wildland Fire, 16: 81-95.

Still SM, Dirr MA, Gartner, JB (1976). Phytotoxic effects of several bark extracts on mung bean and cucumber growth. J. Am. Soc. Hort. Sci. 101:34-37.

Turpie J (2004). The role of resource economics in the control of invasive alien plants in South Africa. SA J. of Sci. 100: 87-93.

UNDP (2003). United Nations Development Programme. Agulhas Biodiversity Initiative. Global Environmental Facility Project document: Cape Action Plan for the Environment. http://www.gefweb.org/Documents/Project_Proposal_for_Endorsm/South_Africa Agulhas Bio Initiative ABI. pdf [27 November 2003].

Volper R, Osswald M, Elstner EF (1995). Effects of cinnamic acid derivatives on indole acetic acid oxidation by peroxidase. Phytochem. 38:1-22.

Wiesman Z, Lavee S (1995). Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. Sci. Hort. 62: 189-198

Wu HC, du Toit ES (2004). Reducing oxidative browning during in vitro establishment of *Protea cynaroides.* Scientia Hort. 100:355-358.

Wu HC, du Toit ES, Reinhardt CF. (2007a). Micrografting of *Protea cynaroides*. Plant Cell Tiss. Cult. 89:23-28.

Wu HC, du Toit ES, Reinhardt CF, Rimando AM, van der Kooy F, Meyer JJM (2007b). The phenolic, 3,4-dihydroxybenzoic acid, is an endogenous regulator of rooting Protea cynaroides. Plant Growth Regul. 52:207-215.

Younge A, Fowkes S (2003). The Cape Action Plan for the Environment: overview of an eco-regional planning process. Biol. Cons. 112: 15-28.

Zimmerman RH (1984). Rooting apple cultivars in vitro: Interaction among light, temperature, phloroglucinol and auxin. Plant Cell Tiss. Cult. 3:01-311.

CHAPTER TWO

A SURVEY OF FARM-LEVEL PRACTICES ON ENDANGERED LEUCADENDRON SPECIES AND THE FUTURE INFLUENCE OF ECOTOURISM DEVELOPMENT ON THE AGULHAS PLAIN.

A SURVEY OF FARM-LEVEL PRACTICES ON ENDANGERED LEUCADENDRON SPECIES AND THE FUTURE INFLUENCE OF ECOTOURISM DEVELOPMENT ON THE AGULHAS PLAIN.

C.P. Laubscher[1], P.A. Ndakidemi[2]*, M.S. Bayat[1], A. Slabbert[1]

[1]Faculty of Business, Cape Peninsula University of Technology, PO Box 652, Cape Town, 8000, South Africa.

[2]Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 652, Cape Town, 8000, South Africa.

*Corresponding author. Charles P. Laubscher, Department of Horticultural Sciences, Cape Peninsula University of Technology, Cape Town, e-mail: laubscherc@cput.ac.za Tel: +2721-460-3198. Fax: +2721-460-3193.

Keywords: Land management; *Leucadendron laxum;* training; permits; propagation.

2.1 ABSTRACT

Large parts of the Agulhas Plain (AP) contribute to the commercial export cut flower industry of South Africa while many threatened and endemic species are destroyed. The Western Cape is the largest area where threatened Red Data species are not known and the illegal harvesting of these species continues. *Leucadendron platyspermum* was the most harvested of the surveyed species with the biggest harvests mainly reported from orchard plantations. Agriculture was perceived to be the lowest threat to the environment, compared to alien invasive species which was reported to be the biggest threat. Fire was seen as the most important factor to enhance the re-growth of species in nature. Permit possession was high; however the misuse of permits and the lack of authorities visiting farms were evident. The ecotourism potential of the Agulhas Plain remains undeveloped, although respondents reacted positively to conservation and the promotion of ecotourism on the Agulhas Plain. Training in the conservation of Red Data species remains low as a lack of information on Red Data species exists. A big concern is that very few Red Data species are propagated to increase the awareness of these species.

2.2 INTRODUCTION

South Africa has become a major tourist destination, with ninety percent of tourists visiting the country for its beautiful scenery and large diversity of plant species (Cowling, 1993). Most of these plant species form part of the Cape Floral Kingdom (CFK) in the Western Cape which is documented as a world heritage site under exceptional habitat threat (Cowling and Richardson, 1995). A total of 51% of tourists visit the Western Cape Province and these trends are expected to grow (Agulhas Biodiversity Initiative, 2008). One region, the Agulhas Plain (AP) which harbours species such as *Leucadendron stelligerum,* forms a large part of the CFK and has been recognised to have one of the most distinctive, diverse and the highest density of endemic flora in the world (Coetzee and Littlejohn, 1994). The potential of future ecotourism in this area remain undeveloped (Cowling and Richardson, 1995), and many parts of the land are threatened with the loss of endemic species. One of the reasons is the continual over-harvesting of cut flowers from threatened plant populations. Over exploitation of these species, such as *L. laxum,* has caused them to be included in the Red Data list of endangered species (Hilton-Taylor, 1996; Mustard et al., 1997; Robyn and Littlejohn, 2002).

Species harvested on the AP have an estimated net income of 1.15 million American dollars per year, with cut flower orchard yields from approximately 120 farms estimated at 200 000 dollars per year (Agulhas Biodiversity Initiative, 2008). This fast developing flower export market (Cowling and Richardson, 1995) has necessitated many Leucadendrons to be cultivated in field-grown orchards (Robyn and Littlejohn, 2002). Unfortunately, many species are continually harvested from the wild. This study was initiated with the concern that these threatened Red Data species on the AP are fast disappearing and that the ecotourism potential would be seriously damaged for future generations. Threats which were identified include the expansion of agriculture on the AP, alien invasive species, fire management, unsustainable harvesting of flowers and coastal development through urbanization (Agulhas Biodiversity Initiative, 2008).

This study aimed to collect data from farmers, cut flower growers and exporters to identify solutions for the conservation of the natural habitat and the growing need to develop ecotourism opportunities on the AP. The information from this study could help to reduce the destruction of endemic and threatened species and could encourage sustainable ecotourism development on the AP.

2.3 METHODOLOGY

2.3.1 Description of the study area

The survey study was conducted within the wild cut flower growing areas of the Eastern, Southern and Western Cape, South Africa to obtain a broader understanding of the core issues which affect the destruction of the natural habitat and the influence it might have on future ecotourism development of the CFK.

2.3.2 Selection of focus group

The South African Protea Producers and Exporters Association (SAPPEX) was selected as a research focus group. Permission was obtained from SAPPEX to ensure the protection of the rights of the survey respondents. Participants were purposely selected to fit the criteria of desirable participants, as many members of SAPPEX (landowners, farmers, producers and exporters) are actively involved in the wild cut flower industry and their contributions would be invaluable to the survey.

2.3.3 Secondary data collection

A literature study was conducted to ascertain and comprehend the scope and depth of the investigation for purpose of this study. Consequently the policies of the Department of Nature Conservation were also reviewed. The focus was on developing ideas through the gathering of information from smaller samples. The application of exploratory research is considered relevant as little documentation exits regarding the activities of producers on cut flower farms. The secondary data collection involved investigation into the current methods and practices of individuals involved in production and agriculture.

2.3.4 Survey questionnaire

A self-administered survey questionnaire was aimed at collecting data, assessing the threats, determining the needs of cut flower producers and ascertaining knowledge, skills, values and attitudes regarding activities and responsibilities in the conservation and protection of four selected threatened Red Data Leucadendron species. The quantitative research approach was adopted. Information was collected using standard open-end questions (Struwig and Stead, 2001; Hancock, 2002) comprised of closed questions with sets of answers to choose from. The questionnaire used 19 statements that contained various selection criteria which were grouped into 7 dimension factors, each concerned with a different variable. The dimensions included: geographical area, Red Data species, cut flowers, training, land management, legal factors and propagation. The questionnaire was pre-tested with a group of horticultural students. After appropriate revisions of the pre-tested instrument, a large-scale questionnaire survey was compiled. Geographical questions were placed in the beginning of the questionnaire to make the respondent feel at ease. Information was carefully assessed to ensure reliability and validity in using representative samples and structured collection procedures to generate data (Struwig and Stead, 2001). All participants were treated in the same way. The sensitivity of privacy and illegal harvesting of cut flowers was considered, as participants would not be willing to supply information freely. All questionnaires were anonymous and participants were informed that the questionnaire was voluntary and responses would be treated in strict confidence to provide more reliable information. The questionnaire was also translated into Afrikaans, as many Afrikaans speaking respondents would be more willing to participate if the questionnaire was in their home language. One hundred and twenty one questionnaires were posted to participants to allow for fair numbers of participation. It was decided that participants could also request electronic copies and return them by e-mail to ensure faster returns. Email questionnaires allowed for 14 days to be returned whereas questionnaires that were posted allowed a return of 30 days.

2.3.5 Statistical analysis

The responses were coded and analysed using a Statistical Package of Social Sciences (SPSS) 14.0 and most of the data collected was analysed using Code Book analysis with descriptive statistics and proportions (Sprent, 1993).

2.4 RESULTS AND DISCUSSION

Of the 121 questionnaires sent, thirty-four respondents (22.6%) returned the questionnaire within one month. The number of respondents was sufficient (n=34) to represent the growers in the study area, as background information of this population was available and the most valuable data were collected. As the number of respondents was lower than expected all members were taken as a sample representing the industry. The number of survey respondents in each area is summarized in Table 2.1. Many cut flower farms are owner managed; therefore more personalized interest and contact responses were received.

2.4.1 Geographical study area

A total of 76.5% of the respondents participated from the Western Cape, 17.6% from the Southern Cape and 2.9% from the Eastern Cape (Table 2.1). Participants were all members of SAPPEX and were either involved in growing or exporting cut flowers. As some respondents reflected small numbers from individual areas (reflective of the actual populations), especially the Eastern Cape, an area-to-area analysis was not feasible. More fittingly, data were aggregated to represent one large geographic region.

2.4.2 Threatened Red Data species

The study reported that *L. platyspermum* is the most harvested (17.6%) Leucadendron compared with the other three threatened Leucadendron species. As was expected, respondents reported that *L. platyspermum* is mainly harvested from planted fields (Table 2.2). Some respondents indicated that this species is not threatened as it occurs over many hectares of the Southern Cape. *L. platyspermum* is highly sought after for its female cones and the possibility exists for respondents to indicate that this species is not over harvested or threatened. It is interesting to see that some respondents indicated that they did not know the Red Data status of *L. platyspermum.* The Protea Atlas (2008) classifies *L. laxum* and *L. stelligerum* as endangered species and *L. elimense* and *L. platyspermum* as vulnerable species. These results are alarming and indicate that many respondents are uninformed in conservation of Red Data species. Only 2.9% of respondents reported harvesting *L. elimense* subsp. *Elimense, L. laxum and L. stelligerum* (Table 2.2). Other Leucadendron species that were picked regularly (64.7%) *were L. acuminate, L. catharinae, L. galpinii, L. glabrum, L. horifolia, L. marii, L. rubrum, L. salicifolium, L. strictum, L. tinctum* and *L. xanthoconus* and over-harvesting of *Berzelia alopercuroides, Brunia laevis, Brunia stokoeii, Erica fastigiata, Erica leucanthera, Erica perspicua, L. elimense, L. laxum* and *Mimetes hirtus* was reported. It was expected to see that over-harvesting of Red Data species continues and that the habitat remains under threat.

Response on threatened populations varied according to category (Table 2.2). Respondents indicated that alien invasive plant species are the biggest threat to Leucadendron populations, although the 20.6% response seems very low (Table 2.2). These results are in contrast with reports that the spread of alien invasive species has been recorded as the second biggest threat to biodiversity on the AP, where 14.7% of the natural habitat is lost and 40% is infested (Agulhas Biodiversity Initiative, 2008). According to Turpie (2004) the overall CFK area is the most heavily invaded with alien species. It was interesting to see that this result was followed by fire (17.6%) from respondents who should have reasonable knowledge that species will recover after fire. The least perceived threats to Red Data populations were farming practices (2.9%) and road works (2.9%), (Table 2.2). These results are in contradiction with the fact that agriculture has been identified as the largest cause of habitat destruction through cereals, dairy pastures, vineyards and cultivated flowers (Lombard et al., 1997). It appears that the respondents do not realize the impact of agriculture on the natural environment.

2.4.3 Cut flower Practices

In Table 2.3 a total of 38.2% of respondents harvest the Red Data Leucadendron species from cultivated orchards. The increase in orchard planting is a result of the Agricultural Research Council (ARC) fynbos unit priority for farmers to cultivate more than 70 commercially viable Proteaceae species for the export market (Dodd and Bell 1993; Coetzee and Littlejohn, 1994).

17.6% of the respondents harvest Leucadendron species from natural stands (Table 2.3). Some respondents (8.8%) also reported harvesting these Leucadendrons species from broadcasted seed fields (Table 2.3). Seed broadcasted into nature will germinate once conditions become favourable. Sustainable flower harvesting levels of some species are unknown and damage to the habitat continues due to over harvesting, poor harvesting techniques and removal of flower heads, the seed carriers of future generations (Agulhas Biodiversity Initiative, 2008).

In the question about quantity of Leucadendrons harvested (Table 2.3), 47.1% of the respondents reported harvesting more than 200 kg per year. Only 2.9% reported to harvest 20-199 kg (Table 2.3). Establishing inventories on volumes harvested by farmers is important to keep record of quantities harvested during each year. At the same time harvester competence can be calculated which in turn will aid in general conservation cultivation (Robyn and Littlejohn, 2002). Robyn and Littlejohn (2002) also reported that the biggest factor in the loss of biodiversity and habitat destruction is the continued over-harvesting of endemic species.

The results from Table 3 show that a total of 17.6% of the respondents stated that they prune while they harvest flowers from natural stands and 23.5% of the respondents reported that they harvest fifty percent of the flowers. 14.7% Claimed to harvest two thirds of the flowers per a plant and 5.9% harvest all the flowers on a plant (Table 2.3). It is alarming to see that some respondents are not practicing sustainable harvesting of cut flowers. Other respondents practised field harvesting, using contractors and plantation harvesting.

As was expected, most Red Data Leucadendrons are exported (61.8%), although 32.4% of the respondents reported their flowers are destined for the local markets (Table 2.3). Some respondents reported that the source of harvested material such as *L. platyspermum* determines its marketable quality, as field harvesting results in lower quality compared with flowers taken from orchards. A general response was provided that illegal harvesting and selling should be arrested, while conservation authorities should do more in communicating and visiting landowners, cut flower sellers and exporters.

A total of 67.6% of the respondents reported no seed harvesting from the four Leucadendron species (Table 2.3). It is interesting to see that 11.8% of the respondents reported they harvest seed from these Leucadendrons to use in broadcasted seed plantings (Table 2.3). According to Coetzee and Littlejohn (1994) approximately 1000 ha of Leucadendrons are cultivated from seed dispersal plants in the natural habitat. Only 2.9% of the respondents collect seed for selling purposes and only 8.8% collect seed to grow new plants (Table 2.3). The collection and selling of seed and growing new plants from seed is an essential part of increasing plant production in other areas. This study supports the Agulhas Biodiversity Initiative (ABI) (2008) suggestion that the cut flower industry should develop as a conservation industry to reduce pressure on endangered species. Sustainable flower harvesting should promote the future development of ecotourism on cut flower farms and alleviate the threat to the biodiversity and increasing land usage on the AP (Cole et al., 2000).

2.4.4 Training in cut flower growing and propagation

It is interesting to see that only 17.6% of the respondents had formal training, 44.1% have completed short courses in cut flower growing and 47% of the respondents were self-trained (Table 2.4). Cut flower short course training used to be one of the principle methods of the Agricultural Research Council in promoting indigenous cut flower enterprises. Farm employee training is important in the protection of the Red Data species. A total of 67.6% of employees were trained in alien invasive species and 35.3% were trained in potential cut flower species. It is alarming to see that only 14.7% of the staff received training on endangered species, and 14.7% of the respondents reported none of these training methods and make use of contract labour for harvesting (Table 2.4). These results indicate a greater need for more specialised training in the identification and protection of Red Data species. Training is important to protect sensitive habitats in locating them and managing them appropriately. Education on farms should be enhanced with training programs and information pamphlets on illegal harvesting and protection of Red Data species.

2.4.5 Land management practices

On the question relating to grazing in natural habitats, a total of 70.6% of the respondents stated that they do not allow livestock to graze in the natural habitat. Only 5.9% allowed grazing on a rotational basis, while 2.9% allowed controlled grazing in the natural habitat (Table 2.5). Cowling (1993) reported that most of the marginal agricultural land on the AP is unsustainable for livestock and that farm land would generate greater wealth if the land was developed for ecotourism activities. Other studies showed that close to half of the cattle farms on the AP rely on natural habitat grazing, where cattle often graze along wetlands (Agulhas Biodiversity Initiative, 2008). These areas where the destruction of endangered species continues are mainly the natural habitat for *L. laxum*. Landowners should create a balance in using plants, animals and natural resources to generating an income on their land (De Villiers, 2002). This revenue could be used in several ways including in the planning and developing the ecotourism activities on the AP.

Fire was reported as the highest (61.8%) factor in enhancing the re-growth of plants (Table 2.5). A total of 23.5% of the respondents said that nature should be allowed to continue its own fire regeneration. Some respondents (5.9%) reported that land should burn every 3-4 years, 6% reported land should burn every 6-7 years, and 14.7% reported fire needed to burn the habitat at intervals longer than 7 years. From our study and reports from other researchers, fires are important in the maintenance of the biodiversity of the CFK (Table 5; Kruger 1983; Cowling 1987; Bond and Van Wilgen 1996). It has been known for many years that planned and unplanned fires in the CFK assist in the germination of various indigenous plant species (Kruger 1983; Cowling 1987; Bond and Van Wilgen 1996). This is due to both the heat of the fires and the chemicals that are present in the smoke of burning plant material (Brown & Duncan, 2006). Unplanned burning can have a large negative impact on post-fire regeneration and therefore the further extinction of species (Cowling and Richardson, 1995; Coetzee and Littlejohn, 1994).

Birds were rated by 47.1% of the respondents as being essential in the reproductive phase of Leucadendron species. It is well known fact that birds are an important part of the CFK reproductive system (Paterson-Jones, 2000). According to Ryan and Bloomer (1999) more than 270 bird species have been recorded on the Agulhas Plain. A total of 38.3% of the respondents reported insects and 35.3% reported rodents to be important in the reproductive phases of Leucadendron (Table 2.5).

2.4.6 Legal factors related to indigenous cut flower species

In Table 6, the knowledge regarding the issuing of cut flower harvesting permits was high. A total of 70.6% of the respondents stated that they were issued with permits successfully (Table 2.6). Only a few respondents (2.9%) had little knowledge of which permits are required for cut flower harvesting, and 5.9% did not know the application procedure for permits. It is apparent that respondents were well informed about permit granting. Earlier documentation by Neiteler (2004) reported that conservation authorities were slow with the granting of permits. This study showed that farm visits by conservation authorities to those who have been granted permits was only 38% (Table 2.6). Our study is in agreement with the ABI, (2008) findings which reported that a lack of capacity exist in the Western Cape Nature Conservation Board (WCNCB) to: a) promote sustainable flower harvesting b) distribute endangered species list and c) to become more vigilant in farm visits. The general lack of information, support from authorities and feedback from cut flower associations were general concerns received by respondents regarding the availability of published information on Red Data species. Permission to use virgin land for any other activity besides production requires a site inspection, permit and possibly an impact study before any activity can commence (Littlejohn, 2002).

It was clear that permit possession was high, with 76.5% respondents having permits to sell protected flora, 58.8% to grow protected flora and 11.8% to cut flora with permission from landowners (Table 2.6). A general concern was raised by some respondents that permits get abused and illegal harvesting of Red Data species is not covered by the permits and reported incidents are not followed up with prosecutions. Vehicles should carry permits, and buyers should ensure that suppliers are in possession of permits (Sappex News, 2002; Littlejohn, 2002). Landowners should be more concerned in sustainable cut flower harvesting to protect and conserve the natural heritage of the CFK.

2.4.7 Propagation techniques

In this question respondents were asked to report how they propagate threatened Red Data species. Respondents reported that propagation of Red Data species was mainly done from seed (20.6%) and cuttings (14.7%), although 41.2% reported that they did not propagate any of these species (Table 2.7). Some respondents (2.9%) indicated other, but did not explain their methods. These results show either a lack of interest or knowledge of propagation of Red Data species. The need for developing and promoting propagation techniques of plants is important to expand plant availability of threatened species as well as cut flower stock material. Seed propagation is necessary when plants are reintroduced in the natural habitat. However, if seed from other areas are introduced into an existing area, new genes can change the biodiversity of that habitat (Littlejohn, 2002). Under such circumstances, vegetative propagation is necessary to retain the characteristics of species, especially those required for cut flower purposes (Hartmann et al., 2002).

Plants that are propagated by farmers are mainly used for new orchard plantings (20.6%), reintroduction in cleared areas (11.8%) and mother stock (11.8%). No plants are propagated to be sold (Table 2.7). Farmers should play a major role in growing endangered plant species and re-introduce cultivated plants into their natural habitat. The results of propagating species remain low and very little propagation is done to reintroduce threatened plant species into the natural habitants. From this background, there is a great need to develop new scientific methods for the vegetative propagation by identifying cheaper and sustainable techniques that could be used by flower growers with little financial resources.

2.5 CONCLUSION

The Agulhas Plain has been perceived as one of the most scenic destinations that can offer a variety of opportunity for unique plant species viewing in the natural habitats. Unfortunately, threatened Red Data species and their habitat are continuing to be destroyed as landowners, local communities and conservations authorities are not vigilant in identifying and conserving these sensitive and vulnerable habitats. It has been postulated that the main reasons for decline in the numbers of Red Data species of the CFK are the expansion of agriculture, lack of propagation skills and the spread of invasive plant species in the natural habitants. Another major constraint was the lack of information on the identification of Red Data species. Unless these problems are addressed, the environmental destruction of the natural habitat and the exploitation of Red Data species will continue and the potential to improve the ecotourism industry of this area will remain undeveloped. There is no question whether foreign and national tourists will visit the Agulhas Plain if the natural habitat of threatened Red Data plant species can be preserved and the current poor infrastructure for ecotourism activities can be developed, as the current economic climate makes travelling to South Africa an affordable destination. The need for scientific development of vegetative propagation exists to promote and save Red Data species such as *L. elimense* subsp. *Elimense, L. laxum, L. platyspermum and L. stelligerum*.

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2.7 REFERENCES

Agulhas Biodiversity Initiative (2008). Agulhas Biodiversity Initiative Research report. http://www.flowervalley.org.za/abi.htm. [5 November 2008].

Bond WJ, Van Wilgen B (1996). Fire and Plants. Chapman and Hall, London.

Brown N, Duncan G (2006). Grow fynbos plants. South African National Biodiversity Institute, Claremont, Cape Town.

Coetzee JH, Littlejohn G (1994). Indigenous South African Flower Industry. Hortagrow. August 1994:9-11.

Cole N, Lombard AT, Cowling RM, Euston-Brown D, Richardson DM, Heijnis CE (2000). Framework for a conservation plan for the Agulhas Plain, Cape Floristic Region, South Africa. Institute for Plant Conservation, University of Cape Town, Cape Town.

Cowling, RM (1987). Fire and its role in coexistence and speciation in Gondwanan shrublands. S. Afr. J. of Sci., 83:106-112.

Cowling R (1993). Ecotourism - What is it and what can it mean for conservation. Veld and Flora, 79(1):3-5.

Cowling R, Richardson D, (1995). Fynbos. South Africa's Unique Floral Kingdom. Fernwood Press, Cape Town, pp. 7-31.

De Villiers T (2002). First Words: A farmer's viewpoint. Veld and Flora, 88(2): 42.

Dodd J, Bell DT (1993). Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. Aust. J. of Bot., 18:281-293.

Hancock B (2002). Trent focus for research and development in primary health care: An introduction to qualitative research. Trent Focus, 1998:1-27.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant Propagation Principles and Practices. 7th Edition. Prentice Hall. New Jersey, pp. 367-374.

Hilton-Taylor C (1996). Red Data List of southern African plants. Strelitzia 4. National Botanical Institute, Pretoria.

Kruger FJ (1983). Plant community diversity and dynamics in relation to fire. In Mediterranean-type Ecosystems. The Role of Nutrients. (Eds FJ Kruger, DT Mitchell, JUM Jarvis), Springer Verlag: Berlin, pp. 446-472.

Littlejohn G (2002). Legal Issues, Planning and Planting. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Elsenburg, Stellenbosch.

Lombard A, Cowling RM, Pressey RL, Mustard PJ (1997). Reserve design on the Agulhas Plain, South Africa: a flexible tool for conservation in a species-rich and fragmented landscape. Cons.Biol., 11:1101-1116.

Mustard P, Cowling R, Albertyn J (1997). Southern Overberg. South African Wild Flower Guide 8. National Botanical Institute. National Book Printers, Cape Town.

Neiteler D (2004). Report on fynbos. IPGA News Letter. Cape Town, South Africa, MAY 2004

Paterson-Jones C (2000). The Protea family of Southern Africa. Struik publishers. Cape Town, pp. 28-124.

Protea Atlas (2008). Current Rarity Status of the Proteaceae 1990. http://protea.worldonline.co.za/redstatus2.htm [30 October 2008].

Robyn A, Littlejohn G (2002). Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production. Elsenburg, South Africa, pp. 22-49.

Ryan PG, Bloomer P (1999). The long-billed lark complex; a species mosaic in South-Western Africa. The Auk, 116: 194-208.

Sappex (2002). Conservation of the Cape Floral Kingdom. Sappex news, 112 :17.

Sprent P (1993). Applied Nonparametric Statistical Methods, 2nd ed Chapman & Hall, London.

Struwig FW, Stead GB (2001). Planning, designing and reporting research. Masker Miller Longman, Cape Town.

Turpie J (2004). The role of resource economics in the control of invasive alien plants in South Africa. S. Afr. J. of Sci., 100: 87-93.

Table 2.1: The distribution of respondents according to their geographical location in South Africa. (n=34)

Province	**Place names**	**Number Respondents (n=34)**	**Percentage Respondents (%)**
Eastern Cape	Kareedouw	1	3.0
Southern Cape	Barrydale, Knysna, Riversdal	6	18.2
Western Cape	Ceres, Citrusdal, Botrivier, Elim, Elgin, Gansbaai, Hermanus, Kleinmond, Porterville, Napier, Somerset Wes, Stanford, Stellenbosch, Riviersondered, Stanford, Worcester	27	78.8
Total		34	100

Table 2.2: Distribution of respondents according to harvesting status, occurrence and categories of threat to Red Data species. (n=34)

Question	**Criteria**	**Yes (%)**	**No (%)**
Do you harvest the following endangered species for cut flower purposes?	*L. elimense* subsp. *elimense*	2.9	97.1
Do you harvest the following endangered species for cut flower purposes?	*L. laxum*	2.9	97.1
Do you harvest the following vulnerable species?	*L. platyspermum*	17.6	82.4
Do you harvest the following vulnerable species?	*L. stelligerum*	2.9	97.1
Do you harvest any other Red Data species?	other Red Data species	64.7	35.3
Are any of these Leucadendron populations threatened by the following practices?	farming practices	2.9	97.1
Would you rate the following to be a threat to Red Data species?	alien plants	20.6	79.4
Would you rate the following as a threat?	fire	17.6	82.4
Would you consider the following to be a threat to Red Data species?	road works	2.9	97.1
Would you agree that these Leucadendron populations are not threatened?	no threat	61.8	38.2

Table 2.3: Distribution of respondents according to their cut flower harvesting status. (n=34)

Question	**Criteria**	**Yes (%)**	**No (%)**
Do you harvest endangered Leucadendron cut flowers from the following areas?	natural stands	17.6	82.4
Do you harvest these cut flowers from the following areas?	broadcasted seeds	8.8	91.2
Do you harvest endangered cut flowers from commercial orchards?	planted orchards	38.2	61.8
Do you harvest these species from any other areas?	other areas	5.9	94.1
What quantity of these species do you harvest?	less than 20kg	0	100
Do you harvest approximately the following quantity?	20 - 199kg	2.9	97.1
Do you harvest more than the following quantity?	more than 200kg	47.1	52.9
When harvesting from natural areas do you practice the following harvesting technique?	cut all flowers that are ready	5.9	94.1
Do you practice the following technique during harvesting?	cut 50% of all flowers	23.5	76.5
Do you prefer to practice the following harvesting technique?	cut 2/3 of all flowers	14.7	85.3
At harvesting do you practice the following technique?	prune while you cut	17.6	82.4
Would you suggest any other than the above harvesting measures?	other	8.8	91.2
Which market do you supply?	local	32.4	67.6
Do you supply the export market?	export	61.8	38.2
Do you supply different markets to the above?	other	2.9	97.1
Do you harvest any seed from Red Data species?	no harvesting	67.6	32.4
Do you harvest any seed for the following use?	broadcast seed	11.8	88.2
Do you harvest any seed for the following use?	collect and sell	2.9	97.1
Do you harvest any seed for the following use?	collect and grow	8.8	91.2

Table 2.4: Distribution of respondents according to their training status. (n=34)

Question	**Criteria**	**Yes (%)**	**No (%)**
Do you have the following training in cut flower harvesting?	formal education	17.6	82.4
Have you received any training from the following?	short courses	44.1	55.9
Is your training mainly the following?	self trained	47	53
Do you have any training different to the above?	other	17.6	82.4
Has your staff been trained in the following?	invasive species identification	67.6	32.4
Has your staff been trained in the identification and conservation of the following?	rare species	14.7	85.3
Does your staff receive training in the following?	potential cut flower species	35.3	64.7
Does your staff have training in none of the above?	none of these	14.7	85.3

Table 2.5: Distribution of respondents according to land management practices. (n=34)

Question	Criteria	Yes (%)	No (%)
Do you allow your livestock to graze in natural areas in the following way?	controlled	2.9	97.1
Do you practice grazing of livestock in natural areas in the following way?	uncontrolled	0	100
Does your livestock graze in the natural habitat in the following way?	on a rotational basis	5.9	94.1
Do you agree that livestock should not graze in the natural habitat?	yes	29.4	70.6
Do you practice grazing of livestock in these areas in any other way?	other	5.9	94.1
Do you think the following factor is responsible for the reproductive phases of Proteaceae species?	fire	61.8	38.2
Does the following factor play a role in the reproductive phases of Proteaceae species?	rodents	35.3	64.7
Does the following factor play a role in the reproductive phases of Proteaceae species?	insects	38.2	61.8
Does the following factor play a role in the reproductive phases of Proteaceae species?	birds	47.1	52.9
If not satisfied with the above do you know of any other reproductive strategies?	other	5.9	94.1
Are you aware that some species may require fire to stimulate germination of seed?	no	29.4	70.6
Would you say that you do not practice controlled fires and that you agree with the following?	allow nature to continue	23.5	76.5
Do you practice controlled fires to stimulate new reproductive growth and do you agree that fields should burn on the following intervals?	every 3-4 years	5.9	94.1
Do you practice controlled fires to stimulate new reproductive growth and do you agree that fields should burn on the following intervals?	every 6-7 years	6	94
Do you practice controlled fires to stimulate new reproductive growth and do you agree that fields should burn on the following intervals?	longer than 7 years	14.7	85.3
If not any of the above do you recommend any other method?	other	20.6	79.4

Table 2.6: Distribution of respondents according to legal factors regarding trading of indigenous plant species. (n=34)

Question	**Criteria**	**Yes (%)**	**No (%)**
Are you satisfied with the way conservation bodies issue harvesting permits?	farm visits from conservation authorities	38	62
Have you been issued with a permit successfully?	permits have been issued successfully	70.6	29.4
Are you aware that you need a permit?	permit possession / awareness	2.9	97.1
Do you know the application procedure for permits?	permit application procedure	5.9	94.1
Do you have the following permit?	permit to sell protected flora	76.5	23.5
Do you have the following permit?	permit to grow protected flora	58.8	41.2
Have you been issued with the following permit?	permit to cut with permission	11.8	88.2

Table 2.7: Distribution of respondents according to propagation practices of Red Data species. (n=34)

Question	**Criteria**	**Yes (%)**	**No (%)**
Do you propagate any Red Data species?	propagation	41.2	58.8
Do you propagate any Red Data species from the following?	seed	20.6	79.4
Do you propagate any Red Data species from the following?	cuttings	14.7	85.3
Do you propagate any Red Data species from the following?	layering	0	100
If not one of the above methods, do you use any other methods?	other	2.9	97.1
Are these propagated plants used for the following?	reintroduction into cleared areas	11.8	88.2
Are your propagated plants used for the following?	selling	0	100
Are these propagated plants used for the following?	planted into cut flower orchards	20.6	79.4
Do you use propagated plants for the following purpose?	as mother stock	11.8	88.2

CHAPTER THREE

ROOTING SUCCESS USING IBA AUXIN ON ENDANGERED LEUCADENDRON LAXUM (PROTEACEAE) IN DIFFERENT ROOTING MEDIUMS

Full Length Research Paper

Rooting success using IBA auxin on endangered Leucadendron laxum (PROTEACEAE) in different rooting mediums

C. P. Laubscher[1] , and P. A. Ndakidemi[2]*

[1]Faculty of Business, Cape Peninsula University of Technology, P. O Box 652, Cape Town 8000, South Africa.

[2]Faculty of Applied Sciences, Cape Peninsula University of Technology, P. O Box 652, Cape Town 8000, South Africa.

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*Corresponding author. E-mail: ndakidemip@cput.ac.za. Tel: +2721-460-3196. Fax: +2721-460-3193.

3.1 Abstract

Leucadendron laxum (Marsh rose Leucadendron) was tested for its rooting ability as an endangered plant species for reintroduction into the natural habitat, using IBA liquid hormone preparations and four growth media. The treatments included a control, 500, 1000, 2000 and 4000 ppm concentrations. Four growth media, namely: bark and polystyrene; peat moss and polystyrene; bark, river sand and polystyrene; and perlite and river sand were tested in an environmentally controlled greenhouse with bottom heat. The experiments used a randomised block design with 10 cuttings per treatment and replicated four times. The results showed that the bark, sand and polystyrene medium had the highest (45%) significant survival rate when supplied with IBA at 500 ppm. The application of 1000 ppm of IBA in bark, sand and polystyrene medium significantly increased the number of roots per cutting (5) and produced the longest roots (4 cm). The bark and polystyrene medium supplied with zero IBA had the largest number of roots per cutting (6.5). The comparison of the four growth media showed that bark and polystyrene was the best medium that produced the highest and most significant callusing formation (30%), % of cuttings that rooted (28%), cuttings with a higher survival rate (57%), and cuttings with a higher number of roots (3.5) and the longest roots (7.35 mm). With regard to various IBA treatments tested across all treatments, the IBA supplied at 1000 ppm produced greater root numbers when compared with all other treatments.

Key words: Hormone treatment, red data species, rooting percentage, vegetative propagation.

Abbreviations: IBA, Indole-3-butyric acid; **ppm**, parts per million; and **SAFEC**, South African Flower Export Council.

3.2 INTRODUCTION

Leucadendron laxum belongs to the Proteaceae family and is mostly found near the most southern point of the African continent, where the Atlantic and Indian Oceans meet. It grows on level, damp ground in the valleys between Hermanus, Bredasdorp and Agulhas (Williams, 1972). The existence of this plant in the wild is not certain, as its habitat is being drained by natural catastrophes such as wild fires and farmers (Mustard et al., 1997; Robyn and Littlejohn, 2002). As a result, *L. laxum* has been named an endangered species in South Africa (Hilton-Taylor, 1996).

Some Leucadendrons are naturally rare owing to reproductive reasons, wild fires and competition with other species, such as the spread of alien invasive species (Paterson-Jones, 2000). One report revealed that *L. laxum* has only 5 000 remaining plants in the natural habitat (Mustard et al., 1997). Improved plant propagation techniques are essential at this point to help save the remaining small plant populations. *L. laxum* is an important plant that is aesthetically pleasing with its bright yellow flowers and attractive cones (Jamieson, 2001). It has a potential role in the flower and tourist industry in South Africa. As a member of the Proteaceae hybrids, these plants can be propagated by vegetative means to maintain the unique characteristics especially cut flower cultivars (Brown and Duncan, 2006) and produce uniform flowering times (Reinten et al., 2002). However, most vegetative techniques applied by Protea growers have not been published (Laubscher, 2000). To our knowledge, the vegetative propagation of *L. laxum* has not previously been described and new documentation would be seen as new research.

The appropriate vegetative propagation techniques for *L. laxum* can speed up the replacement of plants on damaged and reclaimed land and also increase production of cut flower cultivars in nurseries. Improved propagation techniques can also increase royalties and the sale of patented cultivars with an increase in quality and quantity of future export cut flowers. Therefore, faster rooting techniques are important to help save small plant populations such as *L. laxum*, especially after fires (Robyn and Littlejohn, 2002). Improved cultivation and management practices in orchards (SAFEC, 2002) need to be investigated, as threats to the natural habitat continue to escalate. Research evidence suggests that auxins play a central role in the determination of rooting capacity, by enabling the faster production of rooted cutting material which is essential for vegetative propagation (Fogaça and Fett-Neto, 2005). Auxins are known to increase rooting percentage and rooting time together with uniformity of rooting (Hartmann et al., 2002). Under natural conditions, some Proteaceae cuttings are difficult to root, and few species have shown response to auxin treatments (Hartmann et al., 2002).

Research into various auxin concentrations and different rooting media could prove useful to the horticultural industry by enabling the faster production of rooted cutting material. IBA is one form of auxin that is effective in the rooting of a large number of plant species (Hartmann et al., 2002). In some woody species aryl esters and aryl amid of IBA are equal or more effective than acid formulations in root initiation (Hartmann et al., 2002). The use of different concentrations of IBA on *L. laxum* will identify the treatment that produces desirable rooting results. In the literature, there is no available information on the effect of auxin on rooting of *L. laxum* and further experimentation will be documented as new research.

Testing combinations of rooting components should ensure faster and better quality root formation. Components such as bark, peat, polystyrene and river sand should be tested in various combinations. Shredded milled pine bark is a medium widely used in rooting mediums to manage the pH factor, provided the quality is from a good source (Owings, 1996). Polystyrene is a coarse mineral component which provides an air-filled porosity that improves aeration, whereas river sand mostly used in combination with organic materials provides coarseness and drainage. Coarse grade peat moss similar to bark lowers the pH and enhances the water holding capacity of growing mediums (Hartmann et al., 2002).

The aim of this study is therefore to determine whether *L. laxum* responded favourably to different concentrations of IBA rooting hormones and various rooting mediums to ensure faster and more efficient rooting success in pot-plant and cut flower production.

3.3 MATERIALS AND METHODS

3.3.1 Experimental

The experiment was conducted in an environmentally controlled greenhouse, at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa. The clear polycarbonate greenhouse was fitted with a shade screen (40%), where temperature and humidity were monitored on a weekly basis. Midday temperatures fluctuated between 22 -27°C and relative humidity between 39 -86%. Bottom heat (20 -25ºC) and mist bed conditions were supplied (Brown and Duncan, 2006). The irrigation timer was set on 15 s on and 20 min off. The experiment started during the middle of August and continued for 8 weeks. Cutting material of *L. laxum* was collected from selected plant populations in their natural habitat on the Agulhas Plain on the extreme south-western coast of South Africa. The material was kept dry in a sealed plastic bag and transported overnight.

3.3.2 Setup and design

A randomised complete block design involving four growth mediums (bark and polystyrene; peat moss and polystyrene; bark, river sand and polystyrene; and perlite and river sand and four concentrations of IBA (control, 500, 1000, 2000 and 4000 ppm) were used in this study. Altogether 4 × 5 × 10 cuttings were involved in the experiment. The medium had to be light with good drainage and also had to retain some moisture to prevent quick drying; had a pH of 6.5 -7 is preferred (SAFEC, 2002). For each treatment, cuttings were replicated in 10 pots. The experiment was conducted in a controlled greenhouse with misting irrigation and heated beds. Readings of the greenhouse environment, that is, solar radiation, temperature, relative humidity and irrigation were monitored on a weekly basis.

3.3.2 Cuttings, IBA treatment and planting

Cuttings were taken from turgid stems of semi-hardwood stems after shoot elongation (Aug-Nov). Cuttings were taken in dry weather (SAFEC, 2002). For this experiment, terminal cuttings measuring approximately 150 mm long were used. All cutting material was rinsed in Benlate fungicide (10 g/l) before planting. After planting, cuttings were sprayed weekly with Captan (2 g/l) solution (Reinten et al., 2002; SAFEC, 2002). Wilted and infected cuttings were removed over the rooting period (Brown and Duncan, 2006).

Hormone treatments used IBA liquid preparations of 500, 1000, 2000, 4000 ppm. The control remained untreated. The IBA powder was dissolved in 50% ethyl alcohol and made up to the appropriate concentration by adding distilled water (Brown and Duncan, 2006). Prior to planting, the basal 5 mm of cuttings were dipped for 5 s in the rooting hormone (Reinten et al., 2002; Hartmann et al., 2002). Cuttings were then individually planted into new plug foam trays/ pots containing different growth mediums and IBA concentrations.

3.3.3 Data collection and statistical analyses

Samples of cuttings were drawn from the experiment on a weekly basis to monitor results of wounding, hormone treatment, rooting medium, callusing and rooting progress. The factors measured were, average shoot growth, number of cuttings callused, number of cuttings rooted, number of roots on cuttings, length of roots, and the survival rate of cuttings. Shoot growth (mm) and the length of roots (mm) were measured from cutting stage to new growth after 8 weeks. The number of cuttings callused, rooted and survival rates were counted after 8 weeks.

Data was recorded for the percentage of survival rate of cuttings, number of roots per rooted cutting and root length. Data analysis was performed in two different ways. The first consisted of one way analysis of variance for IBA treatments added to each growth medium separately. The second consisted of factorial analysis including the four growth mediums and the five IBA concentrations. Data on number of cuttings that formed callus, the number of cuttings that rooted and the cuttings that survived were transformed into percentages prior to analysis of variance. Data were presented as mean values with predicted standard errors (S.E.). These computations were done with the software program STATISTICA. The Fisher least significance difference (L.S.D.) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of different concentrations of IBA on the four rooting mediums

The four rooting mediums used in our study responded differently to IBA concentrations. The cuttings planted in bark, river sand and polystyrene, and bark and polystyrene rooting mediums were significantly affected by IBA concentrations in certain parameters (Tables 3.1 and 3,2). Plant cuttings inserted in peat moss and polystyrene and perlite and river sand mediums at different IBA concentrations influenced the root formation although their differences were not statistically significant (Tables 3.1 and 3.2). The IBA supplied to bark, river sand and polystyrene medium significantly ($P \leq 0.05$) affected the percentage survival rates, number of roots formed per cutting and root length (Table 3.1). The highest survival rate of cuttings was recorded in the 500 ppm treatment (45%), followed by the 1000 ppm treatment (25%). The higher survival rate observed in this rooting medium is probably due to the well drained conditions of the sand component which encouraged adequate oxygen circulation in the rooting zone during the rooting process (Hartmann et al., 2002). The positive fact that the cuttings survived over the 8 week period in this medium is an indication that rooting percentages could be increased if the rooting period was to be extended. According to Brown and Duncan (2006), rooting of cuttings starts between 6 and 8 weeks, and cuttings should be well rooted within 8 to 16 weeks.

The root length in bark, river sand and polystyrene medium supplied with IBA significantly ($P \leq 0.05$) increased from no reaction in the control to 4 mm in the 1000 ppm treatment (Table 3.1). The root length was significantly reduced to 0.5 mm with successive increases of IBA concentration to 4000 ppm. Taken together (% survival rate, number of roots per cutting and root length) our results indicated that the bark, river sand and polystyrene medium supplied with 1000 ppm IBA was the best treatment as it significantly ($P \leq 0.05$) resulted in successful survival rate of the cuttings (25%), number of roots formed (5) and root length (4 mm) of cuttings (Table 3.1). The successful rooting of *L. laxum* in this study was achieved at 1000 ppm and concentrations above this level inhibited root initiation and development (Table 3.1). The results are in agreement with those reported by Hartmann et al. (2002), who found that auxin concentrations of 500 to 1250 ppm are suitable for softwood cuttings and any levels higher than those found in the plant tissues may cause cell death.

The plant cuttings of *L. laxum* supplied with IBA and inserted in the bark and polystyrene rooting medium was significantly ($P \leq 0.05$) influenced only in the roots formed per cutting (Table 2). The control treatment formed 6.5 roots per cutting, and this was followed by supplying IBA at 500 and 1000 ppm (Table 3.2). Brown and Duncan, (2006), Reinten et al. (2002) and SAFEC (2002) documented the success of bark and polystyrene in rooting Proteaceae. Two other treatments (500 and 1000 ppm) in this medium were also highly significant ($P \leq 0.05$) but resulted in fewer roots (3.5 and 4.3, respectively). The IBA treatments were not as conducive to rooting however, both mediums contained polystyrene which could be linked with the availability of higher aeration in the rooting zone. Rooting numbers also progressed in this medium owing to higher moisture availability of bark in the rooting medium.

3.4.2 Comparisons in growth mediums and IBA concentrations

Among the four mediums tested, bark and polystyrene was significantly ($P \leq 0.01$) superior to all others in influencing all of the measured parameters (Table 3.3). The highest percentage of callus formed (30%), rooting (28%), survival rate (57%), number of roots per cutting (3.5) and rooting length (7.35 cm) was found in the bark and polystyrene medium (Table 3). The peat and polystyrene medium produced the second best overall results (Table 3.3). In comparison with all the mediums used these two components (bark or peat) provided more moisture in the rooting zone and both had polystyrene for aeration as observed in the higher survival rate of the cuttings (Table 3.3). Other investigations have revealed that rooting mediums may have a profound effect on root formation of cuttings (Hartmann *et al.,* 1990). The aeration and water holding capacity of mediums are of paramount importance (SAFEC, 2002). The cuttings planted in bark, river sand and polystyrene and in polystyrene and perlite showed significantly little effect on influencing callus formation, rooting, survival rate, number of roots per cutting and rooting length (Table 3.3). These mediums had relatively low water-holding capacities.

With regard to various IBA treatments tested across all treatments, there was no significant influence to percentage callus formed, percentage rooting, percentage survival rate and rooting length. However, the number of roots per cutting was significantly ($P \leq 0.05$) affected by IBA treatments (Table 3.3). The IBA supplied at 1000 ppm produced greater root numbers as compared with other treatments (Table 3.3). The increase in root numbers after the IBA supply has also been reported in plants such as olive, Dorycnium spp., *Shorea leprosula*, *Leucospermum patersonii*, *Leucospermum patersonii* and *Protea obtusifolia* (Alegre *et al.,* 1998; Aminah *et al.,* 1995; Rodríguez Pérez, 1992; Wiesman and Lavee, 1995). In this study, lower concentrations of IBA were thus more conducive to root formation in Leucadendron. The rooting at higher IBA concentrations was significantly reduced. Too high concentrations of IBA may be toxic to cuttings of some plant species and may reduce cell differentiation in the tissues and finally affect the rooting (Hartmann and Kester, 1983; Hartmann *et al*., 2002).

3.4.3 Growth medium x IBA interaction

The growth medium x IBA interaction was significant ($P \leq 0.05$) only for number of roots per cutting (Figure 3.1). In general, the number of roots per cutting was increased most by IBA concentrations applied to bark and polystyrene and peat and polystyrene mediums. The use of bark or peat in rooting mediums for most Proteaceae is seen as essential as both hold added moisture in the medium (Reinten *et al*., 2002).

3.5 Conclusion

A hormone application of 1000 ppm IBA was successful in rooting *L. laxum*. Both bark, sand and polystyrene and the bark and polystyrene mediums contributed to successful root development of cuttings. However, the bark, sand and polystyrene medium was more successful in the survival rate and root length of cuttings. Further tests on *L. laxum* need to be conducted using different combinations of auxins and different environments to produce maximum quantity and quality rooted plants. The successful production of quality cuttings for the industry is largely defined by the grower who is able to use the correct auxins treatments and rooting mediums to produce quality Proteaceae cuttings which will succeed when planted in the field.

3.6 ACKNOWLEDGEMENTS

We thank Assegaai Bosch Farm on the Agulhas Plain for their donation of *L. laxum* cutting material. We are also grateful to the Department of Horticultural Sciences, Cape Peninsula University of Technology for the use of the nursery facilities and provision of hormones and rooting mediums and Ms A. Jephson for language editing.

3.7 REFERENCES

Alegre J, Toledo JL, Martinez A, Mora O, De Andres EF (1998). Rooting ability of Dorycnium spp. Sci. Horticult. 76: 123-129.

Aminah H, Dick JMCP, Leakey RRB, Grace J, Smith RI (1995). Effect of indole butyric acid (IBA) on stem cuttings of Shorea leprosula. For. Ecol. Manage. 72: 199-206.

Brown N, Duncan G (2006). Manual for growing Proteaceae in greenhouses Joseph Knight in 1809. www.protea.worldonline.co.za/growknight.htm : 14.

Fogaça CM, Fett-Neto AG (2005). Role of auxin and its modulators in the adventitious rooting of Eucalyptus species differing in recalcitrance. Plant Growth Regul. 45:1-10.

Hartmann HT, Kester DE (1983). Plant propagation; principals and Practices. Prentice-Hall International, London.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant Propagation Principles and Practices. 7th Edition. Prentice Hall. New Jersey, pp. 367-374.

Hilton-Taylor C (1996). Red Data List of Southern African Plants. Strelitzia 4. National Botanical Institute, Pretoria.

Jamieson HG (2001). Leucadendron laxum I. Williams. Kirstenbosch Botanic Garden, Cape Town, South Africa.

Laubscher CP (2000). Propagating South African trees. Combined Proceedings International Plant Propagators’ Society 50: 56-65.

Mustard P, Cowling R, Albertyn J (1997). Southern Overberg. South African Wild Flower Guide 8. National Botanical Institute. National Book Printers. South Africa.

Owings AD (1996). Variations in pH from different bark sources. Combined Proceedings International Plant Propagators’ Society. 46: 699.

Paterson-Jones C (2000). The Protea family of Southern Africa. Struik Publishers. Cape Town.

Reinten E, Gertze S, Arends L (2002). Propagation methods. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Elsenburg, South Africa.

Robyn A, Littlejohn G (2002). Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production.

Rodríguez Pérez JA (1992). Propagation by leaf bud cuttings of Leucadendron ‘Safari Sunset’, *Leucospermum cordifolium, Leucospermum patersonii* and *Protea obtusifolia*. Acta. Horticult. 316: 35-46.

South African Flower Export Council (SAFEC) (2002). Preparing orchards for planting of PROTEACEAE. Certificate course in flower export readiness. Unpublished booklet. South Africa.

Steel RGD, Torrie JH (1980). Principles and procedures of statistics: A biometrical approach, Second Edition. McGraw Hill, New York.

Wiesman Z, Lavee S (1995). Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. Sci. Hortic. 62: 189-198.

Williams IJM (1972). A Revision of the Genus Leucadendron (Proteaceae) Contribution of the Bolus Herbarium No. 3.

Table 3.1: Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of Leucadendron laxum grown in bark, river sand and polystyrene medium and peat and polystyrene medium

Concentration	Bark, river sand and polystyrene						Peat moss and polystyrene					
	% Survival Rate		Roots per cutting		Root Length (cm)		% Survival Rate		Roots per cutting		Root Length (cm)	
Control	**10**	**±5.8b**	**0.0**	**±0.0c**	**0.0**	**±0.0c**	60	±21.2	0.25	±0.25	0.25	±0.25
500	**45**	**±15.0a**	**0.0**	**±0.0c**	**0.0**	**±0.0c**	43	±23.2	0.75	±0.75	1.25	±1.25
1000	**25**	**±8.7ab**	**5.0**	**±2.9a**	**4.0**	**±2.3a**	58	±18.4	1.75	±0.75	0.25	±0.25
2000	**5**	**±2.9b**	**0.0**	**±0.0c**	**0.0**	**±0.0c**	65	±16.6	2.00	±1.15	3.75	±2.39
4000	**10**	**±5.8b**	**1.0**	**±0.6b**	**0.5**	**±0.3b**	60	±21.2	2.25	±0.75	1.25	±1.25
F Statistic	5.57*		2.71*		**2.82***		0.18 ns		1.20 ns		1.14 ns	

Values presented are means ± SE. * = significant at P≤ 0.05. Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05

Table 3.2: Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of Leucadendron laxum grown in bark and polystyrene medium and perlite and river sand medium

Concentration	Bark and polystyrene						Perlite and river sand					
	% Survival Rate		Roots per cut		Root Length (cm)		% Survival Rate		Roots per cut		Root Length (cm)	
Control	60	±16.3a	**6.5**	**±2.1a**	2.8	±0.6a	3	±2.5	0.00	±0.00	0.00	±0.00
500	58	±21.8a	**3.5**	**±1.2ab**	8.8	±3.8a	28	±14.9	0.00	±0.00	0.00	±0.00
1000	63	±18.9a	**4.3**	**±0.8ab**	6.5	±2.2a	15	±8.7	0.25	±0.25	0.75	±0.75
2000	40	±21.2a	**1.8**	**±0.6b**	12.5	±4.7a	5	±2.9	0.00	±0.00	0.00	±0.00
4000	65	±12.6a	**1.5**	**±0.3b**	6.3	±1.0a	3	±2.5	0.00	±0.00	0.00	±0.00
F Statistic	0.29 ns		**3.09***		1.48 ns		1.83 ns		1.00 ns		1.00 ns	

Values presented are means ± SE. * = significant at $P \leq 0.05$; ns = not significant. Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$

Table 3.3: Effect of different growth medium and IBA concentrations on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, number of roots per cutting and root length of *Leucadendron laxum*

	% Callus Formed		% Rooted		% Survival rate		Roots/Cutting		Root length	
Main (Mediums)										
Bark and polystyrene	**30**	**±3.9a**	**28**	**±4.6a**	**57**	**±7.6a**	**3.50**	**±0.62a**	**7.35**	**±1.38a**
Peat moss and polystyrene	**19**	**±6.9ab**	**10**	**±3.7b**	**57**	**±8.2a**	**1.40**	**±0.36b**	**1.35**	**±0.61b**
Bark, river sand and polystyrene	**10**	**±3.3b**	**2**	**±0.9bc**	**19**	**±4.8b**	**1.20**	**±0.69bc**	**0.90**	**±0.55bc**
Perlite and river Sand	**7**	**±3.6b**	**1**	**±0.5c**	**11**	**±3.9b**	**0.05**	**±0.05c**	**0.15**	**±0.15c**
F Statistic	**4.49****		**16.2*****		**13.6*****		**11.15*****		**18.8*****	
Sub (IBA- Auxins)										
Control	14	±4.6a	6	±3.3a	33	±9.3a	**1.69**	**±0.85ab**	0.75	±0.34
500	22	±7.3a	6	±3.0a	43	±9.0a	**1.06**	**±0.49b**	2.50	±1.29
1000	21	±7.0a	11	±3.1a	40	±8.4a	**2.81**	**±0.85a**	2.88	±0.99
2000	7	±2.4a	14	±6.8a	29	±8.9a	**0.94**	**±0.38b**	4.06	±1.78
4000	18	±5.0a	14	±4.2a	34	±9.3a	**1.19**	**±0.31b**	2.00	±0.74
F Statistic	1.22 ns		1.15 ns		0.58 ns		**2.54***		2.01 ns	

Values presented are means ± SE. *, **, *** = significant at $P \leq 0.05$, 0.01 and 0.001 respectively. ns = not significant. Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$

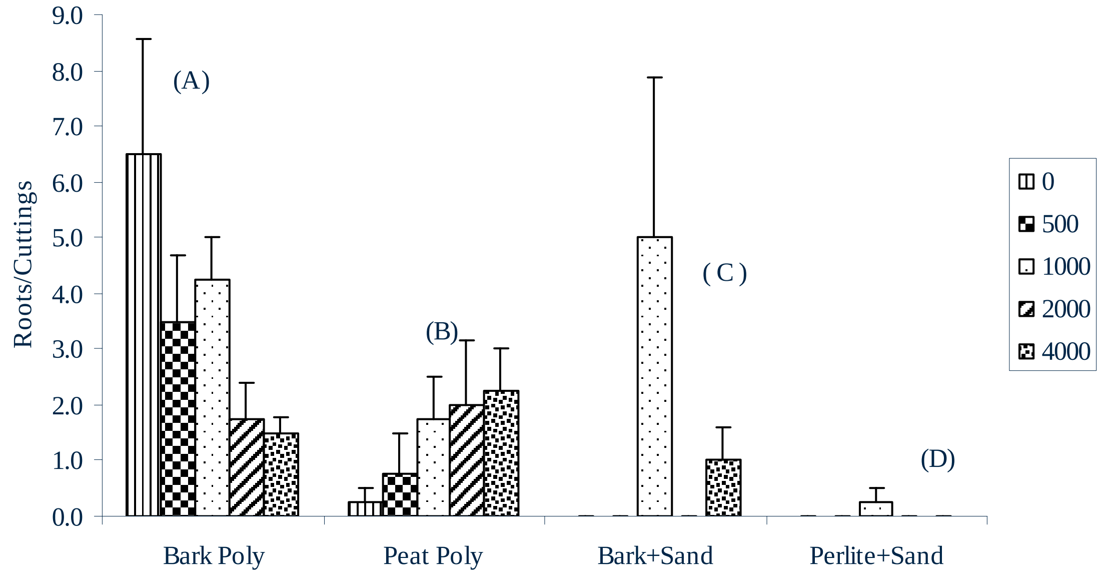

Figure 3.1: Interactive effects: A) Bark and polystyrene and Indole Butyric Acid (IBA) concentration, B) Peat and polystyrene and IBA concentrations, C) Bark, river sand and polystyrene and IBA concentrations, D) Perlite, river sand and IBA concentrations

CHAPTER FOUR

ROOTING RESPONSE UNDER SHADE USING IBA GROWTH REGULATORS AND DIFFERENT GROWTH MEDIUMS ON *LEUCADENDRON LAXUM* (PROTEACEAE) – A COMMERCIAL CUT FLOWER

Full Length Research Paper

Rooting response under shade using IBA growth regulators and different growth mediums on *Leucadendron laxum* (Proteaceae) – A commercial cut flower

C. P. Laubscher[1] , and P. A. Ndakidemi[2]*

[1]Faculty of Business, Cape Peninsula University of Technology, P. O Box 652, Cape Town 8000, South Africa.

[2]Faculty of Applied Sciences, Cape Peninsula University of Technology, P. O Box 652, Cape Town 8000, South Africa.

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*Corresponding author. E-mail: ndakidemip@cput.ac.za

Tel: +27214603196. Fax: +27214603193

4.1 Abstract

The popular cut flower, *Leucadendron laxum* (Marsh rose Leucadendron) was tested for its rooting ability, using various Indole-3-butyric acid (IBA) liquid hormone concentrations with different growth mediums. The IBA treatments included a control, 500, 1000, 2000 and 4000 ppm IBA auxins. Growth mediums tested were a) peat and polystyrene; b) bark and polystyrene; c) bark, sand and polystyrene; and d) perlite and river sand. Basal ends of cuttings were dipped into the IBA solution for a few seconds after preparation, and planted under shade conditions. The experiment was planned in a randomised block design, with 10 cuttings and 3 replicas per treatment. The IBA application in comparison with the control, significantly improved rooting percentages in a) peat and polystyrene and b) bark, river sand and polystyrene mediums. The bark and polystyrene medium was more effective in the stimulation of roots, survival of cuttings, promotion of root length and the number of roots that developed. The overall application of IBA induced more root and shoot formation.

Key words: Auxin, cut flower, growth medium, indole-3-butyric acid, shade environment.

Abbreviations: IBA = Indole-3-butyric acid;
SAFEC = South African Flower Export Council.

4.2 INTRODUCTION

Leucadendron laxum is a member of the Proteaceae family which occurs naturally in damp areas on the Agulhas Plain in the extreme south western coast of southern Africa (Mustard et al., 1997). *L. laxum* is popularly referred to as the marsh rose and is continually being illegally harvested in more than 436 hectares of its natural habitat (Mustard et al., 1997; Robyn and Littlejohn, 2002). Over exploitation of *L. laxum* has caused this species to be listed as endangered in the Cape Floral Kingdom (Hilton-Taylor, 1996).

More than 70 commercial Proteaceae species and cultivars including Leucadendron are already cultivated in field-grown orchards (Brown et al., 1998; Robyn and Littlejohn, 2002). This fast-developing flower export market, which includes the pharmaceutical and medicinal Industry, has exceeded $45 billion per annum on the world market (Cowling and Richardson, 1995). Thirty million flower stems were sold in 2001 and production increased from 8570.6 ha to 22.87% in 2003 and to 27.34% in 2004 (Rabie, 2007, personal communication).

Leucadendron is one of South Africa's leading export floral crops and is mainly harvested on low production fynbos land where it is picked out of the natural veld as part of mixed farming (Dodd and Bell, 1993; Paterson-Jones, 2000; Robyn and Littlejohn, 2002). The male flowers of bright green, yellow and gold shades provide an exotic filler in mixed bouquets known as "Cape Greens", while the female cones are mainly harvested for the dried flower industry (Coetzee and Littlejohn, 1994; Moody, 1995; Berney, 2000; Paterson-Jones, 2000).

The annual demand for *L. laxum* has grown to more than 23,673 cut flower stems and has created an urgent need for the improvement of its propagation and cultivation. Vegetative propagation is a reliable method that would ensure the production of plants with uniform flowering times and duplicate their genetic characteristics (Reinten et al., 2002; Brown and Duncan, 2006). Future sustainable propagation would also aid the replacement of existing species in the natural habitat. Previous research work has shown that *L. laxum* was successfully rooted in controlled greenhouse conditions (Laubscher and Ndakidemi, 2008). No written evidence was found on cutting propagation for *L. laxum* under shade house conditions in South Africa. A study on this would therefore be seen as new research, aimed at preventing the costs of bottom heating. Cheaper built shade structures would be more sustainable for many growers rather than environmentally controlled greenhouse conditions.

Evidence suggests that auxin increases rooting percentages, shortens the rooting period and ensures improved uniformity in plants (Hartmann et al., 2002). Previous research has shown that *L. laxum* responded well to callusing in 500 ppm IBA and the highest number of roots were measured in the 1000 ppm IBA treatment under environmentally controlled greenhouse conditions with bottom heat (Laubscher and Ndakidemi, 2008). While some Proteaceae species favour bottom heat conditions for rooting, other cuttings are difficult to root, and could respond differently to auxin treatments (Hartmann et al., 2002). In this respect auxin formulations supplied at different concentration and growth conditions need further investigation and may improve rooting percentages (Hartmann et al., 2002) and produce desirable rooting results.

Previous results under controlled environment greenhouse conditions proved that using a bark, sand and polystyrene rooting medium resulted in 30% callus formation and 28% rooting and 57% survival rate of *L. laxum* cuttings (Laubscher and Ndakidemi, 2008).

The aim of this current study is therefore to determine whether *L. laxum* could respond favourably by rooting, with different concentrations of IBA and various rooting mediums in a shade house environment without bottom heat. The aim is further to provide faster and more efficient rooting success in cut flower production.

4.3 MATERIALS AND METHODS

The experiment was conducted in a shade tunnel (40% white shade cloth) at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa. The experiment started during the middle of August and continued for 8 weeks. Cutting material of *L. laxum* was collected from various plant populations in the natural habitat on the Agulhas Plain on the extreme southwestern coast of South Africa. The material was kept dry in a sealed plastic bag and transported overnight.

The growth mediums and IBA treatments were randomised in a complete block design with four growth mediums: a) bark and polystyrene; b) peat moss and polystyrene; c) bark, river sand and polystyrene; and d) perlite and river sand. Four concentrations of IBA (control, 500, 1000, 2000 and 4000) were used in this study. Altogether 3 x 5 x 10 cuttings were involved in the experiment. The rooting mediums had good drainage conditions, moisture retention and an average pH of 6.5-7 (SAFEC, 2002). Cuttings were replicated in 10 pots for each treatment. The experiment was set up in a shade house (40% shade) with misting irrigation.

Cuttings were taken from semi-hardwood stems after shoot elongation (Aug-Nov) and not at flowering stage. Stem cuttings of 150 mm long were made and half of the lower leaves removed (SAFEC, 2002; Reinten et al., 2002). The cuttings were rinsed in Benlate fungicide (10 g/l) before planting and Captan (2 g/l) was sprayed weekly to prevent fungal infection

(Reinten et al., 2002; SAFEC, 2002).The hormone treatment used was IBA liquid preparations of 500, 1000, 2000, 4000 ppm. The control remained untreated. IBA powder was dissolved at g/l in 50% ethyl alcohol The basal 5 mm of cuttings were dipped in the rooting hormone for 5 seconds (Hartmann et al., 2002; Reinten et al., 2002; Brown and Duncan, 2006) after which cuttings were individually planted into 200 size plug foam trays.

The tunnel (10 m long) was covered in white shade screen material (40%). The sides of the tunnel were left open to allow air flow during the rooting period (Reinten et al., 2002; SAFEC, 2002). Midday temperatures were measured between 18-25ºC and a relative humidity between 20 and 62%. The irrigation timer was set on 10 s on and 60 min off. Data collected included, average shoot growth, number of cuttings callused, number of cuttings rooted, number of roots on cuttings, length of roots, and the survival rate of cuttings.

Shoot growth (mm) and the length of roots (mm) were measured from cutting stage to new growth after 8 weeks. The number of cuttings callused, rooted and survival rates were also counted after 8 weeks. Data was recorded for the percentage of survival rate of cuttings, number of roots per rooted cutting and root length. Data analysis was performed in two different ways. The first consisted of one-way analysis of variance for IBA treatments added to each growth medium separately. The second consisted of factorial analysis including the four growth mediums and the five IBA concentrations.

Data on the number of cuttings that formed calluses, the number of cuttings that rooted and the cuttings that survived, were transformed into percentages prior to analysis of variance. Data were presented at mean values with predicted standard errors (S.E.). These computations were done with the software program STATISTICA. The Fisher least significance difference (L.S.D.) was used to compare treatment means at $P < 0.05$ level of significance (Steel and Torrie, 1980).

4.4 RESULTS

Tables 4.1 and 4.2 show the effects of different concentrations of IBA on the percentage of cuttings that rooted, cuttings that survived in the rooting mediums, root length and shoot growth of cuttings. The cuttings grown in a) peat and polystyrene; b) bark and polystyrene; c) bark, river sand and polystyrene and d) perlite and river sand mediums all produced different results. The IBA treatments significantly ($P < 0.05$) affected the percentage of cuttings that rooted in a) peat and polystyrene b) bark and polystyrene; and c) bark, river sand and polystyrene (Tables 4.1 - 4.2). The IBA application in comparison with the control, significantly improved rooting percentages in a) peat and polystyrene and b) bark, river sand and polystyrene (Tables 4.1 -

4.2). However, in the bark and polystyrene medium, the rooting percentage was significantly higher in the control treatment compared with other IBA treatments. The higher rooting percentage in both the control (57%) with no addition of IBA and the 4000 ppm IBA treatment (43%) in the bark and polystyrene medium indicated a presence of negative results. These results concur with other studies which reported that IBA applications could also inhibit rooting (Hartmann et al., 2002; Reinten et al., 2002). It is well established that several compounds may act together in the control of several plant functions, including the formation of roots (Volper et al., 1995). It is also worth mentioning that the bark component used in this study originated from pine trees.

Past studies have shown that bark from pine trees is very rich in phenolic compounds, alkaloids and cyanogenic glycosides (Machrafi and Prevost, 2006). These compounds are known to have inhibitory effects on the growth of plants (Still et al., 1976; Rice, 1984; Siqueria et al., 1991). It is likely therefore, that the presence of such phenolic compounds in the bark medium used in our study or possibly other endogenous compounds in combination with the supplied auxins, could have had an synergistic inhibitory effect that reduced the rooting capacity of *L. laxum*. (Table 4.2). Further studies are therefore needed to establish the relationship between the profile of phenolic compounds in the bark medium and different concentrations of growth hormones such as IBA.

The various concentrations of IBA which were supplied to cuttings of *L. laxum* in the bark, river sand and polystyrene medium significantly affected survival and rooting percentages of the cuttings (Table 4.2). IBA supplied at rates between 1000-2000 ppm was significantly superior in stimulating rooting in the bark, river sand and polystyrene rooting medium. It is possible that the lower proportion of bark in the bark, river sand and polystyrene medium induced deleterious effects, compared with the cuttings reported in the bark and polystyrene medium above. The higher IBA level of 4000 ppm suppressed rooting in the *L. laxum* cuttings completely. On the other hand, stem cuttings treated with IBA and grown in a perlite and river sand medium showed a significant improvement ($P < 0.05$) in shoot growth (Table 4.2). The cuttings treated with 2000 and 4000 ppm IBA in a perlite and river sand medium were superior in growth compared with those supplied with other treatments.

The evaluations of IBA concentrations in Table 4.3 show a significant influence on the percentage of rooted cuttings, and their survival and shoot growth. IBA supplied at 500, 2000 and 4000 ppm resulted in significant rooting percentages as compared with the control and 1000 ppm treatment. The percentage survival rate of cuttings in 500 and 1000 ppm IBA treatments showed significantly higher survival rates compared with other treatments (Table 4.3). However, the survival rate declined strongly at the highest rate in the 4000 ppm IBA treatment. The shoot growth was significantly improved at higher levels of IBA treatment ranging from 2000-4000 ppm (Table 4.3). Overall, the results obtained in this study concur with other investigations where the auxin supplied was responsible for root induction and shoot growth in difficult to root plant species, that is, the Proteaceae family (Wu and du Toit 2004; Wu et al., 2007a,b).

4.5 DISCUSSION

Limited studies have been found which document the influence of different concentrations of the IBA auxin in rooting *L. laxum* cuttings (Perez-Frances, 1995). The positive stimulatory effects of growth hormones on rooting stem cuttings have been recorded in other related plant species in South Africa (Wu and du Toit 2004; Wu et al., 2007a, b). Recordings of most results in this study indicated improved rooting, and in some case increases in survival rates and shoot growth of cuttings (Tables 4.1 – 4.3). Therefore, we can assume that the increased exogenous application of the IBA auxin enhanced rooting, root length and number of roots formed in *L. laxum* cuttings. These results are in accordance with the findings of Jones and Hatfield (1976), Zimmerman (1984) and Rout (2006) who proved that the accumulation of auxin in shoots was responsible for successive vegetative propagation of cuttings.

The cuttings of *L. laxum* performed differently when they were grown in different growth mediums and with different IBA concentrations (Table 4.3). Overall, and in comparison with other growth mediums tested, the bark and polystyrene medium was more effective in the stimulation of rooting, survival of cuttings, root length and number of roots formed (Table 4.3). This result was closely followed by the peat and polystyrene and the perlite and sand mediums (Table 4.3). The bark, polystyrene and sand medium produced less appreciable results compared with the other growth mediums tested (Table 4.3). Overall, the highest rooting and survival rate, observed in the bark and polystyrene medium, is an indication that the medium was highly advantageous for *L. laxum* propagation under shade house conditions. Similar results have been reported in other studies involving different plant species (Brown and Duncan, 2006; Reinten et al., 2002; SAFEC, 2002).

A significant interaction was observed between the different growth mediums used in this study, e.g., the effect of a) peat and polystyrene; b) bark and polystyrene; and c) bark, sand and polystyrene and the results of the IBA concentrations on the percentage of rooted cuttings, the percentage survival rate and the shoot growth and root lengths of cuttings (Figures 4.1 – 4.4). In most instances, the overall application of IBA used, in combination with growth mediums, induced more root and shoot formation. *L. laxum* is a member of the

Proteaceae family which is documented as being very difficult to root (Wu et al., 2007a). In this study, the addition of IBA in most growth mediums stimulated root and shoot growth. It is well known that the exogenous supply of auxins in the Proteaceae family is a prerequisite for root induction in cuttings which are difficult to root (Wu et al., 2007b) such as the *L. laxum*. An exogenous supply of auxins has proved to stimulate root and shoot growth in stem cuttings grown in different rooting mediums (Wu et al., 2004; 2007a,b). In the bark polystyrene medium, significant decreases were reported in rooting characteristics by applying IBA (Figure 4.1 and 4.4). It is possible that the synergistic effects between IBA and the phenolic compounds in bark inhibited root formation in stem cuttings of *L. laxum*.

4.6 Conclusion

In conclusion, the results of this study have described the effects of different concentrations of the auxin (IBA) and different growth mediums on the rooting of *L. laxum* under shade house conditions. These results can provide growers with the opportunity to stimulate the rooting of *L. laxum* cuttings faster and at lower costs over a period of 8 - 12 weeks. This study also showed that the growth medium environment determines the survival rate success of cuttings, which in turn results in progressive rooting success. The use of IBA in the rooting of *L. laxum* was variable in different rooting mediums where moisture retention was important. Other endogenous factors in the growth medium of bark, warrants further investigation under different environmental conditions.

4.7 ACKNOWLEDGEMENTS

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4.8 REFERENCES

Berney J (2000). Combined research to benefit Protea growers. Aust. Hort. 98(2): 16-18.

Brown NAC, Kotze D, Botha P (1998). Grow Proteas. A Guide to the Propagation and Cultivation of Some South African Proteaceae. Kirstenbosch Gardening Series, National Botanical Institute, Cape Town, South Africa.

Brown N, Duncan G (2006). Manual for growing proteaceae in greenhouses Joseph Knight in 1809. www.protea.worldonline.co.za/growknight.htm,:14.

Coetzee JH, Littlejohn G (1994). Indigenous South African Flower Industry. Hortagrow. August 1994, pp. 9-11.

Cowling R, Richardson D (1995). Fynbos. South Africa's Unique Floral Kingdom. Fernwood Press, Cape Town, pp. 7-31.

Dodd J, Bell DT (1993). Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. Aust. J. of Bot. 18:281-293.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant Propagation Principles and Practices, Seventh Edition, Prentice Hall, New Jersey, pp. 367-374.

Hilton-Taylor C (1996). Red Data List of Southern African Plants. Strelitzia 4. National Botanical Institute, Pretoria.

Jones OP, Hatfield SGS (1976). Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds. J. Hort. Sci. 51:495-499.

Laubscher CP, Ndakidemi PA (2008). Rooting success using IBA auxin on endangered *Leucadendron laxum* (PROTEACEAE) in different rooting mediums. Afr. J. Biotechnol. 7(19): 3437-3442.

Machrafi Y, Prevost D (2006). Toxicity of phenolic compounds extracted from bark residues of different ages. J. Chem. Ecol. 32:2595-2615.

Moody H (1995). Versatile Leucadendrons and Leucospermums. Aust. Hort. 5: 111-112.

Mustard P, Cowling R, Albertyn J (1997). Southern Overberg. South African Wild Flower Guide 8. National Botanical Institute, National Book Printers, South Africa.

Paterson-Jones C (2000). The Protea family of Southern Africa. Struik Publishers, Cape Town, pp. 28-124.

Perez-Frances JF, Exposito A, Rodriguez JA (1995). Effect of different factors on *in vitro* multiplication of *Leucadendron* 'Safari Sunset' (Proteaceae). Acta Hort. 387: 115-120.

Reinten E, Gertze S, Arends L (2002). Propagation methods. Cultivating fynbos for quality cut flower production. ARC training course manual. Elsenburg, South Africa, pp. 50-70.

Rice EL (1984). Allelopathy. Academic Press, Orlando, pp. 272-274.

Robyn A, Littlejohn G (2002). Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production. Elsenburg, South Africa, pp. 22-49.

Rout GR (2006). Effect of auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. Plant Growth Regul. 48:111-117.

SAFEC (2002). Preparing orchards for planting of Proteaceae. Certificate course in flower export readiness manual. South African Flower Export Council, South Africa.

Siqueria JO, Safir GR, Nair MG (1991). Stimulation of Vesicular-Arbuscular Mycorrhiza Formation and Growth of White Clover by Flavonoid Compounds. New Phytologist 118:87-93.

Steel RGD, Torrie JH (1980). Principles and procedures of statistics: A biometrical approach, Second Edition, McGraw Hill, New York.

Still SM, Dirr MA, Gartner, JB (1976). Phytotoxic effects of several bark extracts on mung bean and cucumber growth. J. Am. Soc. Hort. Sci. 101:34-37.

Volper R, Osswald, M, Elstner, EF (1995). Effects of cinnamic acid derivatives on indole acetic acid oxidation by peroxidase. Phytochem. 38:1-22.

Wu HC, du Toit, ES (2004). Reducing oxidative browning during in vitro establishment of *Protea cynaroides.* Scientia Hort. 100:355-358.

Wu HC, du Toit ES, Reinhardt CF. (2007a). Micrografting of *Protea cynaroides***.** Plant Cell Tiss. Cult. 89:23-28**.**

Wu HC, du Toit ES, Reinhardt CF, Rimando AM, van der Kooy F, Meyer JJM (2007b). The phenolic, 3,4-dihydroxybenzoic acid, is an endogenous regulator of rooting Protea cynaroides. Plant Growth Regul. 52:207-215.

Zimmerman RH (1984). Rooting apple cultivars in vitro: Interaction amongst light, temperature, phloroglucinol and auxin. Plant Cell Tiss. Cult. 3:01-311.

Table 4.1: Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *Leucadendron laxum* grown in peat and polystyrene and bark and polystyrene medium

	Peat Polystrene				Bark Polystrene			
	% Rooted		Root Length		% Rooted		Root Length	
Control	**10**	**±5.8b**	2.33	±1.20a	**57**	**±12.0a**	**8.00**	**±2.08a**
500	**37**	**±6.7a**	5.33	±2.60a	**43**	**±3.3ab**	**2.33**	**±0.33bc**
1000	**20**	**±0.0ab**	5.67	±3.18a	**17**	**±3.3c**	**1.33**	**±0.33c**
2000	**27**	**±8.8ab**	3.67	±1.20a	**23**	**±8.8b**	**1.67**	**±0.33c**
4000	**27**	**±3.3ab**	2.67	±0.33a	**43**	**±3.3ab**	**5.67**	**±1.20ab**
F Stat	**2.9***		0.58 ns		**5.22***		**6.93****	

Values presented are means ± SE. *, **, ns = significant at P≤ 0.05, 0.01, ns = not significant respectively. Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05

Table 4.2: Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *Leucadendron laxum* grown in Bark, River Sand and Polystrene, and Perlite and River Sand medium

	Bark, River Sand and Polystrene						Perlite and River Sand					
	% Rooted		% Survival		Shoot Growth		% Rooted		% Survival		Shoot Growth	
Control	**0**	**±0.0c**	**37**	**±3.3b**	4.3	±1.20a	7	±6.7a	67	±20.3a	**3.7**	**±1.20b**
500	**3**	**±3.3bc**	**60**	**±5.8a**	1.3	±0.67a	20	±0.0a	87	±3.3a	**10.3**	**±1.76ab**
1000	**17**	**±8.8ab**	**60**	**±5.8a**	2.3	±0.33a	7	±6.7a	83	±3.3a	**4.7**	**±1.20b**
2000	**23**	**±3.3a**	**27**	**±3.3b**	6.0	±1.73a	30	±11.5a	80	±11.5a	**16.7**	**±4.18a**
4000	**0**	**±0.0c**	**3**	**±3.3c**	4.3	±1.20a	10	±10.0a	60	±10.0a	**15.3**	**±2.96a**
F Stat	**5.72***		**28.72*****		2.63 ns		1.60 ns		0.98 ns		**5.49***	

Values presented are means ± SE. *, **, ***, ns = significant at P≤ 0.05, 0.01, 0.001, ns = not significant respectively. Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05

Table 4.3: Effect of different growth medium and IBA concentrations on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, shoot growth, root length and the number of roots per cutting of *Leucadendron laxum*

Treatment	Callused		% Rooted		% Survival		Shoot Growth		Root Length		Number of roots/cutting	
Main Treatment												
Peat Polystrene	22	±13.2a	**24**	**±3.2b**	**69**	**±5.6a**	**3.3**	**±0.73b**	**3.9**	**±0.84a**	**5.9**	**±0.98a**
Bark Polystrene	29	±12.5a	**37**	**±4.7a**	**77**	**±4.7a**	**1.4**	**±0.59b**	**3.8**	**±0.81a**	**5.9**	**±0.98a**
Bark/Sand /Polystrene	22	±26.5a	**9**	**±3.1c**	**37**	**±6.0b**	**3.7**	**±0.61b**	**0.9**	**±0.53b**	**1.2**	**±0.54b**
Perlite / Sand	36	±18.0a	**15**	**±3.9c**	**75**	**±5.2a**	**10.1**	**±1.72a**	**1.8**	**±0.46b**	**4.6**	**±1.52a**
F Statistic	1.85 ns		**17.6*****		**18.09*****		**22.76*****		**6.03****		**4.44****	
Sub - Treatment												
Control	26	±21.1a	**18**	**±7.5b**	**61**	**±7.9b**	**3.3**	**±0.81b**	3.0	±1.10a	5.3	±1.73a
500	25	±20.2a	**26**	**±5.0a**	**79**	**±4.2a**	**3.6**	**±1.27b**	3.7	±0.83a	5.8	±1.59a
1000	32	±15.9a	**15**	**±2.9b**	**68**	**±5.6ab**	**2.6**	**±0.63b**	2.2	±0.94a	3.4	±0.68a
2000	33	±20.2a	**26**	**±3.8a**	**58**	**±7.7b**	**7.6**	**±1.99a**	2.0	±0.44a	3.8	±1.06a
4000	20	±16.5a	**20**	**±5.5a**	**57**	**±10.0b**	**6.2**	**±1.82a**	2.3	±0.73a	3.7	±1.13a
F Statistic	0.96 ns		**2.15***		**3.58***		**5.76*****		1.07 ns		0.88 ns	

Values presented are means ± SE. *, **, *** = significant at $P \leq 0.05$, 0.01 and 0.001 respectively. ns = not significant. Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$

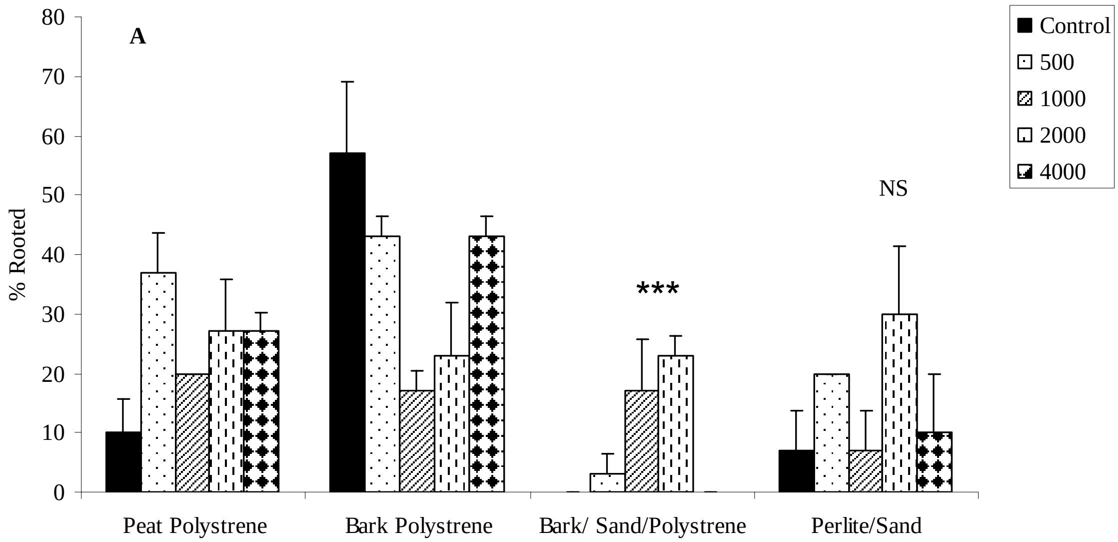

Figure 4.1: Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on % of rooted cuttings. *** = Significant at P ≤ 0.001); NS = Not significant

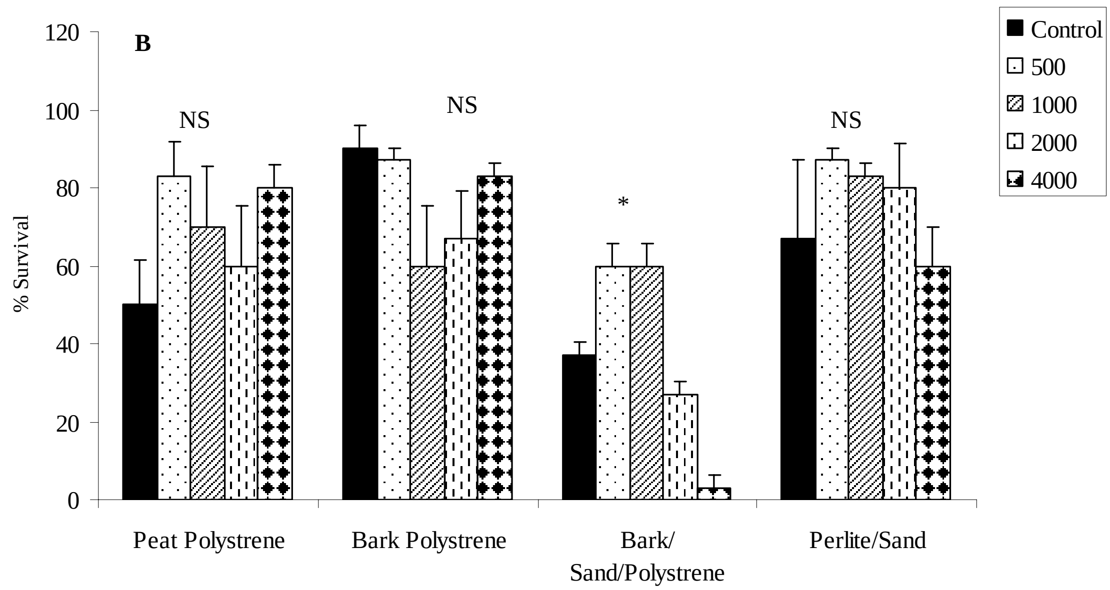

Figure 4.2: Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on % survival of cuttings. * = P $\leq$ 0.05; NS = Not significant

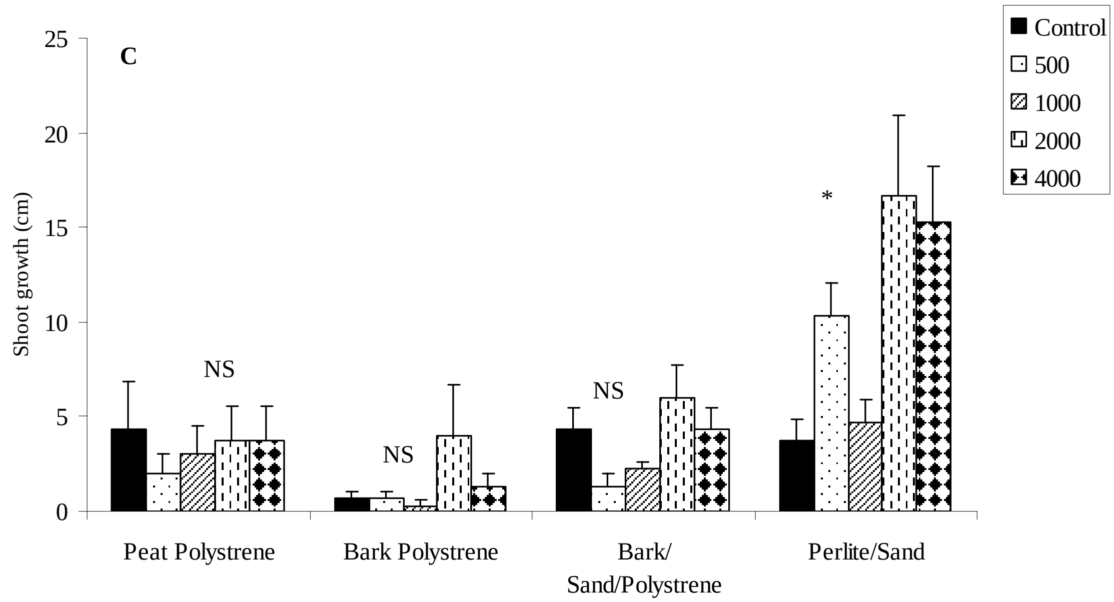

Figure 4.3: Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on shoot growth (cm). * = P $\leq$ 0.05; NS = Not Significant

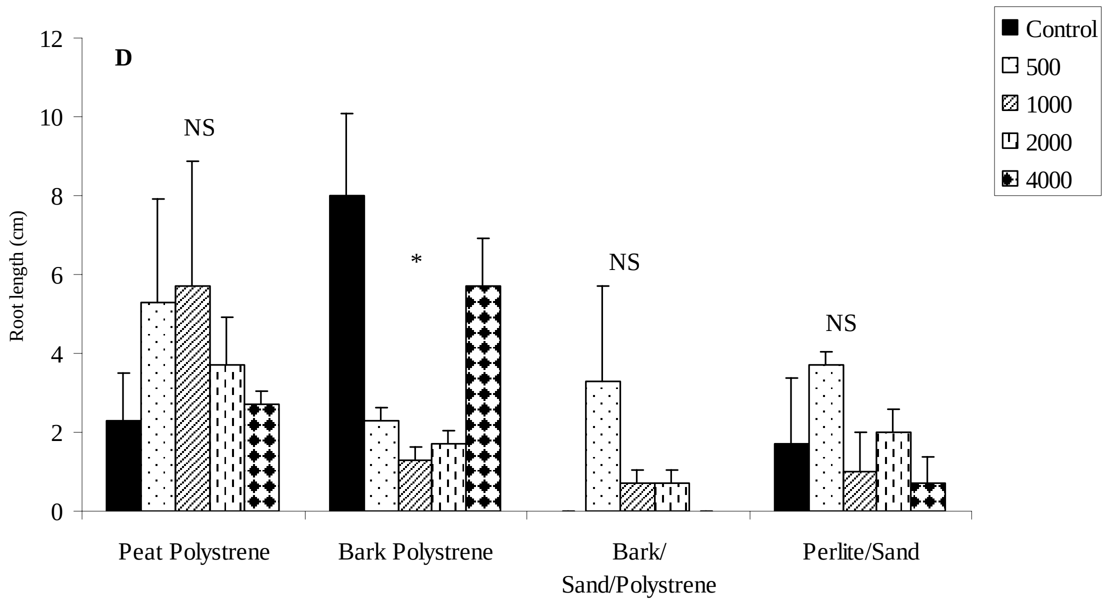

Figure 4.4: Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on root length (cm). * = P $\leq$ 0.05; NS = Not Significant

CHAPTER FIVE

ROOTING AND GROWTH POTENTIAL OF LEUCADENDRON LAXUM (PROTEACEAE) USING DIFFERENT ROOTING MEDIUMS AND INDOLE ACETIC ACID GROWTH REGULATORS

ROOTING AND GROWTH POTENTIAL OF LEUCADENDRON LAXUM (PROTEACEAE) USING DIFFERENT ROOTING MEDIUMS AND INDOLE ACETIC ACID GROWTH REGULATORS

[1,] C.P. LAUBSCHER AND [2]P.A. NDAKIDEMI

[1]Department of Tourism and Hospitality Management, Faculty of Business, [2]Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa.

[1]Correspondence author: C.P. Laubscher, e-mail: laubscherc@cput.ac.za

Keywords: Auxin; flower industry; flowering; potted plants; shoot growth.

Abbreviations: IAA = Indole-3-acetic acid; IBA= Indolebutyric acid; NAA = Naphthaleneacetic acid.

ABSTRACT

Leucadendron laxum (Proteaceae) is a South African plant species with high commercial value as a flowering potted plant. Limited research information on the culture and propagation of this species is available in South Africa. The applications of rooting hormone indole acetic acid (IAA) in various rooting mediums in L. laxum were tested. The treatments included: control, 500, 1000, 2000 and 4000 ppm and four rooting mediums a) bark / polystyrene; b) peat moss / polystyrene; c) bark / river sand / polystyrene; and d) perlite / river sand. A randomised block design with 3 replicates was used. Compared to other mediums, bark and polystyrene had the highest significant results in root and shoot growth, and the percentage that callused, rooted and survived. The IAA treatments at different concentrations had significant effects on rooting, callusing, shoot growth, root lengths and numbers of roots per cutting.

5.2 INTRODUCTION

Leucadendron laxum, is an endangered plant species of the Proteaceae family which naturally occurs on the Agulhas Plain in the most southern tip of Africa (Hilton-Taylor, 1996). This species is also an important component of the indigenous export cut flower industry of Western Cape, South Africa. Its attractive yellow-red flowers and especially the female cones are favoured in mixed bouquets and in the dried flower industry (Dodd & Bell, 1993:281-293; Coetzee & Littlejohn, 1994; Moody, 1995:111-112; Paterson-Jones, 2000; Berney, 2000). The natural growth habit of *L. laxum* has the potential for further commercial flowering pot plant production. No previous studies were found on the pot plant production of this species. A world-wide demand for new and interesting flowering potted plants (Barzilay et al., 1992:117-124) continues to raise a strong interest on the wide biodiversity potential of South African flora (Milandri et al., 2008:239-243). The *L. laxum* is one of the plant species with potential in the flower market. However, most members in this family are categorised as difficult-to-root plants and may require special auxin treatments and environmental conditions to facilitate their propagation (Hartmann et al., 2002:319).

The vegetative propagation of this species is an important factor as faster rooting techniques are necessary for profitable commercial production of high quality products (Robyn & Littlejohn, 2002). Rooted cuttings have the added advantage in that they will flower a year earlier than seed grown plants (Reinten et al., 2002).

Appropriate vegetative propagation techniques for *L. laxum* would increase the production in the controlled greenhouses conditions to supply the demand. The use of rooting hormones such as IAA would prove useful in speeding up the rooting of difficult to root plants such as *L. laxum.* New and more effective auxin formulations are required to increase success in rooting percentages of some difficult-to-root Proteaceae (Hartmann et al., 2002:294-296).
Auxins have an important role in the speeding up uniform rooting and in increasing rooting percentages during vegetative propagation (Hartmann et al., 2002; Fogaça & Fett-Neto, 2005:1-10). While some Proteaceae cuttings are difficult to root, other species have shown positive responses to auxin treatments (Hartmann et al., 2002:319). IAA that occurs naturally in the plants may not be adequate to enhance auxin functions in plants (Hartmann et al., 2002:295). Therefore, testing of different concentrations of IAA on *L. laxum* will identify the treatment that produces the most desirable rooting results. No other studies were found which previously described the use of IAA auxin on the rooting of *L. laxum*.

Research into combinations of different rooting mediums is essential in ensuring faster and better quality root formation. The possibility of improved results might be possible under different conditions or with various auxin treatments. Good quality shredded milled pine bark is recommended for pH control (Owings, 1996:699) or can be substituted with a coarse grade peat moss to enhance further water holding capacity of the growing medium (Hartmann et al., 2002). Polystyrene improves aeration, whereas washed river sand provides coarseness and drainage (Hartmann et al., 2002).

The aim of this study was therefore to determine whether *L. laxum* responded favourably to different concentrations of IAA rooting hormones in various rooting mediums under a controlled greenhouse environment with bottom heating to ensure faster and more efficient rooting success in flowering pot-plant production.

5.3 METHODOLOGY

5.3.1 Experimental

The study was conducted at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa in 2006. An environmentally controlled greenhouse covered in clear polycarbonate greenhouse and fitted with a shade screen (40%) was used. Temperatures and humidity were monitored on a weekly basis with midday temperatures fluctuating between 22-27°C and relative humidity between 39-86%. The irrigation timer was set on 15 sec on and 20 min off. Cutting material of *L. laxum* was collected from selected plant populations in their natural habitat on the Agulhas Plain and kept dry and cool during overnight transportation.

5.3.2 Setup and design

For this study, four growth mediums a) bark / polystyrene; b) peat moss / polystyrene; c) bark / river sand / polystyrene; and d) perlite / river sand were used. Four concentrations of IAA (0, 500, 1000, 2000 & 4000) were set up in a randomised complete block design involving 3 replications and four growth media. The IAA solution was prepared as described by Brown & Duncan 2006:14). The IAA powder was dissolved in 50% ethyl alcohol and made up in the appropriate concentration by adding distilled water. The basal 5 mm of cuttings were dipped for 5 seconds in the IAA rooting hormone (Reinten et al., 2002; Hartmann et al., 2002:371) and cuttings were planted into foam plugs trays containing different growth mediums. The experiment was conducted in a controlled greenhouse with heated beds (20-25 ºC) and mist bed conditions were supplied (Brown, & Duncan, 2006:14). For this study, terminal cuttings measuring approximately 150 mm long were used. All cuttings were taken during dry weather from turgid semi-hardwood stems in the month of November after shoot elongation (SAFEC, 2002). Cuttings were rinsed in Benlate fungicide (10g/l) before planting and sprayed weekly with a Captan (2 g/l) solution after planting to prevent the infection from disease causing organisms (Reinten et al., 2002; SAFEC, 2002). Wilted and infected cuttings were removed over the rooting period.

5.3.3 Data collection and analysis

Cuttings were evaluated on a weekly basis to monitor results of hormone treatment, and influence of rooting medium on rooting progress of *L. laxum*. The parameters measured were, number of cuttings callused, number of cuttings rooted, and the survival rate of cuttings. Shoot growth (mm), length of roots (mm) and number of roots per cutting were measured from the cutting stage to new growth stage after eight weeks. Data on the number of cuttings that formed callus, the number of cuttings that rooted and the cuttings that survived was converted into percentages prior to analysis of variances. Data analysis was performed in two different ways. Firstly, descriptive analytical techniques were employed. Furthermore, factorial analysis including IAA concentrations and growth media was used. These computations were done with the software program STATISTICA. The Fisher least significance difference (L.S.D.) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel, & Torrie, 1980).

5.4 RESULTS AND DISCUSSION

5.4.1 The effect of different growth mediums on rooting responses in *L. laxum*

The different rooting and growth parameters of *L. laxum* were significantly influenced by the four growth mediums used in the study over the period of eight weeks (Table 5.1). The bark / polystyrene medium was the most significantly superior to other growth mediums. This was followed by 1) peat / polystyrene and 2) bark / sand / polystyrene. The results recorded across these two mediums were not statistically different from each other (Table 5.1). The perlite / sand medium was far less successful in producing significant results. The lower response in the perlite / river sand medium could be contributed to the possibility of a courser aggregate with faster draining of water around the root zone area. The percentage increases in rooting characteristics in *L. laxum* by comparing poorly performing medium (perlite / sand) and the best medium (bark / polystyrene) ranged from 4-60; 0-40 and 27-88 for callusing, rooting and survival rate respectively (Table 5.1).

With regards to growth (shoot growth, root length and number of roots per cutting), all parameters except root length (mm) were significantly influenced by growth mediums. The bark / polystyrene medium was significantly superior to all other mediums (Table 5.1). These were followed by 1) peat / polystyrene and 2) bark / sand / polystyrene which showed non significant results amongst them (Table 1). In the perlite / sand medium, there was no growth recorded at all (Table 5.1). The shoot growth and number of roots per cutting increased from 0 – 3.9 mm and 0 – 1.6 respectively when 1) perlite / sand and 2) bark / polystyrene medium were compared.

A number of studies have shown that growth mediums may have profound influences on the rooting of cuttings in different plant species (Leakey *et al*., 1990:247-257; Ofori et al., 1996:39-48). The improved callusing, rooting, % survival rate, shoot growth and number of roots per cutting in bark / polystyrene medium as compared to other mediums indicated that the medium was highly advantageous in the propagation of *L. laxum*. The overall success in the bark / polystyrene medium implied that this medium was highly suitable for the propagation of *L. laxum* due the moisture holding and aerated properties and hence suitable for the propagation of different Proteaceae species. Our results suggested that the appropriate medium with good air: water ratio such as the bark / polystyrene medium is a key to successful rooting (Leakey *et al*., 1990:247-257). Adequate moisture and aeration in the growth mediums are essential in supplying water and enough oxygen for respiration in the cuttings. Similar success in bark / polystyrene medium have been reported in other studies involving other Proteaceae species (Brown, & Duncan, 2006:14; Reinten et al., 2002).

5.4.2 Effect of different concentrations of IAA on rooting and shoot growth of *L. laxum* cuttings

Different rates of IAA applications had some significant effects in callus formation in *L. laxum*. The maximum percentage of callusing (45%) was recorded in cuttings supplied with IAA at 500 ppm (Fig 5.1). However, callusing was significantly inhibited at the highest concentration (4000 ppm) of IAA treatment (Fig 5.1). With regards to rooting percentages, results showed a progressive increase in rooting by increasing IAA supply rates from 1000 - 4000 ppm (Fig 5.1). The highest rooting (21%) was recorded in the cuttings that received the 4000 ppm treatment (Fig 5.1). The IAA treatments did not significantly affect the survival rate of the cuttings (Fig 5.1).

Results on different growth parameters (shoot growth, root length and number of roots per cutting) recorded after 8 weeks showed a significant influence of pre-treating *L. Laxum* cuttings with IAA auxin (Fig 5.2). Supplying IAA at 2000 and 4000 ppm gave the best growth results, with values ranging from 1.8 and 1.9 mm in shoot growth and 1.0 – 1.2 in root numbers respectively (Fig 5.2). Results for root length were not consistent (Fig 5.2). In our study, the overall best rooting and growth occurred at higher IAA treatments (Figures 5.1 – 5.2).

Auxins such as IAA are growth promoting substances that are supplied exogenously to the cuttings to stimulate the meristematic cell division and promote rooting in cuttings (Rout, 2006:111-117). Results from our study showed that the exogenous supply of auxin (IAA) at different rates significantly enhanced different rooting and growth parameters of *L. laxum* (Fig 5.1 & 5.2). Our results are in accordance with other studies in which IAA stimulated rooting characteristics and growth in several plant species (Liu *et al*., 1996:247-257 & 1998:113-118; De Klerk et al., 1997:188-189.). In most cases, higher concentrations of IAA produced better results (Figs 5.1 – 5.2), thus proposing that this hormone was limited in the plant tissues and hence its application to stimulate rooting was essential in *L. laxum* cuttings. Several studies have reported correlations between higher IAA concentrations in plant tissues and rooting in different plant species (Hartmann et al., 2002:295). From our study, it was important to establish the optimum level(s) of IAA necessary to stimulate root development in *L. laxum*. The environmentally controlled greenhouse with bottom heat and misting was conducive to enhance rooting, growth and survival of cuttings. These growth conditions are in agreement with studies in propagating other Proteaceae (Brown, & Duncan, 2006:14; Reinten et al., 2002).

5.5 CONCLUSIONS

The results from this study showed that bark / polystyrene medium was the best significant medium that contributed sufficiently to the root development, survival rate and root length of cuttings. The optimum IAA levels that stimulated rooting in *L. laxum* was established. Generally, as compared to the control treatment, the IAA hormone treatment had significant effects on rooting success of the *L. laxum* cuttings. The successful production of quality cuttings of *L. laxum* for the indigenous cut flower industry can be largely achieved by choosing the appropriate growth medium supported with the correct concentration of the growth hormone.

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5.7 REFERENCES

Barzilay, A., Ben-Jaacov, J., Cohen, A., Ion, A., & Halevy, H. 1992. Mini-gladiolus as a flowering pot plant. *Scientia Horticulturae,* 49:117-124.

Berney, J. 2000. Combined research to benefit Protea growers. *Australian Horticulture,* 98: (2).

Brown, N., & Duncan, G. 2006. Manual for growing Proteaceae in greenhouses Joseph Knight in 1809 http://www.protea.worldonline.co.za/growknight.htm :14.

Coetzee, J.H., & Littlejohn, G. 1994. Indigenous South African Flower Industry. *Hortagrow,* 8.

De Klerk, G., Ter Brugge, J. & Marinova, S. 1997. Effectiveness of IAA, IBA and NAA during adventitious roots, new concepts, new possibilities. *In Vitro Cellular Developmental Biology,* 35:188-189.

Dodd, J. & Bell, D.T. 1993. Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. *Australian Journal of Botany,* 18:281-293.

Fogaça, C.M. & Fett-Neto, A.G. 2005. Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrance. *Plant Growth Regulation,* 45:1-10.

Hartmann, H.T., Kester, D.E., Davies, F.T. & Geneve, R.L. 2002.*Plant Propagation Principles and Practices.* 7th ed. New Jersey, Prentice Hall.

Hilton-Taylor, C. 1996. *Red Data List of Southern African Plants*. Strelitzia 4. Pretoria, National Botanical Institute.

Leakey, R.R.B., Mesén, J.F., Tchoundjeu, Z., Longman, K.A., Dick, J.McP., Newton, A., Martin, A., Grace, J., Monro, R.C. & Muthoka, P.N. 1990. Low technology techniques for the vegetative propagation of tropical trees. *Commonwealth Forestry Review,* 69: 247-257.

Liu, Z-H., Hsiao, I-C. & Pan, Y-W. 1996. Effect of naphthaleneacetic acid on endogenous indole-3-acetic acid, peroxidase and auxin oxidase in hypocotyls cuttings of soybean during root formation. *Botanical Bulletin of Academia Sinica,* 37:247-257.

Liu, Z-H., Wang, W-C. & Yen, Y-S. 1998. Effect of hormone treatment on root formation and endogenous indole-3-acetic acid and polyamine levels of *Glycine max* cultivated *in vitro*. *Botanical Bulletin of Academia Sinica,* 39:113-118.

Milandri, S., Laubscher, C.P. & Ndakidemi, P. 2008. Hydroponic culture of *Gladiolus tristis*: Application of paclobutrazol for flowering and height control. *African Journal of Biotechnology,* 7(3):239-243.

Moody, H. 1995. Versatile Leucadendrons and Leucospermums. *Australian Horticulture,* 5:111-112.

Mustard, P., Cowling, R. & Albertyn, J. 1997. *Southern Overberg. South African Wild Flower Guide 8.* National Botanical Institute. South Africa, National Book Printers.

Ofori, D.A., Newton, A.C., Leakey, R.R.B. & Grace J. 1996. Vegetative propagation of *Milicia* *excelsa* by leafy stem cuttings: effects of auxin concentrations, leaf areas and rooting medium. *Forest Ecology and Management,* 84: 39-48.

Owings, A.D. 1996. Variations in pH from different bark sources. *Combined Proceedings International Plant Propagators' Society,* 46:699.

Paterson-Jones, C. 2000.*The Protea family of Southern Africa*. Cape Town, Struik.

Rebelo, T & Paterson-Jones, C. 2001. *Sasol Proteas: A field guide to Proteas of southern Africa.* 2nd ed. Vlaeberg, Cape Town: Ferwood Press in association with the National Botanical Institute.

Reiten, E., Gertze, S. & Arends, L. 2002. *Propagation methods. Cultivating fynbos for quality cut flower production.* Unpublished training course manual. Elsenburg, South Africa, Agricultural Research Council.

Robyn, A. & Littlejohn, G. 2002. *Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production.* Unpublished report. Elsenburg, South Africa, Agricultural Research Council.

Rout, G.R. 2006. Effects of Auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. *Plant Growth Regulation,* 48: 111-117.

Steel, R.G.D. & Torrie, J.H. 1980. *Principles and procedures of statistics: A biometrical approach.* 2nd ed. New York, McGraw Hill.

Table 5.1: Effect of different growth mediums on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, shoot growth, root length and the number of roots per cutting of *Leucadendron laxum* supplied with IAA

Main treatment	Percentage measured						Growth measured (mm)				Number of roots/cutting	
	% Callused		% Rooted		% Survival		Shoot Growth		Root Length			
Rooting medium:												
Bark/ Polystrene	**60**	**±7.2a**	**40**	**±8.8a**	**88**	**±4.4a**	**3.9**	**±1.0a**	1.0	±0.4a	**1.6**	**±0.5a**
Peat/ Polystrene	**35**	**±6.1b**	**10**	**±4.7b**	**66**	**±8.7a**	**1.1**	**±0.3b**	4.1	±2.5a	**0.7**	**±0.3b**
Bark/Sand /Polystrene	**35**	**±6.1b**	**10**	**±4.7b**	**65**	**±8.5a**	**1.1**	**±0.4b**	4.1	±2.5a	**0.7**	**±0.3b**
Perlite / Sand	**4.0**	**±4.0c**	**0.0**	**±0.0c**	**27**	**±9.4b**	**0.0**	**±0.0c**	0.0	±0.0a	**0.0**	**±0.0c**
F Statistic	**12.4*** **		**7.9*** **		**6.53*** **		**0.001** **		0.27 ns		**0.008** **	

*Notes: Values presented are means ± SE. **, *** indicates statistical significant at P ≤ 0.01 and 0.001 respectively. ns indicates not significant. Means followed by the same letter in the same column are not significantly different from each other at P ≤ 0.05*

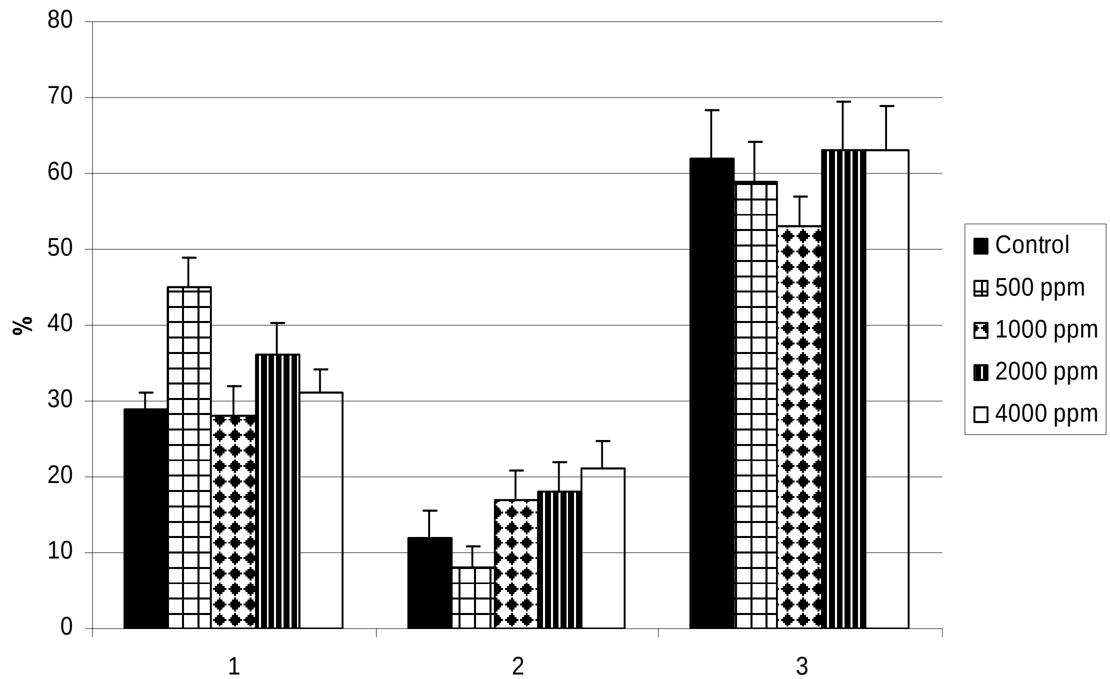

Fig 1. Rooting Parameters: 1 = % Callused; 2 = % Rooted 3 = % Survived.
Bars followed by dissimilar letter(s) are significantly different at P≤0.05.

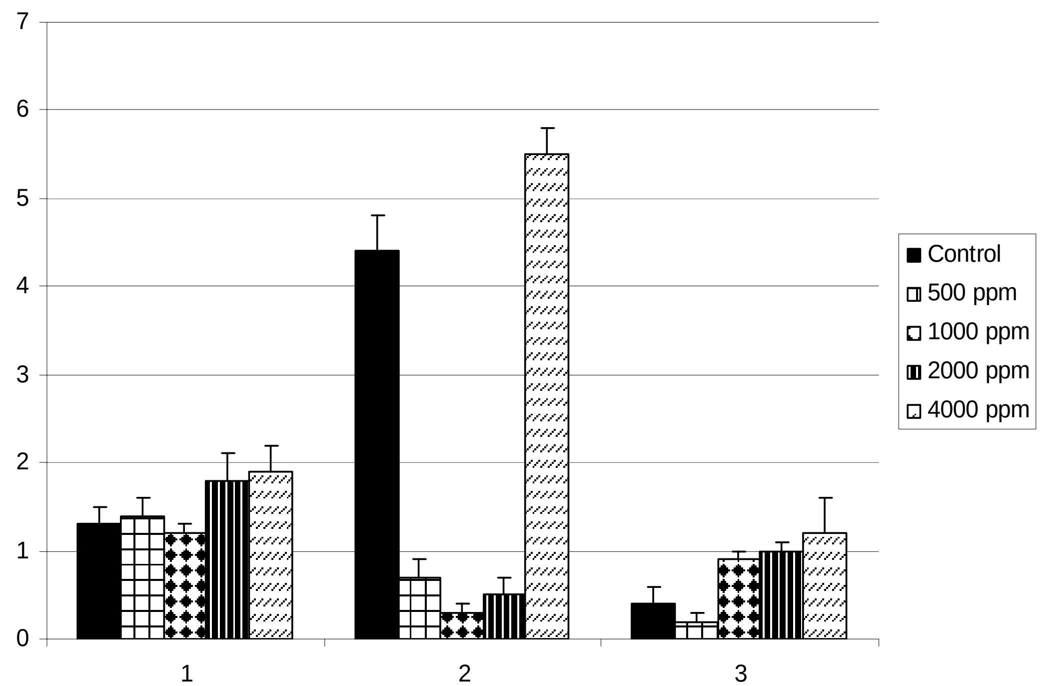

Figure 2: Growth Parameters: 1= Shoot growth (mm); 2 = Root length (mm); 3 = Number of roots per cutting. Bars followed by dissimilar letters are significantly different at P≤0.05

CHAPTER SIX

THE EFFECT OF INDOLE ACETIC ACID GROWTH REGULATOR AND ROOTING MEDIUMS ON ROOTING OF *LEUCADENDRON LAXUM* (PROTEACEAE) IN A SHADE TUNNEL ENVIRONMENT

The Effect of Indole Acetic Acid Growth Regulator and Rooting Mediums on Rooting of *Leucadendron laxum* (Proteaceae) in a Shade Tunnel Environment

[1]C.P. Laubscher and [2]P.A. Ndakidemi

[1]Faculty of Business, [2]Faculty of Applied Sciences,

Cape Peninsula University of Technology, P.O. Box 652, Cape Town, 8000, South Africa

Corresponding Author: P.A. Ndakidemi, Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town, 8000, South Africa

6.1 Abstract:

The effects of different concentrations of IAA exogenously applied to Proteaceae plants of the genus *Leucadendron laxum* on a variety of rooting medium were studied. The aim of the study was to evaluate the rooting response of tip cuttings of nature-grown shrubs using an intermittent mist propagation system in a shaded tunnel. The IAA treatments included a control, 500, 1000, 2000 and 4000 ppm IAA auxins. The growth mediums tested were: a) bark/polystyrene (1:1); b) peat moss/polystyrene (1:1); c) bark/river sand /polystyrene (1:1:1); and d) perlite/river sand (1:1). Each treatment was replicated in ten pots. Overall, the exogenous supply of IAA had a positive effect in the advancement of rooting in *L. laxum* grown in a) peat/polystyrene and b) bark/river sand/polystyrene mediums. With regard to growth mediums, results indicated that the a) bark/polystyrene and b) peat/polystyrene mediums were significantly superior in promoting different aspects of rooting when compared with the other mediums. Evidence from this study was conclusive that the shade tunnel was an economical and successful environment in rooting *L. laxum.*

Abbreviations: IAA = Iindole-3-acetic acid; SAFEC = South African Flower Export Council

Key words: Auxin • Bark • Flower industry • Peat moss • Polystyrene • River sand • Threatened species

6.2 INTRODUCTION

Leucadendron laxum is one of the world's 329 Protea species and is amongst more than 70 commercial cut flowers of Southern Africa [1]. Most Leucadendrons are grown from seed. However, this propagation method is mainly restricted to species grown for retail sales, while vegetative propagation techniques are more widely used in propagating higher quality cultivars and hybrids for commercial cut flower production [2]. Vegetative cuttings also remain cost effective due to the plants flowering a year earlier than seed grown plants [3]. Vegetative propagation techniques for most Proteaceae are broadly described [4] and give little reference to the propagation of individual species.

Certain plant species in the Proteaceae family remain difficult to root [4], and some such as *L. elimense* subsp. *Elimense,* do not propagate vegetatively [5]. The application of auxins such as IAA may induce economically viable results in Proteaceae by increasing rooting percentages, although the aryl esters of IAA are equal or more effective than acid formulations in root initiation [4]. Additional experiments with auxin formulations in order to increase rooting percentages of woody species remain necessary for the development of commercial crops [4]. Further research on the use of different concentrations of IAA on *L. laxum* in shade conditions will show which treatment can provide faster and more desirable rooting results of vegetative cuttings.

The function of the rooting medium should not be excluded during rooting trials. Rooting mediums are essential in increasing rooting percentages, as the selection and combination of medium components are of importance in the rooting success of any vegetative propagation [6]. The combination of suitable rooting components is essential in providing adequate aeration and drainage to ensure faster and better quality root development. These requirements can be obtained from components such as bark, peat, polystyrene and river sand. Milled pine bark is a medium widely used and must be of good quality [7]. A coarse grade peat moss will enhance the water holding capacity of the medium [4]. Polystyrene will improve aeration and coarse grade, washed river sand will improve the water drainage [8]. The ideal rooting environment is necessary to provide the optimum conditions for successful rooting. The use of a shade tunnel will reduce electricity usage and the maintenance and construction costs compared to environmentally controlled greenhouses. Vegetative propagation techniques were chosen because they provide uniform flowering times and replicate the same characteristics and growth habit as parent stock plants [9,3]. The results of this study will indicate new study, as the propagation of *L. laxum* and the use of IAA auxins and shaded rooting conditions in Proteaceae have never been recorded.

This study aimed at testing the rooting potential of *L. laxum* in shade tunnel conditions using various strengths of IAA auxin applications and different growth mediums.

6.3 MATERIALS AND METHODS

The experiment was conducted at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa. The experiment lasted for 8 weeks from mid August to mid October. *L. laxum* cutting material was sourced from nature grown populations on the Agulhas Plain and was kept dry and transported overnight to Cape Town.

The IAA treatments and growth mediums were randomised in a complete block design of four concentrations of IAA (control, 500, 1000, 2000 and 4000 ppm) and four growth mediums: a) bark / polystyrene (1:1); b) peat moss / polystyrene (1:1); c) bark / river sand / polystyrene (1:1:1); and d) perlite / river sand (1:1). Each treatment was replicated in ten pots. Tip cuttings were made 150 mm long from semi-hardwood stems [9, 10, 3, 11] and half of the lowest leaves were removed [12, 7]. The cuttings were rinsed in Benlate fungicide (10g/l) before planting and sprayed with Captab fungicide (2g/l) weekly to prevent fungal infections [3, 11]. Wilted and infected cuttings were removed over the rooting period [9]. Adequate drainage was provided to prevent *Phytophtora* fungal infection of roots.

IAA powder was dissolved at g/l in 50% ethyl alcohol. The basal 5 mm of cuttings were dipped for 5 seconds in the rooting hormone [9, 4, 3] and then planted into foam plug trays and placed in a white shade (40%) tunnel. Midday temperatures were measured between 18 - 25ºC and a relative humidity between 20 and 62% was recorded. The irrigation timer was set on 10 sec on and 60 min off.

Data were recorded on the percentage of callused cuttings, rooted cuttings and survival rates. Quality of growth was assessed on the numbers of roots, their length and the length of shoots obtained. These results were transformed into data percentages prior to analysis of variance. Data analysis was performed in two different ways. The first method consisted of one way analysis of variance for IAA treatments added to each growth medium separately. The second method consisted of factorial analysis including the four growth mediums and the five IAA concentrations. Data were presented as mean values with predicted standard errors (S.E.). These computations were done with the software program STATISTICA. The Fisher least significance difference (L.S.D.) was used to compare treatment means at $P<0.05$ level of significance [12].

6.4 RESULTS AND DISCUSSION

6.4.1 Effects of IAA treatments in a) peat / polystyrene and b) bark / river sand / polystyrene mediums: Statistical analysis of data showed that the addition of different IAA treatments in *L. laxum* had evident significant rooting effect in the a) peat / polystyrene and b) the bark / river sand / polystyrene mediums (Table 6.1). In the bark / river sand / polystyrene medium, the IAA supplied at 500 and 4000 ppm significantly ($P \leq 0.05$) produced more roots (3.3 and 5.7, respectively) per cutting than all other treatments (Table 6.1). Furthermore, the callusing percentage was significantly increased ($P \leq 0.05$) in the IAA treatment of the peat / polystyrene medium (Table 6.1). The only significant callusing (30 %) of the *L laxum* cuttings was observed in the 4000 ppm IAA treatment (Table 6.1). The root length of *L laxum* was also significantly increased ($P \leq 0.01$) in the 500 and the 4000 ppm IAA treatments, with measurements of 2.0 and 1.7 mm**,** respectively (Table 6.1). Overall, according to the results in (Table 6.1), the IAA had a positive effect in advancement of rooting in *L. laxum* grown in a) peat / polystyrene and b) the bark / river sand / polystyrene mediums. The overall success of the IAA treatments on the number of roots, callusing and root length in these two mediums are in agreement with Hartmann *et al*. [4] who reported successful effect of IAA in root promotion and development. Similar success in bark / river sand / polystyrene medium indicated a suitable rooting medium for Proteaceae. These results are in agreement with Brown and Duncan [9] and Reinten *et al*. [3] where cuttings required a well drained and light rooting medium.

6.4.2 The effects of growth mediums on callusing, rooting, survival, shoot growth, root length and number of roots: According to the results presented in Table 6.2, the a) bark / polystyrene and b) peat / polystyrene mediums were significantly superior growth mediums compared with the rest used in this study. These two mediums had the best similar performances in callusing, rooting, survival, shoot growth, root length and number of roots as compared with a) bark / river sand / polystyrene and b) the perlite / river sand (Table 6.2). For instance, the highest significant ($P \leq 0.001$) rooting percentage in *L. laxum* was observed in the

a) bark / polystyrene and b) the peat / polystyrene mediums with the highest rooting percentages of 31 and 29%, respectively. The a) bark / river sand / polystyrene and b) the perlite / river sand rooting percentages were significantly much lower (8 and 4%) than the first two mediums (Table 6.2). The survival rate of the cuttings was significantly different ($P \leq 0.001$) in all four mediums, but more so in the a) bark / polystyrene and b) the peat / polystyrene mediums (81 and 79%). The *L. laxum* cuttings propagated in the a) bark / river sand / polystyrene and b) the perlite / river sand showed lower survival rates of 28 and 29 percent respectively (Table 6.2). It is therefore likely that the higher survival measurements in the a) bark / polystyrene and b) the peat / polystyrene mediums were due to the superior moisture holding capacity of the bark and peat and the drainage supplied by the polystyrene compared with the other two mediums used. The fact that a higher survival was recorded in these mediums is an indication that a higher rooting percentage was possible. The rooting period of 8 weeks used in might have been too short. Brown and Duncan [9] reported a rooting period for Proteaceae to be between 8 and 12 weeks, while Kibbler *et al*. [13] suggested that an extended rooting period increases survival and rooting percentages. Extending the rooting period could produce different results. This should be considered in future studies.

Shoot growth was significantly ($P \leq 0.05$) influenced by growth mediums. The a) bark / river sand / polystyrene and b) peat / polystyrene medium were superior with shoots growing to an average of 1.07 and 0.53 mm, respectively (Table 6.2). The a) bark / polystyrene and b) peat / polystyrene mediums showed less successful shoot growth of 0.40 and 0.20 mm respectively (Table 2). The success in shoot growth in *L. laxum* using bark / peat is in agreement with Matkin [8]. The addition of polystyrene and river sand in some of the mediums provided more aeration and drainage. Species which are slower to root need to stay longer in the medium while the medium must continually supply the optimum rooting requirements to ensure optimum conditions.

The measurements of root length on the cuttings of *L laxum* showed significant differences ($P \leq 0.001$) with mediums. Values ranging from 3.0 - 2.87 mm were recorded in a) the peat / polystyrene and b) the bark / polystyrene mediums respectively (Table 6.2). This was followed by significantly lower values (1.0 - 0.60 mm) in a) bark / river sand / polystyrene and b) the perlite / river sand mediums, respectively. The root numbers per cutting were significantly ($P \leq 0.001$) influenced by growth mediums (Table 6.2). The a) bark / polystyrene and the b) peat / polystyrene medium were significantly better in developing roots per cutting (4.4 - 3.73) as compared with other mediums. Less significant results in the number roots formed on the stem cuttings was recorded in the a) bark / river sand / polystyrene and b) perlite / river sand medium mediums (2.27 - 0.93, Table 6.2). These results are not in agreement with results reported by

Lamb [14], where other species rooted faster in a similar rooting medium. This lower success rate in perlite / river sand could be contributed to the possibility of faster draining capacity of the sand or the fact that perlite water retention of 23 percent [8] was too high for rooting *L. laxum* cuttings.

Results from this study clearly show that rooting of *L. laxum* was significantly influenced by rooting medium. Overall, the a) bark / polystyrene and b) peat / polystyrene mediums were more responsive to rooting and shooting characteristics than the other two mediums tested (Table 6.2). The overall results in these two rooting mediums may be attributed to their good aeration characteristics and good water holding capacity [15, 16]. Taken together, these two characteristics are documented to positively favour the rooting physiology in different plant species [17, 18].

6.4.3 The effect of IAA concentrations on callusing, rooting, survival, shoot growth, root length and number of roots: As shown in Table 6.2 all IAA treatments, including the control, did not significantly influence the percentage of cuttings that callused and survived, and shoot growth in *L. laxum.* However, the IAA treatments significantly ($P \leq 0.05$) influenced the percentage rooting, root length and the number of rooted cuttings in *L. laxum*. The addition of 500 ppm of IAA was the most significant treatment in increasing the rooting percentages (23%) of cuttings (Table 6.2). These results were closely followed by the 4000 and 2000 treatments which obtained 22 and 20% respectively. The control and 1000 ppm IAA treatment were the lowest in influencing rooting, with 12 and 13% respectively (Table 6.2). Results from this study showed that IAA was successful in rooting *L. laxum.* This is in agreement with other research findings which accredited auxins such as IAA in playing a significant role in the stimulation of rooting characteristics in cuttings of different plant species [4]. The rooting efficiency may be influenced by the exogenous supply of auxins, especially in difficult to root plants such as those belonging to Proteaceae family including *L laxum*. Auxins have been reported to increase the number of roots formed per cutting in different plant species [19].

In the data obtained in Table 6.2, the root length and number of roots per cutting were significantly ($P \leq 0.05$) affected by all IAA treatments as compared with the control. Root length in these treatments measured between 0.67 in the control to 2.58 in the cuttings supplied with IAA at 500 ppm. The number of roots per cutting counted between 0.92 in the control and 4.33 in the treatment supplied with 500 ppm. Overall, for the number of roots per cutting and root length, the IAA treatment applied at any level produced positive results which were not significantly different from each other when compared with the control treatment. This suggests that the IAA treatments were equally effective in influencing these two parameters. The effects of these IAA treatments are well known to show a significant impact in rooting other plant species [20, 1]. This study also concurs with Hartmann et al. [4] theory that a quick dip application of auxin is an effective method of applying auxins to cuttings. Furthermore, shade tunnel conditions were proved sufficient in providing a stable rooting environment for *L. laxum*.

6.5 CONCLUSION

The preliminary results from the study indicated that the possibilities exist for the successful vegetative propagation of *L. laxum* under cheaper shaded tunnel conditions that may be affordable to many flower producers in the farming communities with limited greenhouse facilities. In such areas, the use of this approach in combination with the appropriate doses of IAA and rooting mediums should be able to promote rooting of the difficult to root plant specie such as *L. laxum*. Additionally, this may add value to the rapid multiplication and hence the domestication of this plant species which is listed as endangered in South Africa.

The present study provides new scientific opportunities for the vegetative propagation of *L laxum,* the rooting possibilities of which were previously undocumented and which remains threatened in South Africa. The reported technology which makes use of a shaded tunnel can be cost effective, sustainable and could be used by flower growers with little resources.

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6.7 REFERENCES

1. Dodd, J., and D.T. Bell, 1993. Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. Australian Journal of Botany, 18: 281-293.

2. Robyn, A. and G. Littlejohn, 2002. Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production, pp: 22-48.

3. Reinten, E., S. Gertze and L. Arends, 2002. Propagation methods. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Elsenburg, South Africa, pp: 50-70.

4. Hartmann, H.T., D.E. Kester, F.T. Davies and R.L. Geneve, 2002. Plant Propagation Principles and Practices. 7th Edition. New Jersey, Prentice Hall, pp: 367-374.

5. Tansley, S.A. and C.R. Brown, 2000. RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): Implications for their conservation**.** Biological Conservation, 95: 39-48.

6. Laubscher, C.P., 1999. Rooting techniques for selected tree species. M Thesis. Unpublished. Cape Technikon, Cape Town.

7. Owings, A.D., 1996. Variations in pH from different bark sources. In the Combined Proceedings of the 1996 International Plant Propagators' Society, 46: 699.

8. Matkin, O.A., 2008. Perlite Vs. Polystyrene In Potting Mixes. The Schundler Company 150 Edison, NJ 08817 http://www.schundler.com/polystyrene.htm.

9. Brown, N., and G. Duncan, 2006. Manual for Growing Proteaceae in Greenhouses Joseph Knight in 1809 www.protea.worldonline.co.za/growknight.htm :14.

10. Littlejohn, G., 2002. Legal Issues, Planning and Planting. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Agricultural Research Council, Elsenburg, South Africa pp: 82-90.

11. S.A. Flower Export Council, 2002. Preparing orchards for planting of Proteaceae. Certificate course in flower export readiness. Unpublished booklet. Elsenburg, South Africa.

12. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach, Second Edition. New York, McGraw Hill.

13. Kibbler, H., M.E. Johnston and R.R. Williams, 2004. Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell: 2 Seasonal influences of temperature, rainfall, flowering and auxins on the the stock plant. Scientia Horticulturae, 102: 343-358.

14. Lamb, J.G.D., 1972. The use of Peat in Propagating Hardy Nursery Stock at Kinsealy. Acta Horticulturae, 26: 125-128.

15. Leakey, R.R.B., J.F. Mesén, Z. Tchoundjeu, K.A. Longman, J.McP. Dick, A. Newton, A. Martin, J. Grace, R.C. Monro and P.N. Muthoka, 1990. Low technology techniques for the vegetative propagation of tropical trees. Commonwealth Forestry Review, 69: 247-257.

16. Ofori, D.A., A.C. Newton, R.R.B. Leakey and J. Grace, 1996. Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentrations, leaf areas and rooting medium. Forest Ecology and Management, 84: 39-48.

17. Grange, R.I., and K. Loach, 1983. The water economy of un-rooted leafy cuttings. Journal of Horticultural Science, 58 (10): 1-17.

18. Newton, A.S., J.F. Mesén, J. McP Dick and R.R.B. Leakey, 1992. Low-technology propagation of tropical trees: rooting physiology and its practical implications. In: Mass production Technology for genetically Improved Fast Growing Forest Tree Species, vol 2 AFOCEL, Nangis, pp: 417-324.

19. Aminah, H., J.Mcp Dick, R.R.B. Leakey, J. Grace and R.I. Smith, 1995. Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. Ecology. Management, 72:199-206.

20. Baraldi, R., G. Bertazza, S. Predierii, A. Bregoli and J.D. Cohen, 1993. Uptake and metabolism of Indole-3-butyric acid during the in vitro rooting phase in pear cultivar (*Pyrus comunis*). Acta Horticulturae, 329: 289-291.

Table 6.1: Effect of different IAA concentrations on number of roots per cutting, percentage callus formed, and root length of *Leucadendron laxum* grown in peat / polystyrene and bark / river sand / polystyrene medium

	Peat / Polystyrene	**Bark / River sand / Poly**		
	Number of roots (cutting)	**%Callused**	**Root Length**	**Number of roots (cutting)**
Control	**1 ±0.58b**	**10 ±5.8b**	**0.7 ±0.33b**	**1.0 ±0.58b**
500	**4 ±2.00a**	**13 ±3.3b**	**2.0 ±0.00a**	**3.3 ±0.88a**
1000	**4 ±1.53a**	**3 ±3.3b**	**0.7 ±.33b**	**1.3 ±0.67b**
2000	**3 ±0.58a**	**13 ±6.7b**	**0.0 ±.00b**	**0.0 ±0.00b**
4000	**3 ±1.53a**	**30 ±5.8 a**	**1.7 ±0.33a**	**5.7 ±2.19a**
F Statistic	**0.80***	**3.63***	**10.0****	**4.0***

Values presented are means ± SE. *, ** = significant at $P \leq 0.05$ and 0.01 respectively.
Means followed by the same letter are not significantly different from each other at $P \leq 0.05$

Table 6.2: Effect of different growth medium and IAA concentrations on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, shoot growth, root length and the number of roots per cutting of *Leucadendron laxum*

Treatment	**% Callused**	**% Rooted**	**% Survival**	**Shoot Growth**	**Root Length**	**Number of roots/cutting**
Main Treatment						
Bark Polystyrene	**42 ±9.2a**	**29 ±4.8a**	**81 ±4.5a**	**0.40 ±0.13b**	**2.87 ±0.52a**	**4.40 ±0.96a**
Peat Polystyrene	**31 ±6.9ab**	**31 ±4.0a**	**79 ±3.2a**	**0.53 ±0.17ab**	**3.00 ±0.59a**	**3.73 ±0.50ab**
Bark River Sand Poly	**14 ±3.1bc**	**8 ±1.7b**	**28 ±4.7b**	**1.07 ±0.28a**	**1.00 ±0.22b**	**2.27 ±0.69bc**
Perlite River Sand	**8 ±2.4c**	**4 ±1.3b**	**29 ±6.4b**	**0.20 ±0.11b**	**0.60 ±0.21b**	**0.93 ±0.47c**
F Statistic	**5.3****	**18.2*****	**37.4*****	**3.53***	**9.22*****	**6.66*****
Sub - Treatment						
Control	18 ±6.5a	**12 ±3.9b**	58 ±9.6a	0.33 ±0.14a	**0.67 ±0.19b**	**0.92 ±0.29b**
500	27 ±9.8a	**23 ±6.9a**	58 ±8.9a	0.75 ±0.25a	**2.58 ±0.54a**	**4.33 ±0.81a**
1000	20 ±7.7a	**13 ±4.1b**	52 ±10.2a	0.33 ±0.14a	**2.00 ±0.75a**	**2.58 ±0.90a**
2000	27 ±9.2a	**20 ±5.5ab**	53 ±10.4a	0.58 ±0.19a	**1.75 ±0.52ab**	**3.00 ±1.06a**
4000	28 ±5.5a	**22 ±3.7ab**	50 ±7.5a	0.75 ±0.33a	**2.33 ±0.54a**	**3.33 ±0.74a**
F Statistic	0.32 ns	**2.2***	0.46 ns	0.90 ns	**2.62***	**2.48***

Values presented are means ± SE. **, *** = significant at P ≤ 0.01 and 0.001 respectively. ns = not significant. Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05

CHAPTER SEVEN

GENERAL CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 LITERATURE REVIEW AND INTRODUCTION

The introductory chapter established a rationale for the study with a review of literature, to provide an understanding of the conceptual framework and theoretical underpinning of the threat of Red Data Proteaceae on the Agulhas Plain. The study strived to identify new knowledge that may be used to solve conservation and propagation problems, thereby increasing the conservation of Red Data species and promoting ecotourism on the Agulhas Plain. This reference study showed that threatened Proteaceae species such as *L. laxum* are continually being destroyed through illegal cut flower harvesting in the wild. These important plant species have the potential to attract international tourists to the Western Cape. The few existing ecotourism facilities provided evidence that the development potential of ecotourism in the area has not been fully realised. Furthermore, interviews with farmers on the Agulhas Plain revealed that little information is available on Red Data species and that propagation of these species has not been considered. The reference study therefore investigated current propagation techniques of Proteaceae and found now propagation recordings on Red Data species of the Agulhas Plain. The study suggests that factors responsible for the deterioration of the environment, the loss of species and the lack of ecotourism development on the Agulhas Plain should be investigated. Furthermore ecotourism facilities need to be established. It is necessary to investigate the management practices of landowners, such as wild flower harvesting, orchard plantings, fire and alien plant control, and staff training to conserve the Agulhas Plain. Improved propagation techniques for Proteaceae species, such as the use of auxin applications as well as optimum rooting environment and mediums, could help advance the growing and replacement of threatened species. Future studies should be aimed at developing new propagation techniques and the replanting of threatened species in the wild.

7.2 PRACTICES INVOLVING ENDANGERED LEUCADENDRON SPECIES AND THE FUTURE INFLUENCE OF ECOTOURISM DEVELOPMENT ON THE AGULHAS PLAIN

Chapter 2 investigated the threats to endemic plant species on the Agulhas Plain where many species are harvested for commercial export cut flowers. The aim of the study was to collect data which could be linked to the destruction of flora and its impact on the development of ecotourism on the Agulhas Plain. The study found that the ecotourism potential of the Cape Floral Kingdom has not been explored and that the potential for future development exists. The Western Cape is the largest area where Red Data species occur and where illegal harvesting of these species is taking place. The study found that very little training of individuals and farm workers is carried out. There seems to be a lack of knowledge of the Red Data species in the area, as well as methods that could be used to conserve these species. Farmers continue to graze animals in threatened natural habitats where flowering plants are destroyed before seed set can occur. Fire was identified as the second biggest threat to the biodiversity of the area. Fires are often started by the irresponsible actions of landowners or their employees and as a result many hectares of land are destroyed. This factor also has a devastating effect on ecotourism on the Agulhas Plain. Furthermore, this study found that permits necessary for the cutting and trading of flowers are misused. Even though the issuing of permits ranks high it was clearly evident that the lack of conservation authorities visiting farms is a big problem. This lack of action causes further abuse of permits, flower harvesting and habitat destruction. The study documented that landowners are positive with regards to conservation and the promotion of ecotourism on the Agulhas Plain, although a lack of information and guidance from authorities exists. The lack of tourist facilities was also evident, even though the Agulhas Plain is perceived as one of the most scenic destinations to view unique plant species in pristine natural habitats. There is no question that foreign tourists would visit the Agulhas Plain if the natural habitat of threatened Red Data plant species could be preserved and the current poor infrastructure for ecotourism activities upgraded and developed. This chapter concluded with the findings that there is no data available on propagation methods of selected Red Data species such as *L. laxum*. The study suggested the testing of auxins, rooting mediums and propagation facilities using randomised block designs in the rooting and shooting of Proteaceae. The propagation of these species could help save highly threatened species in sensitive habitats and develop more sustainable agriculture practices which will support future ecotourism on the Agulhas Plain. The study established a great need for the scientific development of vegetative propagation of Red Data species such as *L. laxum* on the Agulhas Plain as few Red Data species are propagated and very little is done to increase the awareness of these species. In propagating these plants species especially where only a few populations or even a few plants exist, they can be reintroduced in nature.

7.3 ROOTING SUCCESS USING IBA AUXIN ON ENDANGERED *Leucadendron laxum* (PROTEACEAE) IN DIFFERENT ROOTING MEDIUMS

Chapter 3 investigated the rooting ability of *L. laxum* (Marsh Rose Leucadendron), an endangered plant species on the AP, for reintroduction into the natural habitat. The study tested IBA liquid hormone preparations and four growth mediums. The treatments included a control, 500, 1000, 2000 and 4000 ppm concentrations. The four growth mediums, namely: a) bark / polystyrene; b) peat moss / polystyrene; c) bark / river sand / polystyrene; and d) perlite / river sand were tested in an environmentally controlled greenhouse with bottom heat. The experiments used a randomised block design with 10 cuttings per treatment, replicated four times. The results showed that the bark / sand / polystyrene medium had the highest (45%) significant survival rate when supplied with IBA at 500 ppm. The application of 1000 ppm of IBA in bark / sand / polystyrene medium significantly increased the number of roots per cutting (5) and produced the longest roots (4 cm). The bark / polystyrene medium supplied with zero IBA had the largest number of roots per cutting (6.5). The comparison of the four growth media showed that bark / polystyrene was the best medium, producing the highest and most significant amount of callus formation (30%), highest percentage of cuttings that rooted (28%), cuttings with a higher survival rate (57%), and cuttings with a higher number of roots (3.5) and the longest roots (7.35 mm). With regard to various IBA treatments tested across all treatments, the IBA supplied at 1000 ppm produced greater root numbers when compared with the other treatments.

The study concluded that a hormone application of 1000 ppm IBA was successful in rooting *L. laxum*. The bark / sand / polystyrene and the bark / polystyrene mediums contributed to successful root development of cuttings. However, the bark / sand / polystyrene medium was most successful in increasing the survival rate and root length of cuttings. As no other evidence could be found on the propagation of endangered species on the Agulhas Plain, this study recommends that further tests on *L. laxum* need to be conducted using various combinations of auxins and in different environments to increase the quantity and quality rooted plants. The successful production of quality cuttings for the industry is largely defined by the grower who is able to use the correct auxins treatments and rooting mediums to produce quality Proteaceae cuttings which will succeed when planted in the field.

7.4 ROOTING RESPONSE UNDER SHADE USING IBA GROWTH REGULATORS AND DIFFERENT GROWTH MEDIUMS ON *Leucadendron laxum* (PROTEACEAE) – A COMMERCIAL CUT FLOWER

Chapter 4 described a study on the rooting potential of *L laxum,* a popular cut flower on the Agulhas Plain. The study tested various Indole-3-butyric acid (IBA) liquid hormone concentrations in combination with different growth mediums. The IBA treatments included a control and 500, 1000, 2000 and 4000 ppm IBA auxin applications. Growth mediums tested were a) peat / polystyrene; b) bark / polystyrene; c) bark / sand / polystyrene; and d) perlite / river sand. Basal ends of cuttings were dipped into the IBA solution for a few seconds after preparation, and the treated cuttings were planted under shade conditions. The experiment was planned in a randomised block design, with 10 cuttings and 3 replications per treatment. The IBA application, in comparison with the control, significantly improved rooting percentages in the peat / polystyrene and bark / river sand / polystyrene mediums. The bark / polystyrene medium was more effective in the stimulation of roots, survival of cuttings, promotion of root length and the number of roots that developed, as compared to the other mediums. Overall, the application of IBA induced more root and shoot formation.

In conclusion, the results of this study have demonstrated the effects of various concentrations of the auxin IBA and different growth mediums on the rooting of *L. laxum* under shade house conditions. These results can provide growers with the information necessary to stimulate the rooting of *L. laxum* cuttings faster and at lower costs over a period of 8 - 12 weeks. This study also indicated that the growth medium environment determines the survival rate success of cuttings, which in turn results in progressive rooting success. The use of IBA in the rooting of *L. laxum* was variable in different rooting mediums where moisture retention was important. Other endogenous factors in the growth medium of bark, warrants further investigation under different environmental conditions when propagating Proteaceae.

7.5 ROOTING AND GROWTH POTENTIAL OF *Leucadendron laxum* (PROTEACEAE) USING DIFFERENT ROOTING MEDIUMS AND INDOLE ACETIC ACID GROWTH REGULATORS

Chapter 5 identified *L. laxum,* an endangered Red Data plant species on the Agulhas Plain, as having a high commercial value as a flowering potted plant. Limited research information on the culture and propagation of this species was found. This study therefore investigated the applications of the rooting hormone indole acetic acid (IAA) in various rooting mediums. The IAA treatments for the study included a control and 500, 1000, 2000 and 4000 ppm concentrations. Four rooting mediums were used: a) bark / polystyrene; b) peat moss / polystyrene; c) bark / river sand / polystyrene; and d) perlite / river sand. The study used a randomised block design with 3 replications.

The study found that the bark and polystyrene medium had the most significant results in root and shoot growth, and percentage of plants that callused, rooted and survived. The different concentrations of IAA treatments had significant effects on rooting, callusing, shoot growth, root lengths and numbers of roots per cutting. This study concluded that the bark / polystyrene medium was the medium that contributed most to the root development, survival rate and root length of cuttings. These results established that the optimum IAA levels stimulated rooting in *L. laxum*. Generally, as compared to the control treatment, the IAA hormone treatment had significant effects on rooting success of the *L. laxum* cuttings. The conclusion of this study is that the successful production of quality cuttings of *L. laxum* for the indigenous cut flower industry can largely be achieved by choosing the appropriate growth medium supported with the correct concentration of the growth hormone.

7.6 THE EFFECT OF INDOLE ACETIC ACID GROWTH REGULATOR AND ROOTING MEDIUMS ON ROOTING OF *Leucadendron laxum* (PROTEACEAE) IN A SHADE TUNNEL ENVIRONMENT

Chapter 6 tested the rooting ability of *L. laxum* under shade house conditions. The effects of different concentrations of IAA exogenously applied to Proteaceae plants of the genus *L. laxum* on a variety of rooting medium were studied. The aim of the study was to evaluate the rooting response of tip cuttings of nature-grown shrubs using an intermittent mist propagation system in a shaded tunnel. The IAA treatments included a control, 500, 1000, 2000 and 4000 ppm IAA auxins. The growth mediums tested were: a) bark/polystyrene (1:1); b) peat moss / polystyrene (1:1); c) bark / river sand / polystyrene (1:1:1); and d) perlite / river sand (1:1). Each treatment was replicated in ten pots. Overall, the exogenous supply of IAA had a positive effect on the rooting of *L. laxum* grown in peat / polystyrene and bark / river sand / polystyrene mediums. With regard to growth mediums, results indicated that the bark / polystyrene and peat / polystyrene mediums were significantly superior in promoting the various aspects of rooting when compared with the other mediums. Evidence from this study was conclusive that the shade tunnel was an economical and successful environment in rooting *L. laxum.*

The preliminary results of the study indicated that the possibilities exist for the successful vegetative propagation of *L. laxum* under the cheaper shaded tunnel conditions that may be affordable to many flower producers in the farming communities with limited greenhouse facilities. In such areas, the use of this approach (in combination with the appropriate rooting mediums and doses of IAA) should result in the promotion of rooting of difficult to root species such as *L. laxum*. Additionally, this may add value to the rapid multiplication and hence the domestication of this plant species, which is listed as endangered in South Africa.

The present study provides new scientific opportunities for the vegetative propagation of *L laxum,* which remains threatened in South Africa and whose rooting possibilities were previously undocumented. The reported technology which makes use of a shaded tunnel can be cost effective, sustainable and could be used by flower growers with little resources.

7.7 MAJOR FINDINGS OF THE STUDY

Chapter 2 found that many Red Data species on the Agulhas Plain are continually being destroyed through illegal flower harvesting and permits are abused. The study found that there is clear evidence of a lack of visits to farms by conservation authorities, even though high rates of permit possession exist. There is a lack of knowledge of the Red Data species in the area, as well as methods that could be used to conserve these species. The study reported that landowners, local communities and conservation authorities are not vigilant in identifying and conserving sensitive and vulnerable areas and that very little training of individuals and farm workers are in place. Farmers continue to graze animals in the natural habitat where many threatened species occur. Fire was identified as the second biggest threat to the biodiversity of the area. This factor also has a devastating effect on ecotourism development on the Agulhas Plain. The study found that the ecotourism potential of the Agulhas Plain has not been explored and that a current poor infrastructure of ecotourism activities and facilities is evident. The study found that landowners are positive with regards to conservation and the promotion of ecotourism on the Agulhas Plain, although a lack of information and guidance from authorities exists. The study established a great need for the scientific development of vegetative propagation of Red Data species such as *L. laxum* on the Agulhas Plain. These propagation methods could increase the awareness of these species and help reintroduce them back into the wild.

Chapter 3 investigated the rooting ability of *L. laxum* using IBA auxins. The results showed that the application of 1000 ppm of IBA in bark / sand / polystyrene medium significantly increased the number of roots per cutting and produced the longest roots. The bark / polystyrene medium produced the highest and most significant amount of callus formation, highest percentage of cuttings that rooted, cuttings with a higher survival rate, and cuttings with a higher number of roots and the longest roots.

Chapter 4 found that the IBA application, in comparison with the control, significantly improved rooting percentages of *L laxum* under shade house conditions. The bark / polystyrene medium was more effective in the stimulation of roots, survival of cuttings, promotion of root length and the number of roots that developed, as compared to the other mediums. Overall, the application of IBA induced more root and shoot formation. This study also indicated that the growth medium environment determines the survival rate success of cuttings, which in turn results in progressive rooting success. The use of IBA in the rooting of *L. laxum* was variable in different rooting mediums where moisture retention was important.

Chapter 5 identified *L. laxum,* as having a high commercial value with limited research information available. A bark and polystyrene medium had the most significant results in root and shoot growth, and percentage of plants that callused, rooted and survived. The different concentrations of IAA treatments had significant effects on rooting, callusing, shoot growth, root lengths and numbers of roots per cutting. This study concluded that the bark / polystyrene medium was the medium that contributed most to the root development, survival rate and root length of cuttings. These results established that the optimum IAA levels stimulated rooting in *L. laxum*. Generally, as compared to the control treatment, the IAA hormone treatment had significant effects on rooting success of the *L. laxum* cuttings.

Chapter 6 reported that the exogenous supply of IAA had a positive effect on the rooting of *L. laxum.* The results indicated that the bark / polystyrene and peat / polystyrene mediums were significantly superior in promoting root formation when compared with the other mediums. The shade tunnel was an economical and successful environment in rooting *L. laxum* and indicated that that the possibilities exist for the successful vegetative propagation under less expensive shaded tunnel conditions.

This final chapter provided a summary of the research activities and the findings. The ensuing recommendations culminate in a conservation and propagation policy for Red Data species on the Agulhas Plain. This study was aimed at identifying new knowledge that may be used to address the various challenges facing Red Data species conservation on the Agulhas Plain. This national heritage is of major importance for ecotourism development of the region. It is hoped that the findings of this study will support the cut flower industry in propagating and cultivating endangered Red Data species through sustainable conservation.

7.8 RECOMMENDATIONS

This study recommends that the destruction of Red Data species, in particular those with few remaining populations and highly endemic species, should receive greater conservation attention. Additional research studies are necessary to investigate the conservation of these highly sensitive species. Furthermore local communities should be encouraged to supply the need for local tourist based products to increase employment in the region. These could include the development of flower routes, indicating existing Red Data populations, similar to the famous wine routes in other parts of the Western Cape should be developed. These can also include visits to working flower farms. More printed information should be distributed in the area and in tourism offices to educate local people and tourists regarding the threats to these species. Further research is necessary to find ways to support landowners financially in the control of invasive plant species, conservation management of threatened Red Data species and preventative fire control. Policing of illegal harvesting of Red Data species, over harvesting and permit abuse should be improved.

Questions relating to the propagation of other Red Data species should be addressed in future research projects, including environmental control, auxin applications and other practical methods. The results from this study are valuable in assisting in propagating other species. However, further studies on the rooting of endangered species such as *L. elimense, L. platyspermum* and *L. stelligerum* are recommended to encourage the re-establishment of these plants in the wild. In addition to developing propagation techniques, farmers need to investigate the horticultural potential of many of these indigenous species. This recommendation will increase the awareness of the plight of endangered species in South Africa. The flora of the Cape Floral Kingdom holds great commercial potential for the future. The preservation of this flora is highly important for the future development of sustainable national and international ecotourism on the Agulhas Plain.

CHAPTER EIGHT

REFERENCES

REFERENCES

ABI, 2008. *Agulhas Biodiversity Initiative research report.* http://www.flowervalley.org.za/abi.html [5 November 2008].

Alegre, J. Toledo, J.L. Martinez, A. Mora, O. & De Andres, E.F. 1998. Rooting ability of Dorycnium species. *Scientia Horticulturae*, 76: 123-129.

Aminah, H. Dick, JMc.P. Leakey, R.R.B. Grace, J. & Smith, R.I. 1995. Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. Ecology Management, 72:199-206.

Baraldi, R. G. Bertazza, S. Predierii, A. Bregoli & Cohen, J.D. 1993. Uptake and metabolism of Indole-3-butyric acid during the in vitro rooting phase in pear cultivar (*Pyrus comunis*). *Acta Horticulturae*, 329: 289-291.

Barzilay, A. Ben-Jaacov, J. Cohen, A. Ion, A. & Halevy, H. 1992. Mini-gladiolus as a flowering pot plant. *Scientia Horticulturae,* 49: 117-124.

Berney, J. 2000. Combined research to benefit Protea growers. *Australian Horticulture,* 98(2): 16-18.

Bond, W.J. & Van Wilgen, B. 1996. *Fire and Plants.* London: Chapman and Hall.

Brown, N.A.C. Kotze, D. & Botha, P. 1998. Grow Proteas. *A Guide to the Propagation and Cultivation of Some South African Proteaceae. Kirstenbosch Gardening Series.* Cape Town: National Botanical Institute.

Brown, N. & Duncan, G. 2006a. *Manual for growing Proteaceae in greenhouses Joseph Knight in 1809.* http://www.protea.worldonline.co.za/growknight.htm :14.

Brown, N. & Duncan, G. 2006b. *Grow fynbos plants*. South African National Biodiversity Institute, Claremont, Cape Town.

Coetzee, J.H. & Littlejohn, G. 1994. Indigenous South African Flower Industry. *Hortagrow,* 8: 9-11.

Cole, N. Lombard, A.T. Cowling, R.M. Euston-Brown, D. Richardson, D.M. & Heijnis, C.E. 2000. *Framework for a conservation plant for the Agulhas Plain, Cape Floristic Region, South Africa.* Institute for Plant Conservation, Cape Town: UCT.

Cooper, K.H. 2003. A future for SA environmental conservation. *Park and Grounds*. May: 50-52.

Cowling, R.M. 1987. Fire and its role in coexistence and speciation in Gondwanan shrublands. *South African Journal of Science,* 83: 106-112.

Cowling, R. 1993. Ecotourism - What is it and what can it mean for conservation. *Veld and Flora,* 79(1): 3-5.

Cowling, R. Richardson, D. 1995. *Fynbos. South Africa's Unique Floral Kingdom.* Cape Town: Fernwood Press: 7-31.

De Klerk, G. Ter Brugge, J. & Marinova, S. 1997. Effectiveness of IAA, IBA and NAA during adventitious roots, new concepts, new possibilities. *In Vitro Cellular Developmental Biology,* 35: 188-189.

De Villiers, T. 2002. First Words: A farmer's viewpoint. *Veld and Flora,* 88(2): 42.

Dodd, J. & Bell, D.T. 1993. Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. *Australian Journal of Botany,* 18: 281-293.

Flower Valley 2008. *Annual Report.* http://www.givengain.com/cause_data/images/1866/FlowerValleyReport2008.pdf [9 February 2009].

Fogaça, C.M. & Fett-Neto, A.G. 2005. Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrance. *Plant Growth Regulation,* 45: 1-10.

Grange, R.I. & Loach, K. 1983. The water economy of un-rooted leafy cuttings. *Journal of Horticultural Science,* 58(10): 1-17.

Hancock, B. 2002. Trent focus for research and development in primary health care: An introduction to qualitative research. *Trent Focus,* 1998: 1-27.

Hartmann, H.T. & Kester, D.E. 1983. *Plant propagation; principals and practices.* London: Prentice-Hall International.

Hartmann, H.T. Kester, D.E. Davies, F.T. & Geneve, R.L. 2002*. Plant propagation principles and practices.* 7th ed. New Jersey: Prentice Hall: 367-374.

Hilton-Taylor, C. 1996. *Red Data List of Southern African Plants*. *Strelitzia 4*. Pretoria: National Botanical Institute.

Hughes, C. 2002. The potential impact of viticulture expansion on biodiversity in the Cape Floristic Region. MSc Thesis, University of Cape Town, Cape Town.

Jamieson, H.G. 2001. *Leucadendron laxum. I. Williams.* Cape Town: Kirstenbosch Botanic Garden.

Jeffery, R. 2003. The challenges for the defence force. *Equus*, March 9(1): 8-9.

Jones, O.P. Hatfield, S.G.S. 1976. Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds. *Journal of Horticultural Sciences,* 51: 495-499.

Kibbler, H. Johnston, M.E.& Williams, R.R. 2004. Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell: 2 Seasonal influences of temperature, rainfall, flowering and auxins on the stock plant. *Scientia Horticulturae,* 102: 343-358.

Kruger, F.J. 1983. Plant community diversity and dynamics in relation to fire. In Mediterranean-type Ecosystems. In Kruger, F.J. Mitchell, D.T. & Javis, J.U.M. (eds). *The Role of Nutrients*. Berlin: Springer Verlag: 446-472.

Lamb, J.G.D. 1972. The use of Peat in Propagating Hardy Nursery Stock at Kinsealy. *Acta Horticulturae,* 26: 125-128.

Laubscher, C.P. 1999. Rooting techniques for selected tree species. Unpublished M Thesis, Cape Technikon, Cape Town.

Laubscher, C.P. 2000. Propagating South African trees. *Combined Proceedings of International Plant Propagators' Society,* 50: 56-65.

Laubscher, C.P. & Ndakidemi, P.A. 2008. Rooting success using IBA auxin on endangered *Leucadendron laxum* (PROTEACEAE) in different rooting mediums. *African Journal of Biotechnology,* 7(19): 3437-3442.

Leakey, R.R.B. Mesén, J.F. Tchoundjeu, Z. Longman, K.A. Dick, J.McP. Newton, A. Martin, A. Grace, J. Monro, R.C. & Muthoka, P.N. 1990. Low technology techniques for the vegetative propagation of tropical trees. *Commonwealth Forestry Review,* 69: 247-257.

Littlejohn, G. 2002. Legal Issues, Planning and Planting. Cultivating fynbos for quality cut flower production. Unpublished training course manual. Agricultural Research Council, Stellenbosch: 82-90.

Liu, Z-H. Hsiao, I-C. & Pan, Y-W. 1996. Effect of naphthaleneacetic acid on endogenous indole-3-acetic acid, peroxidase and auxin oxidase in hypocotyls cuttings of soybean during root formation. *Botanical Bulletin of Academia Sinica,* 37: 247-257.

Liu, Z-H. Wang, W-C. & Yen, Y-S. 1998. Effect of hormone treatment on root formation and endogenous indole-3-acetic acid and polyamine levels of *Glycine max* cultivated *in vitro*. *Botanical Bulletin of Academia Sinica,* 39: 113-118.

Lochner, P. Weaver, A. Gerderblom, C. Peart, R. Sandwith, T. & Fowkes, S. 2003. Aligning the diverse: The development of a biodiversity conservation strategy for the Cape Floristic Region. *Biological Conservation,* 112: 29-43.

Lombard, A., Cowling, R.M. Pressey, R.L. & Mustard, P.J. 1997. Reserve design on the Agulhas Plain, South Africa: a flexible tool for conservation in a species-rich and fragmented landscape. *Conservation Biology,* 11: 1101-1116.

Machrafi, Y. & Prevost, D. 2006. Toxicity of phenolic compounds extracted from bark residues of different ages. *Journal of Chemical Ecology*, 32: 2595-2615.

Matkin, O.A. 2008. *Perlite Vs. Polystyrene In Potting Mixes.* The Schundler Company, 150 Edison, NJ 08817 http://www.schundler.com/polystyrene.htm.

McVeigh, S. 1998. Fabulous fynbos a favourite for the small-scale farmer. *Farmers Weekly,* 4: 30-32.

Middlemann, M. 2004. Fynbos industry statistics - Statistics appendix. *Sappex News*, 120.

Midgeley, J.J. 1989. Season of burn of serotinous Proteaceae: acritical review and further data. *South African Journal of Botany,* 55: 165-170.

Milandri, S. Laubscher, C.P. & Ndakidemi, P. 2008. Hydroponic culture of *Gladiolus tristis*: Application of paclobutrazol for flowering and height control. *African Journal of Biotechnology,* 7(3): 239-243.

Moody, H. 1995. Versatile Leucadendrons and Leucospermums. *Australian Horticulture,* 5: 111-112.

Mustard, P. Cowling, R. & Albertyn, J. 1997. *Southern Overberg. South African Wild Flower Guide 8.* National Botanical Institute. Cape Town: National Book Printers.

Neiteler, D. 2004. Report on fynbos. *IPGA News Letter*. May 2004.

Newton, A.S. Mesén, J.F. Dick, J. McP. & Leakey, R.R.B. 1992. Low-technology propagation of tropical trees: rooting physiology and its practical implications. In: Mass production Technology for genetically Improved Fast Growing Forest Tree Species, *Afocel*, 2: 417-324.

Ofori, D.A. Newton, A.C. Leakey, R.R.B. & Grace, J. 1996. Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentrations, leaf areas and rooting medium. *Forest Ecology and Management,* 84: 39-48.

Owings, A.D. 1996. Variations in pH from different bark sources. *Combined Proceedings International Plant Propagators' Society,* 46: 699.

Paterson-Jones, C. 2000. *The Protea family of Southern Africa.* Cape Town: Struik publishers: 28-124.

Patterson, T. 2005. Sappex develops a code of practice. Talking Flowers Africa. *Journal for floriculture in Southern Africa*. July 2005.

Perez-Frances, J.F. Exposito, A. & Rodriguez, J.A. 1995. Effect of different factors on in vitro multiplication of *Leucadendron* 'Safari Sunset' (Proteaceae). Acta Horticulturae, 387**:** 115-120.

Privet, S. 2002. Putting fynbos to work on the Agulhas Plain. *Veld and Flora,* 88(2): 50-51.

Protea Atlas, 2008. *Current Rarity Status of the Proteaceae 1990*. http://protea.worldonline.co.za/redstatus2.htm [30 October 2008].

Rabie M (2002). Export statistics for season June 2001 to July 2002. (sappex@hermanus.co.za). [15 June 2004].

Rabie M (2003). Research/ Navorsing – "Think Tank / Dinkskrum". (sappex@hermanus.co.za). [15 June 2004].

Rebelo, A.G. 1995. *Proteas. A Field Guide to the Proteas of Southern Africa.* Cape Town: Fernwood Press.

Rebelo, A.G. 1997. Conservation. In Cowling, R.M. Richardson, D.M. & Pierce, S.M. (eds). *Vegetation of Southern Africa.* Cambridge: Cambridge University Press: 571-590.

Rebelo, T. 2001. *Sasol Proteas: A field guide to Proteas of Southern Africa.* 2nd ed. Cape Town: Fernwood Press.

Reinten, E. Gertze, S. & Arends, L. 2002. Propagation methods. Cultivating fynbos for quality cut flower production. Unpublished manual, Agricultural Research Council, Stellenbosch: 50-70.

Rice, E.L. 1984. *Allelopathy.* Orland: Academic Press: 272-274.

Robyn, A. & Littlejohn, G. 2002. Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production. Unpublished manual, Agricultural Research Council, Stellenbosch: 22-49.

Rodríguez Pérez, J.A. 1992. Propagation by leaf bud cuttings of *Leucadendron* 'Safari Sunset', *Leucospermum cordifolium*, *Leucospermum patersonii* and *Protea obtusifolia*. Acta Horticulturae, 316: 35-46.

Rouget, M. Richardson, D.M. Cowling, R.M. Lloyd, J.W. & Lombard, A.T. 2003. Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region. *Biological Conservation,* 112: 63-95.

Rout, G.R. 2006. Effects of auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. *Plant Growth Regulation,* 48: 111-117.

Ryan, P.G. & Bloomer, P. 1999. The long-billed lark complex; a species mosaic in South-Western Africa. *The Auk,* 116: 194-208.

SAFEC. 2002. *Preparing orchards for planting of PROTEACEAE.* Unpublished manual, South African Flower Council. Johannesburg.

SAPPEX. 2002. Conservation of the Cape Floral Kingdom. *Sappex News,* 112: 17.

Seydack, A.H.W. Bekker, S.J. & Marshall, A.H. 2007. Shrubland fire regime scenarios in the Swartberg Mountain Range, South Africa: implications for fire management. *International Journal of Wildland Fire,* 16: 81-95.

Siqueria, J.O. Safir, G.R. & Nair, M.G. 1991. Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white Clover by flavonoid compounds. *New Phytologist,* 118: 87-93.

Sprent, P. 1993. *Applied Nonparametric Statistical Methods.* 2nd ed. London: Chapman & Hall.

Steel, R.G.D. & Torrie, J.H. 1980. *Principles and procedures of statistics: A biometrical approach.* 2nd ed. New York: McGraw Hill.

Still,S.M. Dirr, M.A. & Gartner, J.B. 1976. Phytotoxic effects of several bark extracts on mung bean and cucumber growth. *Journal of the American Society for Horticultural Science,* 101: 34-37.

Struwig, F.W. & Stead, G.B. 2001. *Planning, designing and reporting research.* Cape Town: Masker Miller Longman.

Tansley, S.A. & Brown, C.R. 2000. RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): Implications for their conservation**.** *Biological Conservation,* 95: 39-48.

Turpie, J. 2004. The role of resource economics in the control of invasive alien plants in South Africa. *South African Journal of Science,* 100: 87-93.

United Nations Development Programme, 2003. *Agulhas Biodiversity Initiative. Global Environmental Facility Project document: Cape Action Plan for the Environment.* http://www.gefweb.org/Documents/Project_Proposal_for_Endorsm/South_Africa Agulhas Bio Initiative ABI. pdf [27 November 2003].

Volper R, Osswald, M, Elstner, EF (1995). Effects of cinnamic acid derivatives on indole acetic acid oxidation by peroxidase. *Phytochemistry,* 38: 1-22.

Wiesman, Z. & Lavee, S. 1995. Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. *Scientia Horticulturae,* 62: 189-198.

Williams, I.J.M. 1972. A Revision of the Genus Leucadendron (Proteaceae). Contribution of the Bolus Herbarium No. 3. Cape Town: South African Botanical Institute.

Wu, H.C. & Du Toit, E.S. 2004. Reducing oxidative browning during in vitro establishment of *Protea cynaroides. Scientia Horticulturae,* 100: 355-358.

Wu, H.C. Du Toit, E.S. & Reinhardt, C.F. 2007a. Micrografting of *Protea cynaroides*. *Plant Cell Tissue Culture,* 89: 23-28.

Wu, H.C. Du Toit, E.S. Reinhardt, C.F. Rimando, A.M. van der Kooy, F. & Meyer, J.J.M. 2007b. The phenolic, 3,4-dihydroxybenzoic acid, is an endogenous regulator of rooting *Protea cynaroides. Plant Growth Regulation,* 52: 207-215.

Younge, A. & Fowkes, S. 2003. The Cape Action Plan for the Environment: overview of an eco-regional planning process. *Biological Conservation,* 112: 15-28.

Zimmerman, R.H. 1984. Rooting apple cultivars in vitro: Interaction amongst light, temperature, phloroglucinol and auxin. *Plant Cell Tissue Culture,* 3: 01-311.

LIST OF APPENDICES

APPENDIX A

5 October 2005

Dear Farmer/Grower/Exporter

RE: CURRENT RESEARCH ON RED DATA LEUCADENDRON SPECIES AT THE CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

The department Environmental Sciences of the Cape Peninsula University of Technology is currently doing a research project on the status of Red Data Proteaceae species in agricultural areas in the Western Cape. Leucadendrons for example, form a vital part of the Cape fynbos with about 79 species, most of which occur in the Western Cape. Presently more than 15 species, together with several subspecies, are endangered as their habitat is being threatened.

The attached questionnaire is aimed at assessing the state of the fynbos industry, farming methods and the state of conservation in your region and to find ways in which these can be improved. It is by no means a personal investigation into agricultural practices, but rather a study aimed at finding ways in which to work together in order to protect and conserve our precious heritage. Personal details are of no relevance unless you are interested in receiving the outcome and statistics of the questionnaire.

The questionnaire will take a few minutes to complete. Most of the questions require you to choose between several options which you can mark with a cross. Please take the time to fill in 'other' with your relevant answer. Your assistance in completing the questionnaire is vitally important in helping preserve our indigenous plants.

Thank you for taking the time, your assistance is greatly appreciated.

Yours sincerely

C P Laubscher
Dept of Environmental Sciences
Cape Peninsula University of Technology

Date of return **EMAIL 15 October 2005**.
POST 30 October 2005

Please return the questionnaire by post (self addressed envelope enclosed)
Email laubscherc@cput.ac.za or at tel. no. 021 4603198 if you would like to receive a hard or email copy.

5 Oktober 2005

Geagte Boer/Kweker/Uitvoerder

RE: HUIDIGE NAVORSING VAN ROOI DATA LEUCADENDRON SPESIES BY DIE KAAPSE SKIEREILAND UNIVERSITEIT VAN TEGNOLOGIE

Die department van Omgewingswetenskappe by die Kaapse Skiereiland Universiteit van Tegnologie is tans besig met 'n navorsings projek oor die Rooi Data status van Proteaceae spesies in landbougebiede in die Wes-Kaap. Leucadendrons by voorbeeld vorm 'n belangrike deel van die Kaapse fynbos met omtrent 79 spesies, waarvan die meeste in die Wes-Kaap voorkom. Huidiglik is meer as 15 spesies en subspesies bedreig omdat die natuurlike omgewing bedreig word.

Die aangehegte vraelys is daarop gemik om die stand van die fynbos industrie, boerderymetodes en die stand van bewaring in u omgewing te ondersoek en om maniere te vind hoe hierdie aspekte verbeter kan word. Dit is nie 'n persoonlike ordersoek van boerderypraktyke nie, maar eerder 'n studie gemik om maniere te vind om saam te werk met die doel om ons belanglike erfenis te bewaar en te beskerm. Persoonlike besonderhede is nie belangrik nie behalwe as u sou belangstel om die uitslag van die statistieke van die vraelys te ontvang.

Die vraelys sal slegs 'n paar minute neem om te voltooi. Vir die meeste van die vrae moet u kies tussen veskillende opsies en dit met 'n kruisie merk. Neem asb die tyd om 'ander' in te vul met u antwoord. U hulp om die vraelys te voltooi is uiters belangrik om ons inheemse plante te help bewaar.

Dankie dat u die tyd geneem het, u hulp word baie waardeer.

Die uwe

C P Laubscher
Dept van Omgewingswetenskappe
Kaapse Skiereiland Universiteit van Tegnologie

Datum van terugstuur **E-POS 15 Oktober 2005**.
POS 30 Oktober 2005

Stuur asb die vraelys terug met die pos (self geadresseerde koevert ingesluit). E-pos asb laubscherc@cput.ac.za of by tel. no. 021 4603198 as u 'n harde of e-pos afskrif wil ontvang.

APPENDIX B

AGHULHAS PLAIN QUESTIONNAIRE / VRAELYS

(Research purposes only/Navorsings doeleindes alleenlik)

THE QUESTIONNAIRE WILL TAKE A FEW MINUTES TO COMPLETE. PLEASE MAKE YOUR CHOICE WITH A CROSS/ DIE VRAELYS SAL SLEGS 'N PAAR MINUTE NEEM OM TE VOLTOOI. MERK ASB U KEUSE MET 'N KRUISIE.
If other, please stipulate/ indien ander, stipuleer asseblief.

1. **In which region is your farm situated/ In watter streek is u plaas geleë?**

WESTERN CAPE/ WES-KAAP		SOUTHERN CAPE/ SUID-KAAP		EASTERN CAPE/ OOS-KAAP	
CLOSES TOWN NAASTE DORP					

2. **The purpose of fynbos use on your farm / Die doel van fynbos op u plaas word gebruik?**

CUT FLOWERS SNYBLOMME	**Y**	**N**	PROPAGATION/ VOORTPLANTING	**Y**	**N**	TRAINING/EDUCATION/ OPLEIDING/OPVOEDING	**Y**	**N**	TOURISM/ TOERISME	**Y**	**N**
OTHER/ANDER											

3. **Have you considered the benefits of ecotourism/ Het u al die voordele van eko-toerisme oorweeg?**

RESERVES/ RESERVATE	**Y** / **N**	WALKS/ STAP ROETES	**Y** / **N**	HERITAGE SITES/ERFENIS AREAS	**Y** / **N**	GOVERNMENT GRANTS/REGERING SKENKINGS	**Y** / **N**	NONE/ GEEN	
OTHER/ ANDER									

4. **Do you pick any of the following endangered Leucadendron species for cut flower purposes/ Pluk u enige van die volgende bedreigde Leucadendron spesies vir snyblom doeleindes?**

L.ELIMENSE SUBSP. ELIMENSE	**Y**	**N**	L. LAXUM	**Y**	**N**	L. PLATYSPERMUM	**Y**	**N**	L. STELLIGERUM	**Y**	**N**
OTHER/ANDER											

5. **Are any of these Leucadendron populations threatened by the following/ Word enige van die Leucadendron populasies deur die volgende bedreig?**

FARMING PRACTICES/ BOERDERY AKTIWITEITE	**Y** / **N**	ALIEN PLANTS/ INDRINGER PLANTE	**Y** / **N**	FIRE/ VUUR	**Y** / **N**	ROAD WORKS/ PAD WERKE	**Y** / **N**	TELKOM/ ESKOM	**Y** / **N**	NONE / GEEN		
OTHER/ANDER												

6. **On which terrain do these Leucadendrons occur/ Op watter areas kom hierdie Leucadendrons voor?**

MOUNTAIN/BERG	**Y**	**N**	WETLAND/VLEI	**Y**	**N**	VALLEY/VALLEI	**Y**	**N**	EVEN/ GELYK	**Y**	**N**
OTHER/ANDER											

7. **What type of soil is found where these Leucadendron species occur and please indicate the pH/ Watter tipe grond word gevind waar hierdie Leucadendron spesies groei en aandui asb die pH aan?**

CLAY/KLEI	**Y**	**N**	SANDY/SANDERIG	**Y**	**N**	LOAMY/LEEMERIG	**Y**	**N**	pH	

8. **From which areas on your farm do you harvest these these Leucadendrons cut flowers/ Van watter areas oes u hierdie Leucadendrons snyblomme?**

NATURAL STANDS/ NATUURLIKE PLANTEGROEI	Y / N	BROADCASTED SEEDS/ DIREK GESAAIDE SAAD	Y / N	PLANTED ORCHARDS/ GEPLANTE BOORDE	Y / N	NONE/ GEEN	
OTHER/ANDER							

9. **What quantity do you harvest/ Watter hoeveelheid oes u?**

100 - 199KG	Y	N	MORE THAN 200KG/ MEER AS 200KG	Y	N	NONE/GEEN	
OTHER/ANDER							

10. **Which markets do you supply/Watter mark verskaf u?**

LOCAL/PLAASLIK	Y	N	EXPORT/UITVOER	Y	N	NONE/GEEN	
OTHER/ANDER							

11. **Do you harvest any seed from any of these Leucadendrons and, what are the seeds used for/ Oes u enige saad van hierdie Leucadendrons en waarvoor word die saad gebruik?**

NO HARVESTING	Y / N	BROADCAST SEED/ DIREKTE SAAI	Y / N	COLLECT AND SELL/ VERSAMEL EN VERKOOP	Y / N	COLLECT AND GROW/ VERSAMEL EN GROEI	Y / N
OTHER/ANDER							

12. Which of the following reproductive phases for Leucadendrons are you aware of/ Watter van die volgende reproduseerende fases vir Leucadendrons is u bewus van?

FIRE/VUUR	**Y**	**N**	RODENTS/KNAAGDIERE	**Y**	**N**	INSECTS/INSEKTE	**Y**	**N**	BIRDS/VOëLS	**Y**	**N**
OTHER/ ANDER											

13. Are you aware that some species may require fire to stimulate germination and do you practice controlled fires to stimulate new reproductive growth/ Is u bewus dat sommige spesies vuur stimulering tydens ontkieming benodig en beoefen u beheerde brande om nuwe groei te stimuleer?

NONE/ GEEN		ALLOW NATURE TO CONTINUE/ LAAT AAN NATUUR OOR	**Y** / **N**	EVERY 3-4 YEARS/ ELKE 3-4 JAAR	**Y** / **N**	EVERY 6-7 YEARS/ ELKE 6-7 JAAR	**Y** / **N**	LONGER THAN 7 YEARS/ LANGER AS 7 JAAR	**Y** / **N**
OTHER/ANDER									

14. Do you have any training in the growing of cut flowers/ Het u enige opleiding in die kweek van snyblomme?

FORMAL EDUCATION/ FORMELE OPVOEDING	**Y** / **N**	SHORT COURSES/KORT KURSUSSE	**Y** / **N**	SELF TRAINED/ SELF GELEER	**Y** / **N**
OTHER/ANDER					

15. **Do your staff receive training in invasive species, indigenous species/ Kry u personeel opleiding in indringer spesies, inheemse spesies?**

INVASIVE SPECIES IDENTIFICATION/ INDRINGER SPESIE IDENTIFIKASIE	**Y** / **N**	RARE SPECIES/ SKAARS SPESIES	**Y** / **N**	POTENTIAL CUT FLOWER SPECIES/ MOONTLIKE SNYBLOM SPESIES	**Y** / **N**	NONE OF THESE/ GEEN VAN HIERDIE	
OTHER/ANDER							

16. **When cutting flowers from natural stands/wild areas which of the following pruning methods do you use/ Wanneer u snyblomme uit die veld sny, watter van die volgende snoeimetodes gebruik u?**

CUT ALL FLOWERS THAT ARE READY/ SNY AL DIE BLOMME WAT REG IS	**Y** / **N**	CUT 50% OF ALL FLOWERS/ SNY SLEGS 50% VAN ALL DIE BLOMME	**Y** / **N**	CUT 2/3 OF ALL FLOWERS/ SNY 2/3 VAN AL DIE BLOMME	**Y** / **N**	PRUNE WHILE YOU CUT/ SNOEI TERWYL U OES	**Y** / **N**
OTHER/ANDER							

17. **Do you allow your livestock to graze in wild areas/ Word u lewende hawe toegelaat om in die veld te wei?**

CONTROLLED/ WORD GEKONTROLEER	**Y** / **N**	UNCONTROLLED/ NIE GEKONTROLEER	**Y** / **N**	ON ROTATIONAL BASIS/OP ‘N ROTASIE BASIS	**Y** / **N**	NONE / GEEN	
OTHER/ANDER							

18. **Has a conservation body visited you farm to record Red Data species/ Het 'n bewaringsliggaam al u plaas besoek om Rooi Data spesies te dokumenteer?**

<table>
<tr><td rowspan="2">NATURE CONSERVATION/ NATUURBEWARING</td><td>Y</td><td rowspan="2">PROTEA ATLAS</td><td>Y</td><td rowspan="2">SANBI</td><td>Y</td><td rowspan="2">CUT FLOWER ASSOCIATION/ SNYBLOM VERENIGING</td><td>Y</td><td rowspan="2">NONE/ GEEN</td><td rowspan="2"></td></tr>
<tr><td>N</td><td>N</td><td>N</td><td>N</td></tr>
<tr><td>OTHER/ANDER</td><td colspan="9"></td></tr>
</table>

19. **Have you reported any occurrences of threatened Red Data species to these bodies and if so, what was their response/ Het u al voorvalle van bedreiging van Rooi Data spesies gerapportteer, en indien wat was hul reaksie?**

<table>
<tr><td rowspan="2">NO/NEE</td><td rowspan="2"></td><td rowspan="2">IMMEDIATE RESPONSE/ ONMIDDELIKE REAKSIE</td><td>Y</td><td rowspan="2">SLOW BUT INTERESTED/ STADIG MAAR GEINTERESSEERD</td><td>Y</td><td rowspan="2">NO RESPONSE/ GEEN REAKSIE</td><td>Y</td></tr>
<tr><td>N</td><td>N</td><td>N</td></tr>
<tr><td>Which body/ Watter liggaam?</td><td colspan="7"></td></tr>
</table>

20. **What is your attitude regarding protecting Red Data Proteaceae species on your farm/ Wat is u houding oor die beskerming van Rooi Data Proteaceae species op u plaas?**

<table>
<tr><td rowspan="2">HIGHLY CONCERNED/ HOOGS BEKOMMERD</td><td>Y</td><td rowspan="2">CONCERNED/ BEKOMMERD</td><td>Y</td><td rowspan="2">MODERATELY CONCERNED/ MATIG BEKOMMERD</td><td>Y</td><td rowspan="2">NOT CONCERNED/ NIE BEKOMMERD</td><td>Y</td></tr>
<tr><td>N</td><td>N</td><td>N</td><td>N</td></tr>
<tr><td>OTHER/ANDER</td><td colspan="7"></td></tr>
</table>

21. What are your major concerns regarding published Red Data information/ Wat is u hoof bekommernisse ten opsigte van gepubliseerde Rooi Data informasie?

INFORMATION NOT READILY AVAILABLE/ INFORMASIE NIE REDELIK BESKIBAAR NIE	**Y**	LITTLE SUPPORT FROM CONSERVATION AUTHORITIES/ MIN ONDERSTEUNING VAN BEWARINGSOWERHEDE	**Y**	NOT ENOUGH FEEDBACK FROM CUT FLOWER ASSOCIATIONS/ NIE GENOEG KOMMUNIKASIE VAN BLOMVERENIGINGS	**Y**
	N		**N**		**N**
OTHER/ANDER					

22. In the cultivation of new areas, how do you protect rare/ endangered plant species/ Met die voorbereiding van nuwe groei areas, hoe beskerm u spesies wat skaars/in gevaar is?

ASSESSMENT/ OPNAME	**Y**	**N**	RELOCATION/ HERVESTIGING	**Y**	**N**	DEVELOP BOTANICALLY RICH AREAS/ ONTWIKKEL BOTANIESE RYK GEBIEDE	**Y**	**N**
OTHER/ANDER								

23. How do you propagate Red Data species/ Hoe kweek u Rooi Data spesies?

SEED/ SAAD	**Y**	**N**	CUTTINGS/ STEGGIES	**Y**	**N**	LAYERING/ INLEêRS	**Y**	**N**	NONE/ GEEN	
OTHER/ ANDER										

24. What are these plants used for/ Waarvoor word hierdie plante gebruik?

REINTRODUCTION INTO CLEARED AREAS /HERVESTIG IN SKOON GEMAAKTE AREAS	**Y**	SOLD/ VERKOOP	**Y**	PLANTED INTO ORCHARDS FOR CUT FLOWERS/ GEPLANT IN BOORDE VIR SNYBLOMME	**Y**	USED AS MOTHERSTOCK FOR CUT FLOWERS/ GEBRUIK AS MOEDERPLANTE VIR SNYBLOMME	**Y**
	N		**N**		**N**		**N**
OTHER/ANDER							

25. Are you satisfied with the way conservation bodies issue picking permits/ Is u tevrede met die manier waarop natuurbewaringsliggame plukpermitte uitreik?

HAS YOUR FARM BEEN VISITED/WAS U PLAAS AL BESOEK	**Y**	YOU HAVE BEEN ISSUED A PERMIT SUCCESSFULLY/U IS SUKSESSVOL MET DIE UITREIKING VAN N PERMIT	**Y**	YOU ARE UNAWARE THAT YOU NEED A PERMIT/ U IS ONBEWUS DAT U 'N PERMIT BENODIG	**Y**	YOU DO NOT KNOW THE PROSEDURE FOR APPLICATIONS/ U KEN NIE DIE PROSEDURE VIR AANSOEKE NIE	**Y**
	N		**N**		**N**		**N**
OTHER/ANDER							

26. Which permit or licence do you have/Watter permit of lisensie het u?

LICENCE TO SELL PROTECTED FLORA/LISENSIE OM BESKERMDE FLORA TE VERKOOP	**Y**	LICENCE TO GROW PROTECTED FLORA/ LISENSIE OM BESKERMDE FLORA TE GROEI	**Y**	LICENCE TO CUT WITH PERMISSION OF LANDOWNER/LISENSIE OM TE SNY MET PERMISSIE VAN GRONDEIENAAR	**Y**
	N		**N**		**N**
OTHER/ANDER					

27. In your opinion do you feel that / Volgens u mening dink u dat?

NATURAL VELD GETS BURNED IRRESPONSIBLY/ NATUURLIKE VELD WORD ONVERANTWOORDELIK GEBRAND	**Y**	DESTRUCTION OF NATURAL HABITATS CONTINUES/ VERWOESTING VAN NATUURLIKE VERBLYF WORD VOORTGESIT	**Y**	CHEMICALS ARE USED IN NATURAL AREAS/ CHEMIESE STOWWE WORD GEBRUIK IN NATUURLIKE AREAS	**Y**	INTRODUCTION OF CULTIVARS WILL DESTROY THE NATURAL BIODIVERSITY / INVOERING VAN KULTIVARS SAL DIE NATUURLIKE BIODIVERSITEIT VERWOES	**Y**	AGRICULTURE CONTINUES TO EXPAND INTO NATURAL FYNBOS AREAS /LANDBOU AANHOU OM UITEBREI IN NATUURLIKE AREAS	**Y**
	N		**N**		**N**		**N**		**N**
OTHER/ANDER									

28. Are you aware of any organisations which help you to protect indigenous species/ Is u bewus van enige organisasie wat help om inheemse spesies te beskerm?

NONE/GEEN	**Y**	SOUTH AFRICAN NATIONAL BOTANICAL INSTITUTE/SUID AFRIKAANSE BOTANIESE INSTITUUT	**Y**	INSTITUTE FOR PLANT CONSERVATION, UCT/ INSTITUUT VIR PLANT BEWARING, UK	**Y**	CAPE NATURE CONSERVATION/ KAAPSE NATUURBEWARING	**Y**
	N		**N**		**N**		**N**
OTHER/ANDER							

29. Have you had your land assessed by a conservation body before you practised burning and for what purpose/ Het u al u grond deur 'n natuurbewaringsliggaam laat inspekteer voor u gebrand het en vir watter doel?

NO ASSESSMENT/ GEEN INSPEKSIE		FIRE HAZARDS/ VUUR RISIKO		FIREBREAKS/ VUURSTOOK		BURNING METHOD/ BRAND METODE		TYPES OF PLANTS TO BE BURNED/ TIPE PLANTE WAT GEBRAND MOET WORD		ENVIRONMENTAL IMPACT OF BURN/ OMGEWINGS IMPAK VAN BRAND	
	Y		**Y**		**Y**		**Y**		**Y**		**Y**
	N		**N**		**N**		**N**		**N**		**N**
OTHER/ANDER											

30. Which bodies/authorities have supported you or your neighbours during veld fires/ Watter liggaame /outoriteite het u en bure ondersteun gedurende veldbrande?

LOCAL MUNICIPALITIES/ PLAASLIKE MUNISIPALITEITE		NEIGHBOURING FARMS/ NABURIGE PLASE		AIR FORCE/ LUGMAG	
	Y		**Y**		**Y**
	N		**N**		**N**
OTHER/ANDER					

31. **Do you need help in clearing alien vegetation from the following bodies/ Benodig u hulp om indringer plantegroei te bekamp van een die volgende liggaame?**

GOVERNMENT/ REGERING	**Y**	WORKING FOR WATER/ WERK VIR WATER	**Y**	MUNICIPALITY/ MUNISIPALITEIT	**Y**	CAPE NATURE CONSERVATION/ KAAPSE NATUURBEWARING	**Y**
	N		**N**		**N**		**N**
OTHER/ ANDER							

32. **Have you received help in the past with clearing alien vegetation from any of the following bodies/ Het u al hulp in die verlede gekry met die skoonmaak van indringerplante van die volgende liggame?**

WORKING FOR WATER/ WERK VIR WATER	**Y**	LOCAL MUNICIPALITY/ PLAASLIKE MUNISIPALITEIT	**Y**	ROAD WORKS/ PADWERKE	**Y**
	N		**N**		**N**
OTHER/ ANDER					

33. **Are you practicing alien clearing/ Roei u indringerplante uit?**

ONLY IN WILD AREAS/ SLEGS IN NATUURLIKE VELD	**Y**	ONLY IN CULTIVATED AREAS/SLEGS IN BEWERKTE GEBIEDE	**Y**	ALL AREAS OF YOUR LAND/OP AL DIE AREAS VAN U GROND	**Y**	NONE /GEEN	
	N		**N**		**N**		
OTHER/ANDER							

34. Which methods do you use/ Watter metodes gebruik u?

CUT AND LEAVE/ SNY EN LOS	**Y**	CUT AND BURN/ SNY EN BRAND	**Y**	BURN STANDING/ SLEGS BRAND	**Y**	CHEMICAL CONTROL/ CHEMIESE BEHEER	**Y**	BIOLOGICAL CONTROL/ BIOLOGIESE BEHEER	**Y**
	N		**N**		**N**		**N**		**N**
OTHER/ANDER									

35. Which of the following invasive species is the most problematic on your land/ Watter van die volgende indringer spesies is die mees problematies of u grond?

BLACK WATTLE	**Y**	ROOI KRANS	**Y**	PORT JACKSON	**Y**	STINK BEAN	**Y**	HAKEA	**Y**	AUSTRALIAN MYRTLE	**Y**
	N		**N**		**N**		**N**		**N**		**N**
OTHER/ANDER											

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE.
DANKIE DAT U TYD GENEEM HET OM HIERDIE VRAELYS TE VOLTOOI.

PLEASE EMAIL TO/E-POS ASB AAN laubscherc@cput.ac.za **by 15 OCTOBER 2005** or
PLEASE POST TO/POS ASB AAN Mr. C P Laubscher, Dept of Environmental Sciences, Cape Peninsula University of Technology, PO Box 652, Cape Town, 8000 **by 30 OCTOBER 2005**

PLEASE INDICATE YOUR EMAIL ADDRESS IF YOU REQUIRE FEEDBACK ON RESULTS. GEE ASB U E-POS ADRES AS U TERUGVOERING VAN DIE RESULTATE VEREIS.	

APPENDIX C

APPENDIX D

APPENDIX E

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Full Length Research Paper

Rooting success using IBA auxin on endangered *Leucadendron laxum* (PROTEACEAE) in different rooting mediums

C. P. Laubscher and P. A. Ndakidemi*

Faculty of Applied Sciences, Cape Peninsula University of Technology, P. O. Box 652, Cape Town 8000, South Africa.

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Leucadendron laxum **(Marsh rose Leucadendron) was tested for its rooting ability as an endangered plant species for reintroduction into the natural habitat, using IBA liquid hormone preparations and four growth media. The treatments included a control, 500, 1000, 2000 and 4000 ppm concentrations. Four growth media, namely: bark and polystyrene; peat moss and polystyrene; bark, river sand and polystyrene; and perlite and river sand were tested in an environmentally controlled greenhouse with bottom heat. The experiments used a randomised block design with 10 cuttings per treatment and 4 replicated four times. The results showed that the bark, sand and polystyrene medium had the highest (45%) significant survival rate when supplied with IBA at 500 ppm. The application of 1000 ppm of IBA in bark, sand and polystyrene medium significantly increased the number of roots per cutting (5) and produced the longest roots (4 cm). The bark and polystyrene medium supplied with zero IBA had the largest number of roots per cutting (6.5). The comparison of the four growth media showed that bark and polystyrene was the best medium that produced the highest and most significant callusing formation (30%), % of cuttings that rooted (28%), cuttings with a higher survival rate (57%), and cuttings with a higher number of roots (3.5) and the longest roots (7.35 mm). With regard to various IBA treatments tested across all treatments, the IBA supplied at 1000 ppm produced greater root numbers when compared with all other treatments.**

Key words: Hormone treatment, red data species, rooting percentage, vegetative propagation.

INTRODUCTION

Leucadendron laxum belongs to the Proteaceae family and is mostly found near the most southern point of the African continent, where the Atlantic and Indian Oceans meet. It grows on level, damp ground in the valleys between Hermanus, Bredasdorp and Agulhas (Williams, 1972). The existence of this plant in the wild is not certain, as its habitat is being drained by natural catastrophes such as wild fires and farmers (Mustard et al., 1997; Robyn and Littlejohn, 2002). As a result, *L. laxum* has been named an endangered species in South Africa (Hilton-Taylor, 1996).

Some Leucadendrons are naturally rare owing to reproductive reasons, wild fires and competition with other species, such as the spread of alien invasive species (Paterson-Jones, 2000). One report revealed that *L. laxum* has only 5 000 remaining plants in the natural habitat (Mustard et al., 1997). Improved plant propagation techniques are essential at this point to help save the remaining small plant populations.

L. laxum is an important plant that is aesthetically pleasing with its bright yellow flowers and attractive cones (Jamieson, 2001). It has a potential role in the flower and tourist industry in South Africa. As a member of the Proteaceae hybrids, these plants can be propagated by vegetative means to maintain the unique characteristics especially cut flower cultivars (Brown and Duncan, 2006) and produce uniform flowering times (Reinten et al., 2002). However, most vegetative techniques applied by Protea growers have not been published (Laubscher, 2000). To our knowledge, the vegetative

*Corresponding author. E-mail: ndakidemip@cput.ac.za. Tel: +2721-460-3196. Fax: +2721-460-3193.

Abbreviations: IBA, Indole-3-butyric acid; **ppm**, parts per million; and **SAFEC**, South African Flower Export Council.

propagation of *L. laxum* has not previously been described and new documentation would be seen as new research.

The appropriate vegetative propagation techniques for *L. laxum* can speed up the replacement of plants on damaged and reclaimed land and also increase production of cut flower cultivars in nurseries. Improved propagation techniques can also increase royalties and the sale of patented cultivars with an increase in quality and quantity of future export cut flowers. Therefore, faster rooting techniques are important to help save small plant populations such as *L. laxum*, especially after fires (Robyn and Littlejohn, 2002). Improved cultivation and management practices in orchards (SAFEC, 2002) need to be investigated, as threats to the natural habitat continue to escalate.

Research evidence suggests that auxins play a central role in the determination of rooting capacity, by enabling the faster production of rooted cutting material which is essential for vegetative propagation (Fogaça and Fett-Neto, 2005). Auxins are known to increase rooting percentage and rooting time together with uniformity of rooting (Hartmann et al., 2002). Under natural conditions, some Proteaceae cuttings are difficult to root, and few species have shown response to auxin treatments (Hartmann et al., 2002).

Research into various auxin concentrations and different rooting media could prove useful to the horticultural industry by enabling the faster production of rooted cutting material. IBA is one form of auxin that is effective in the rooting of a large number of plant species (Hartmann et al., 2002). In some woody species aryl esters and aryl amid of IBA are equal or more effective than acid formulations in root initiation (Hartmann et al., 2002). The use of different concentrations of IBA on *L. laxum* will identify the treatment that produces desirable rooting results. In the literature, there is no available information on the effect of auxin on rooting of *L. laxum* and further experimentation will be documented as new research.

Testing combinations of rooting components should ensure faster and better quality root formation. Components such as bark, peat, polystyrene and river sand should be tested in various combinations. Shredded milled pine bark is a medium widely used in rooting mediums to manage the pH factor, provided the quality is from a good source (Owings, 1996). Polystyrene is a coarse mineral component which provides an air-filled porosity that improves aeration, whereas river sand mostly used in combination with organic materials provides coarseness and drainage. Coarse grade peat moss similar to bark lowers the pH and enhances the water holding capacity of growing mediums (Hartmann et al., 2002).

The aim of this study is therefore to determine whether *L. laxum* responded favourably to different concentrations of IBA rooting hormones and various rooting mediums to ensure faster and more efficient rooting success in pot-plant and cut flower production.

MATERIALS AND METHODS

Experimental

The experiment was conducted in an environmentally controlled greenhouse, at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa. The clear polycarbonate greenhouse was fitted with a shade screen (40%), where temperature and humidity were monitored on a weekly basis. Midday temperatures fluctuated between 22 - 27°C and relative humidity between 39 - 86%. Bottom heat (20 - 25ºC) and mist bed conditions were supplied (Brown and Duncan, 2006). The irrigation timer was set on 15 s on and 20 min off.

The experiment started during the middle of August and continued for 8 weeks (Brown and Duncan, 2006). Cutting material of *L. laxum* was collected from selected plant populations in their natural habitat on the Agulhas Plain on the extreme south-western coast of South Africa. The material was kept dry in a sealed plastic bag and transported overnight.

Setup and design

A randomised complete block design involving four growth mediums (bark and polystyrene; peat moss and polystyrene; bark, river sand and polystyrene; and perlite and river sand and four concentrations of IBA (control, 500, 1000, 2000 and 4000 ppm) were used in this study. Altogether $4 \times 5 \times 10$ cuttings were involved in the experiment. The medium had to be light with good drainage and also had to retain some moisture to prevent quick drying; had a pH of 6.5 - 7 is preferred (SAFEC, 2002).

For each treatment, cuttings were replicated in 10 pots. The experiment was conducted in a controlled greenhouse with misting irrigation and heated beds. Readings of the greenhouse environment, that is, solar radiation, temperature, relative humidity and irrigation were monitored on a weekly basis.

Cuttings, IBA treatment and planting

Cuttings were taken from turgid stems of semi-hardwood stems after shoot elongation (Aug-Nov). Cuttings were taken in dry weather (SAFEC, 2002). For this experiment, terminal cuttings measuring approximately 150 mm long were used. All cutting material was rinsed in Benlate fungicide (10 g/l) before planting. After planting, cuttings were sprayed weekly with Captan (2 g/l) solution (Reinten et al., 2002; SAFEC, 2002). Wilted and infected cuttings were removed over the rooting period (Brown and Duncan, 2006).

Hormone treatments used IBA liquid preparations of 500, 1000, 2000, 4000 ppm. The control remained untreated. The IBA powder was dissolved in 50% ethyl alcohol and made up to the appropriate concentration by adding distilled water (Brown and Duncan, 2006). Prior to planting, the basal 5 mm of cuttings were dipped for 5 s in the rooting hormone (Reinten et al., 2002; Hartmann et al., 2002). Cuttings were then individually planted into new plug foam trays/ pots containing different growth mediums and IBA concentrations.

Data collection and statistical analyses

Samples of cuttings were drawn from the experiment on a weekly basis to monitor results of wounding, hormone treatment, rooting medium, callusing and rooting progress. The factors measured were, average shoot growth, number of cuttings callused, number of cuttings rooted, number of roots on cuttings, length of roots, and

Table 1. Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *L. laxum* grown in bark, river sand and polystyrene medium and peat and polystyrene medium.

Conc. (ppm)	Bark, river sand and polystyrene			Peat moss and polystyrene		
	% Survival rate	Roots per cutting	Root length (cm)	% Survival rate	Roots per cutting	Root length (cm)
Control	10 ± 5.8b	0.0 ± 0.0c	0.0 ± 0.0c	60 ± 21.2	0.25 ± 0.25	0.25 ± 0.25
500	45 ± 15.0a	0.0 ± 0.0c	0.0 ± 0.0c	43 ± 23.2	0.75 ± 0.75	1.25 ± 1.25
1000	25 ± 8.7ab	5.0 ± 2.9a	4.0 ± 2.3a	58 ± 18.4	1.75 ± 0.75	0.25 ± 0.25
2000	5 ± 2.9b	0.0 ± 0.0c	0.0 ± 0.0c	65 ± 16.6	2.00 ± 1.15	3.75 ± 2.39
4000	10 ± 5.8b	1.0 ± 0.6b	0.5 ± 0.3b	60 ± 21.2	2.25 ± 0.75	1.25 ± 1.25
F Statistic	5.57*	2.71*	2.82*	0.18 ns	1.20 ns	1.14 ns

Values presented are means ± SE.
* = Significant at P ≤ 0.05.
Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05.

the survival rate of cuttings. Shoot growth (mm) and the length of roots (mm) were measured from cutting stage to new growth after 8 weeks. The number of cuttings callused, rooted and survival rates were counted after 8 weeks.

Data was recorded for the percentage of survival rate of cuttings, number of roots per rooted cutting and root length. Data analysis was performed in two different ways. The first consisted of one way analysis of variance for IBA treatments added to each growth medium separately. The second consisted of factorial analysis including the four growth mediums and the five IBA concentrations. Data on number of cuttings that formed callus, the number of cuttings that rooted and the cuttings that survived were transformed into percentages prior to analysis of variance. Data were presented as mean values with predicted standard errors (S.E.). These computations were done with the software program STATISTICA. The Fisher least significance difference (L.S.D.) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of different concentrations of IBA on the four rooting mediums

The four rooting mediums used in our study responded differently to IBA concentrations. The cuttings planted in bark, river sand and polystyrene, and bark and polystyrene rooting mediums were significantly affected by IBA concentrations in certain parameters (Tables 1 and 2). Plant cuttings inserted in peat moss and polystyrene and perlite and river sand mediums at different IBA concentrations influenced the root formation although their differences were not statistically significant (Tables 1 and 2). The IBA supplied to bark, river sand and polystyrene medium significantly ($P \leq 0.05$) affected the percentage survival rates, number of roots formed per cutting and root length (Table 1). The highest survival rate of cuttings was recorded in the 500 ppm treatment (45%), followed by the 1000 ppm treatment (25%). The higher survival rate observed in this rooting medium is probably due to the well drained conditions of the sand component which encouraged adequate oxygen circulation in the rooting zone during the rooting process (Hartmann et al., 2002). The positive fact that the cuttings survived over the 8 week period in this medium is an indication that rooting percentages could be increased if the rooting period was to be extended. According to Brown and Duncan (2006), rooting of cuttings starts between 6 and 8 weeks, and cuttings should be well rooted within 8 to 16 weeks.

The root length in bark, river sand and polystyrene medium supplied with IBA significantly ($P \leq 0.05$) increased from no reaction in the control to 4 mm in the 1000 ppm treatment (Table 1). The root length was significantly reduced to 0.5 mm with successive increases of IBA concentration to 4000 ppm. Taken together (% survival rate, number of roots per cutting and root length) our results indicated that the bark, river sand and polystyrene medium supplied with 1000 ppm IBA was the best treatment as it significantly ($P \leq 0.05$) resulted in successful survival rate of the cuttings (25%), number of roots formed (5) and root length (4 mm) of cuttings (Table 1). The successful rooting of *L. laxum* in this study was achieved at 1000 ppm and concentrations above this level inhibited root initiation and development (Table 1). The results are in agreement with those reported by Hartmann et al. (2002), who found that auxin concentrations of 500 to 1250 ppm are suitable for softwood cuttings and any levels higher than those found in the plant tissues may cause cell death.

The plant cuttings of *L. laxum* supplied with IBA and inserted in the bark and polystyrene rooting medium was significantly ($P \leq 0.05$) influenced only in the roots formed per cutting (Table 2). The control treatment formed 6.5 roots per cutting, and this was followed by supplying IBA at 500 and 1000 ppm (Table 2). Brown and Duncan, (2006), Reinten et al. (2002) and SAFEC (2002) documented the success of bark and polystyrene in rooting Proteaceae. Two other treatments (500 and 1000 ppm) in this medium were also highly significant ($P \leq 0.05$) but resulted in fewer roots (3.5 and 4.3, respectively). The IBA treatments were not as conducive to

Table 2. Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *L. laxum* grown in bark and polystyrene medium and perlite and river sand medium.

Concentration	Bark and polystyrene			Perlite and river sand		
	% Survival rate	Roots per cutting	Root length (cm)	% Survival rate	Roots per cutting	Root length (cm)
Control	60 ± 16.3a	6.5 ± 2.1a	2.8 ± 0.6a	3 ± 2.5	0.00 ± 0.00	0.00 ± 0.00
500	58 ± 21.8a	3.5 ± 1.2ab	8.8 ± 3.8a	28 ± 14.9	0.00 ± 0.00	0.00 ± 0.00
1000	63 ± 18.9a	4.3 ± 0.8ab	6.5 ± 2.2a	15 ± 8.7	0.25 ± 0.25	0.75 ± 0.75
2000	40 ± 21.2a	1.8 ± 0.6b	12.5 ± 4.7a	5 ± 2.9	0.00 ± 0.00	0.00 ± 0.00
4000	65 ± 12.6a	1.5 ± 0.3b	6.3 ± 1.0a	3 ± 2.5	0.00 ± 0.00	0.00 ± 0.00
F Statistic	0.29 ns	3.09*	1.48 ns	1.83 ns	1.00 ns	1.00 ns

Values presented are means ± SE.
* = significant at P ≤ 0.05; ns = not significant.
Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05.

Table 3. Effect of different growth medium and IBA concentrations on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, number of roots per cutting and root length of *L. laxum*.

Main (Mediums)	% Callus formed	% Rooted	% Survival rate	Roots/ cutting	Root Length
Bark and polystyrene	30 ± 3.9a	28 ± 4.6a	57 ± 7.6a	3.50 ± 0.62a	7.35 ± 1.38a
Peat moss and polystyrene	19 ± 6.9ab	10 ± 3.7b	57 ± 8.2a	1.40 ± 0.36b	1.35 ± 0.61b
Bark, river sand and polystyrene	10 ± 3.3b	2 ± 0.9bc	19 ± 4.8b	1.20 ± 0.69bc	0.90 ± 0.55bc
Perlite and river Sand	7 ± 3.6b	1 ± 0.5c	11 ± 3.9b	0.05 ± 0.05c	0.15 ± 0.15c
F Statistic	4.49**	16.2***	13.6***	11.15***	18.8***
Sub (IBA- Auxins)					
Control	14 ± 4.6a	6 ± 3.3a	33 ± 9.3a	1.69 ± 0.85ab	0.75 ± 0.34
500	22 ± 7.3a	6 ± 3.0a	43 ± 9.0a	1.06 ± 0.49b	2.50 ± 1.29
1000	21 ± 7.0a	11 ± 3.1a	40 ± 8.4a	2.81 ± 0.85a	2.88 ± 0.99
2000	7 ± 2.4a	14 ± 6.8a	29 ± 8.9a	0.94 ± 0.38b	4.06 ± 1.78
4000	18 ± 5.0a	14 ± 4.2a	34 ± 9.3a	1.19 ± 0.31b	2.00 ± 0.74
F Statistic	1.22 ns	1.15 ns	0.58 ns	2.54*	2.01 ns

Values presented are means ± SE.
*, **, *** = significant at P ≤ 0.05, 0.01 and 0.001 respectively. ns = not significant.
Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05.

rooting however, both mediums contained polystyrene which could be linked with the availability of higher aeration in the rooting zone. Rooting numbers also progressed in this medium owing to higher moisture availability of bark in the rooting medium.

Comparisons in growth mediums and IBA concentrations

Among the four mediums tested, bark and polystyrene was significantly ($P \leq 0.01$) superior to all others in influencing all of the measured parameters (Table 3). The highest percentage of callus formed (30%), rooting (28%), survival rate (57%), number of roots per cutting (3.5) and rooting length (7.35 cm) was found in the bark and polystyrene medium (Table 3). The peat and polystyrene medium produced the second best overall results (Table 3). In comparison with all the mediums used, these two components (bark or peat) provided more moisture in the rooting zone and both had polystyrene for aeration as observed in the higher survival rate of the cuttings (Table 3). Other investigations have revealed that rooting mediums may have a profound effect on root formation of cuttings (Hartman et al., 1990). The aeration and water holding capacity of mediums are of paramount importance (SAFEC, 2002). The cuttings planted in bark, river sand and polystyrene and in polystyrene and perlite showed significantly little effect on influencing callus formation, rooting, survival rate, number of roots per cutting and rooting length (Table 3). These mediums had relatively low water-holding capacities.

With regard to various IBA treatments tested across all treatments, there was no significant influence to percentage callus formed, percentage rooting, percentage

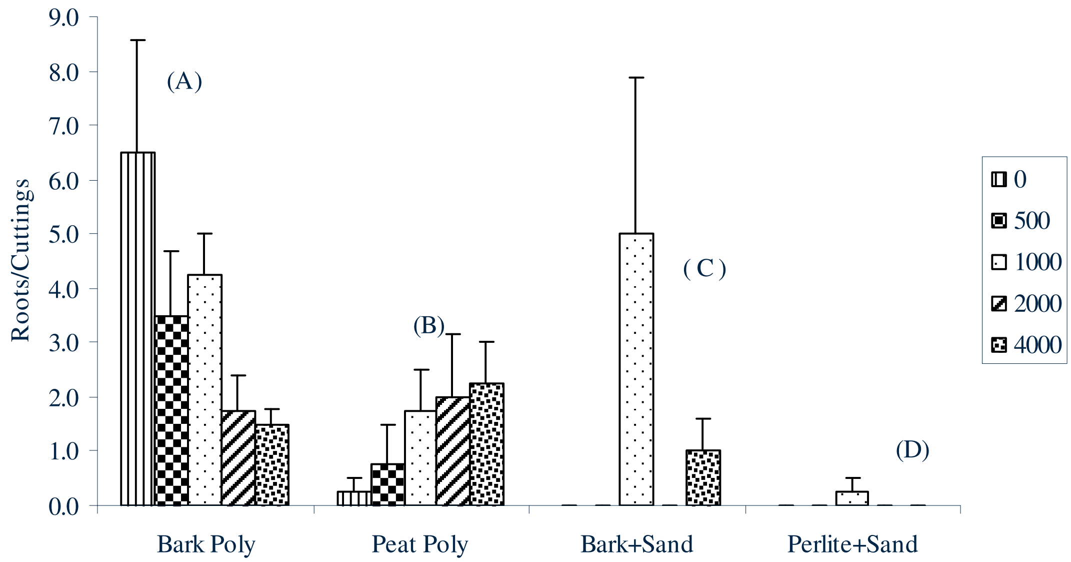

Figure 1. Interactive effects: A) Bark and polystyrene and indole butyric acid (IBA) concentration, B) peat and polystyrene and IBA concentrations, C) bark, river sand and polystyrene and IBA concentrations, D) perlite, river sand and IBA concentrations.

survival rate and rooting length. However, the number of roots per cutting was significantly ($P \leq 0.05$) affected by IBA treatments (Table 3). The IBA supplied at 1000 ppm produced greater root numbers as compared with other treatments (Table 3). The increase in root numbers after the IBA supply has also been reported in plants such as olive, *Dorycnium* spp., *Shorea leprosula*, *Leucospermum patersonii, Leucospermum patersonii* and *Protea obtusifolia* (Alegre et al., 1998; Aminah et al., 1995; Rodríguez Pérez, 1992; Wiesman and Lavee, 1995). In this study, lower concentrations of IBA were thus more conducive to root formation in *Leucadendron*. The rooting at higher IBA concentrations was significantly reduced. Too high concentrations of IBA may be toxic to cuttings of some plant species and may reduce cell differentiation in the tissues and finally affect the rooting (Hartmann and Kester, 1983; Hartmann et al., 2002).

Growth medium x IBA interaction

The growth medium x IBA interaction was significant ($P \leq 0.05$) only for number of roots per cutting (Figure 1). In general, the number of roots per cutting was increased most by IBA concentrations applied to bark and polystyrene and peat and polystyrene mediums. The use of bark or peat in rooting mediums for most Proteaceae is seen as essential as both hold added moisture in the medium (Reinten et al., 2002).

Conclusion

A hormone application of 1000 ppm IBA was successful in rooting *L. laxum.* Both bark, sand and polystyrene and the bark and polystyrene mediums contributed to successful root development of cuttings. However, the bark, sand and polystyrene medium was more successful in the survival rate and root length of cuttings. Further tests on *L. laxum* need to be conducted using different combinations of auxins and different environments to produce maximum quantity and quality rooted plants. The successful production of quality cuttings for the industry is largely defined by the grower who is able to use the correct auxins treatments and rooting mediums to produce quality Proteaceae cuttings which will succeed when planted in the field.

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REFERENCES

Alegre J, Toledo JL, Martinez A, Mora O, De Andres EF (1998). Rooting ability of *Dorycnium* spp. Sci. Horticult. 76: 123-129.

Aminah H, Dick JMCP, Leakey RRB, Grace J, Smith RI (1995). Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. For. Ecol. Manage. 72: 199-206

Brown N, Duncan G (2006). Manual for growing Proteaceae in greenhouses Joseph Knight in 1809 www.protea.worldonline.co.za/growknight.htm, : 14.

Fogaça CM, Fett-Neto AG (2005). Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrance. Plant Growth Regul. 45: 1-10.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant Propagation Principles and Practices. 7th Edition. Prentice Hall. New Jersey, pp. 367-374.

Hartmann HT, Kester DE (1983). Plant propagation; principals and Practices. Prentice-Hall International, London.

Hilton-Taylor C (1996). Red Data List of Southern African Plants. Strelitzia 4. National Bonanical Institute, Pretoria.

Jamieson HG (2001). *Leucadendron laxum* I. Williams. Kirstenbosch Botanic Garden, Cape Town, South Africa.

Laubscher CP (2000). Propagating South African trees. Combined Proceedings International Plant Propagators' Society 50: 56-65.

Mustard P, Cowling R, Albertyn J (1997). Southern Overberg. South African Wild Flower Guide 8. National Botanical Institute. National Book Printers. South Africa.

Owings AD (1996). Variations in pH from different bark sources. Combined Proceedings International Plant Propagators' Society. 46: 699.

Paterson-Jones C (2002). The Protea family of Southern Africa. Struik Publishers. Cape Town.

South African Flower Export Council (SAFEC) (2002). Preparing orchards for planting of PROTEACEAE. Certificate course in flower export readiness. Unpublished booklet. South Africa.

Steel RGD, Torrie JH (1980). Principles and procedures of statistics: A biometrical approach, Second Edition. McGraw Hill, New York.

Reinten E, Gertze S, Arends L (2002). Propagation methods. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Elsenburg, South Africa.

Robyn A, Littlejohn G (2002). Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production.

Rodríguez Pérez JA (1992). Propagation by leaf bud cuttings of *Leucadendron* 'Safari Sunset', *Leucospermum cordifolium*, *Leucospermum patersonii* and *Protea obtusifolia*. Acta. Horticult. 316: 35-46.

Wiesman Z, Lavee S (1995). Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. Sci. Hortic. 62: 189-198.

Williams IJM (1972). A Revision of the Genus *Leucadendron* (Proteaceae) Contribution of the Bolus Herbarium No. 3.

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Full Length Research Paper

Rooting response under shade using IBA growth regulators and different growth mediums on *Leucadendron laxum* (Proteaceae) – A commercial cut flower

C. P. Laubscher[1], and P. A. Ndakidemi[2]*

[1]Faculty of Business, Cape Peninsula University of Technology, P. O Box 652, Cape Town 8000, South Africa.
[2]Faculty of Applied Sciences, Cape Peninsula University of Technology, P. O Box 652, Cape Town 8000, South Africa.

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The popular cut flower, *Leucadendron laxum* (Marsh rose Leucadendron) was tested for its rooting ability, using various Indole-3-butyric acid (IBA) liquid hormone concentrations with different growth mediums. The IBA treatments included a control, 500, 1000, 2000 and 4000 ppm IBA auxins. Growth mediums tested were a) peat and polystyrene; b) bark and polystyrene; c) bark, sand and polystyrene; and d) perlite and river sand. Basal ends of cuttings were dipped into the IBA solution for a few seconds after preparation, and planted under shade conditions. The experiment was planned in a randomised block design, with 10 cuttings and 3 replicas per treatment. The IBA application in comparison with the control, significantly improved rooting percentages in a) peat and polystyrene and b) bark, river sand and polystyrene mediums. The bark and polystyrene medium was more effective in the stimulation of roots, survival of cuttings, promotion of root length and the number of roots that developed. The overall application of IBA induced more root and shoot formation.

Key words: Auxin, cut flower, growth medium, indole-3-butyric acid, shade environment.

INTRODUCTION

Leucadendron laxum is a member of the Proteaceae family which occurs naturally in damp areas on the Agulhas Plain in the extreme south western coast of southern Africa (Mustard et al., 1997). *L. laxum* is popularly referred to as the marsh rose and is continually being illegally harvested in more than 436 hectares of its natural habitat (Mustard et al., 1997; Robyn and Littlejohn, 2002). Over exploitation of *L. laxum* has caused this species to be listed as endangered in the Cape Floral Kingdom (Hilton-Taylor, 1996).

More than 70 commercial Proteaceae species and cultivars including Leucadendron are already cultivated in field-grown orchards (Brown et al., 1998; Robyn and Littlejohn, 2002). This fast-developing flower export market, which includes the pharmaceutical and medicinal Industry, has exceeded $45 billion per annum on the world market (Cowling and Richardson, 1995). Thirty million flower stems were sold in 2001 and production increased from 8570.6 ha to 22.87% in 2003 and to 27.34% in 2004 (Rabie, 2007, personal communication).

Leucadendron is one of South Africa's leading export floral crops and is mainly harvested on low production fynbos land where it is picked out of the natural veld as part of mixed farming (Dodd and Bell, 1993; Paterson-Jones, 2000; Robyn and Littlejohn, 2002). The male flowers of bright green, yellow and gold shades provide an exotic filler in mixed bouquets known as "Cape Greens", while the female cones are mainly harvested for the dried flower industry (Coetzee and Littlejohn, 1994; Moody, 1995; Berney, 2000; Paterson-Jones, 2000).

The annual demand for *L. laxum* has grown to more

*Corresponding author. E-mail: ndakidemip@cput.ac.za
Tel: +27214603196. Fax: +27214603193

Abbreviations: IBA = Indole-3-butyric acid; SAFEC = South African Flower Export Council.

than 23,673 cut flower stems and has created an urgent need for the improvement of its propagation and cultivation. Vegetative propagation is a reliable method that would ensure the production of plants with uniform flowering times and duplicate their genetic characteristics (Reinten et al., 2002; Brown and Duncan, 2006). Future sustainable propagation would also aid the replacement of existing species in the natural habitat.

Previous research work has shown that *L. laxum* was successfully rooted in controlled greenhouse conditions (Laubscher and Ndakidemi, 2008). No written evidence was found on cutting propagation for *L. laxum* under shade house conditions in South Africa. A study on this would therefore be seen as new research, aimed at preventing the costs of bottom heating. Cheaper built shade structures would be more sustainable for many growers rather than environmentally controlled greenhouse conditions.

Evidence suggests that auxin increases rooting percentages, shortens the rooting period and ensures improved uniformity in plants (Hartmann et al., 2002). Previous research has shown that *L. laxum* responded well to callusing in 500 ppm IBA and the highest number of roots were measured in the 1000 ppm IBA treatment under environmentally controlled greenhouse conditions with bottom heat (Laubscher and Ndakidemi, 2008). While some Proteaceae species favour bottom heat conditions for rooting, other cuttings are difficult to root, and could respond differently to auxin treatments (Hartmann et al., 2002). In this respect auxin formulations supplied at different concentration and growth conditions need further investigation and may improve rooting percentages (Hartmann et al., 2002) and produce desirable rooting results.

Previous results under controlled environment greenhouse conditions proved that using a bark, sand and polystyrene rooting medium resulted in 30% callus formation and 28% rooting and 57% survival rate of *L. laxum* cuttings (Laubscher and Ndakidemi, 2008).

The aim of this current study is therefore to determine whether *L. laxum* could respond favourably by rooting, with different concentrations of IBA and various rooting mediums in a shade house environment without bottom heat. The aim is further to provide faster and more efficient rooting success in cut flower production.

MATERIALS AND METHODS

The experiment was conducted in a shade tunnel (40% white shade cloth) at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa. The experiment started during the middle of August and continued for 8 weeks. Cutting material of *L. laxum* was collected from various plant populations in the natural habitat on the Agulhas Plain on the extreme south-western coast of South Africa. The material was kept dry in a sealed plastic bag and transported overnight.

The growth mediums and IBA treatments were randomised in a complete block design with four growth mediums: a) bark and polystyrene; b) peat moss and polystyrene; c) bark, river sand and polystyrene; and d) perlite and river sand. Four concentrations of IBA (control, 500, 1000, 2000 and 4000) were used in this study. Altogether 3 x 5 x 10 cuttings were involved in the experiment. The rooting mediums had good drainage conditions, moisture retention and an average pH of 6.5-7 (SAFEC, 2002). Cuttings were replicated in 10 pots for each treatment. The experiment was set up in a shade house (40% shade) with misting irrigation.

Cuttings were taken from semi-hardwood stems after shoot elongation (Aug-Nov) and not at flowering stage. Stem cuttings of 150 mm long were made and half of the lower leaves removed (SAFEC, 2002; Reinten et al., 2002). The cuttings were rinsed in Benlate fungicide (10 g/l) before planting and Captan (2 g/l) was sprayed weekly to prevent fungal infection (Reinten et al., 2002; SAFEC, 2002).

The hormone treatment used was IBA liquid preparations of 500, 1000, 2000, 4000 ppm. The control remained untreated. IBA powder was dissolved at g/l in 50% ethyl alcohol The basal 5 mm of cuttings were dipped in the rooting hormone for 5 seconds (Hartmann et al., 2002; Reinten et al., 2002; Brown and Duncan, 2006) after which cuttings were individually planted into 200 size plug foam trays.

The tunnel (10 m long) was covered in white shade screen material (40%). The sides of the tunnel were left open to allow air flow during the rooting period (Reinten et al., 2002; SAFEC, 2002). Midday temperatures were measured between 18-25ºC and a relative humidity between 20 and 62%. The irrigation timer was set on 10 s on and 60 min off.

Data collected included, average shoot growth, number of cuttings callused, number of cuttings rooted, number of roots on cuttings, length of roots, and the survival rate of cuttings.

Shoot growth (mm) and the length of roots (mm) were measured from cutting stage to new growth after 8 weeks. The number of cuttings callused, rooted and survival rates were also counted after 8 weeks. Data was recorded for the percentage of survival rate of cuttings, number of roots per rooted cutting and root length. Data analysis was performed in two different ways. The first consisted of one-way analysis of variance for IBA treatments added to each growth medium separately. The second consisted of factorial analysis including the four growth mediums and the five IBA concentrations.

Data on the number of cuttings that formed calluses, the number of cuttings that rooted and the cuttings that survived, were transformed into percentages prior to analysis of variance. Data were presented at mean values with predicted standard errors (S.E.). These computations were done with the software program STATISTICA. The Fisher least significance difference (L.S.D.) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

RESULTS

Tables 1 and 2 show the effects of different concentrations of IBA on the percentage of cuttings that rooted, cuttings that survived in the rooting mediums, root length and shoot growth of cuttings. The cuttings grown in a) peat and polystyrene; b) bark and polystyrene; c) bark, river sand and polystyrene and d) perlite and river sand mediums all produced different results. The IBA treatments significantly ($P \leq 0.05$) affected the percentage of cuttings that rooted in a) peat and polystyrene b) bark and polystyrene; and c) bark, river sand and polystyrene (Tables 1-2). The IBA application in comparison with the control, significantly improved rooting percentages in a) peat and polystyrene and b) bark, river sand and polysty-

Table 1. Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *Leucadendron laxum* grown in peat and polystyrene and bark and polystyrene medium.

	Peat Polystrene				Bark Polystrene			
	% Rooted		Root Length		% Rooted		Root Length	
Control	**10**	**±5.8b**	2.33	±1.20a	**57**	**±12.0a**	**8.00**	**±2.08a**
500	**37**	**±6.7a**	5.33	±2.60a	**43**	**±3.3ab**	**2.33**	**±0.33bc**
1000	**20**	**±0.0ab**	5.67	±3.18a	**17**	**±3.3c**	**1.33**	**±0.33c**
2000	**27**	**±8.8ab**	3.67	±1.20a	**23**	**±8.8b**	**1.67**	**±0.33c**
4000	**27**	**±3.3ab**	2.67	±0.33a	**43**	**±3.3ab**	**5.67**	**±1.20ab**
F Stat	**2.9***		0.58 ns		**5.22***		**6.93****	

Values presented are means ± SE. *, **, ns = significant at P≤ 0.05, 0.01, ns = not significant respectively. Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05.

Table 2. Effect of different concentrations of IBA on percentage survival rates of cuttings, number of roots per cutting and root length of *Leucadendron laxum* grown in Bark, River Sand and Polystrene, and Perlite and River Sand medium.

	Bark, River Sand and Polystrene						Perlite and River Sand					
	% Rooted		% Survival		Shoot Growth		% Rooted		% Survival		Shoot Growth	
Control	0	±0.0c	37	±3.3b	4.3	±1.20a	7	±6.7a	67	±20.3a	3.7	±1.20b
500	3	±3.3bc	60	±5.8a	1.3	±0.67a	20	±0.0a	87	±3.3a	10.3	±1.76ab
1000	17	±8.8ab	60	±5.8a	2.3	±0.33a	7	±6.7a	83	±3.3a	4.7	±1.20b
2000	23	±3.3a	27	±3.3b	6.0	±1.73a	30	±11.5a	80	±11.5a	16.7	±4.18a
4000	0	±0.0c	3	±3.3c	4.3	±1.20a	10	±10.0a	60	±10.0a	15.3	±2.96a
F Stat	**5.72***		**28.72*****		2.63 ns		1.60 ns		0.98 ns		**5.49***	

Values presented are means ± SE. *, **, ***, ns = significant at P≤ 0.05, 0.01, 0.001, ns = not significant respectively. Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05.

rene (Tables 1-2). However, in the bark andpolystyrene medium, the rooting percentage was significantly higher in the control treatment compared with other IBA treatments. The higher rooting percentage in both the control (57%) with no addition of IBA and the 4000 ppm IBA treatment (43%) in the bark and polystyrene medium indicated a presence of negative results. These results concur with other studies which reported that IBA applications could also inhibit rooting (Hartmann et al., 2002; Reinten et al., 2002). It is well established that several compounds may act together in the control of several plant functions, including the formation of roots (Volper et al., 1995). It is also worth mentioning that the bark component used in this study originated from pine trees. Past studies have shown that bark from pine trees is very rich in phenolic compounds, alkaloids and cyanogenic glycosides (Machrafi and Prevost, 2006). These compounds are known to have inhibitory effects on the growth of plants (Still et al., 1976; Rice, 1984; Siqueria et al., 1991). It is likely therefore, that the presence of such phenolic compounds in the bark medium used in our study or possibly other endogenous compounds in combination with the supplied auxins, could have had an synergistic inhibitory effect that reduced the rooting capacity of *L. laxum*. (Table 2). Further studies are therefore needed to establish the relationship between the profile of phenolic compounds in the bark medium and different concentrations of growth hormones such as IBA.

The various concentrations of IBA which were supplied to cuttings of *L. laxum* in the bark, river sand and polystyrene medium significantly affected survival and rooting percentages of the cuttings (Table 2). IBA supplied at rates between 1000-2000 ppm was significantly superior in stimulating rooting in the bark, river sand and polystyrene rooting medium. It is possible that the lower proportion of bark in the bark, river sand and polystyrene medium induced deleterious effects, compared with the cuttings reported in the bark and polystyrene medium above. The higher IBA level of 4000 ppm suppressed rooting in the *L. laxum* cuttings completely. On the other hand, stem cuttings treated with IBA and grown in a perlite and river sand medium showed a significant improvement ($P \leq 0.05$) in shoot growth (Table 2). The cuttings treated with 2000 and 4000 ppm IBA in a perlite and river sand medium were superior in growth compared with those supplied with other treatments.

The evaluation of IBA concentrations in Table 3 shows a significant influence on the percentage of rooted cutt-

Table 3. Effect of different growth medium and IBA concentrations on percentage callus formed, % of cuttings that rooted, % survival rates of cuttings, shoot growth, root length and the number of roots per cutting of *Leucadendron laxum*.

Treatment	Callused		% Rooted		% Survival		Shoot Growth		Root Length		Number of roots/cutting	
Main Treatment												
Peat Polystrene	22	±13.2a	**24**	**±3.2b**	**69**	**±5.6a**	**3.3**	**±0.73b**	**3.9**	**±0.84a**	**5.9**	**±0.98a**
Bark Polystrene	29	±12.5a	**37**	**±4.7a**	**77**	**±4.7a**	**1.4**	**±0.59b**	**3.8**	**±0.81a**	**5.9**	**±0.98a**
Bark/Sand /Polystrene	22	±26.5a	**9**	**±3.1c**	**37**	**±6.0b**	**3.7**	**±0.61b**	**0.9**	**±0.53b**	**1.2**	**±0.54b**
Perlite / Sand	36	±18.0a	**15**	**±3.9c**	**75**	**±5.2a**	**10.1**	**±1.72a**	**1.8**	**±0.46b**	**4.6**	**±1.52a**
F Statistic	1.85 ns		**17.6*** **		**18.09*** **		**22.76*** **		**6.03** **		**4.44** **	
Sub - Treatment												
Control	26	±21.1a	**18**	**±7.5b**	**61**	**±7.9b**	**3.3**	**±0.81b**	3.0	±1.10a	5.3	±1.73a
500	25	±20.2a	**26**	**±5.0a**	**79**	**±4.2a**	**3.6**	**±1.27b**	3.7	±0.83a	5.8	±1.59a
1000	32	±15.9a	**15**	**±2.9b**	**68**	**±5.6ab**	**2.6**	**±0.63b**	2.2	±0.94a	3.4	±0.68a
2000	33	±20.2a	**26**	**±3.8a**	**58**	**±7.7b**	**7.6**	**±1.99a**	2.0	±0.44a	3.8	±1.06a
4000	20	±16.5a	**20**	**±5.5a**	**57**	**±10.0b**	**6.2**	**±1.82a**	2.3	±0.73a	3.7	±1.13a
F Statistic	0.96 ns		**2.15***		**3.58***		**5.76*** **		1.07 ns		0.88 ns	

Values presented are means ± SE. *, **, *** = significant at P ≤ 0.05, 0.01 and 0.001 respectively. ns = not significant. Means followed by the same letter(s) are not significantly different from each other at P ≤ 0.05.

ings, and their survival and shoot growth. IBA supplied at 500, 2000 and 4000 ppm resulted in significant rooting percentages as compared with the control and 1000 ppm treatment. The percentage survival rate of cuttings in 500 and 1000 ppm IBA treatments showed significantly higher survival rates compared with other treatments (Table 3). However, the survival rate declined strongly at the highest rate in the 4000 ppm IBA treatment. The shoot growth was significantly improved at higher levels of IBA treatment ranging from 2000-4000 ppm (Table 3). Overall, the results obtained in this study concur with other investigations where the auxin supplied was responsible for root induction and shoot growth in difficult to root plant species, that is, the Proteaceae family (Wu and du Toit 2004; Wu et al., 2007a,b).

DISCUSSION

Limited studies have been found which document the influence of different concentrations of the IBA auxin in rooting *L. laxum* cuttings (Perez-Frances, 1995). The positive stimulatory effects of growth hormones on rooting stem cuttings have been recorded in other related plant species in South Africa (Wu and du Toit 2004; Wu et al., 2007a, b). Recordings of most results in this study indicated improved rooting, and in some cases an increase in survival rates and shoot growth of cuttings (Tables 1-3). Therefore, we can assume that the increased exogenous application of the IBA auxin enhanced rooting, root length and number of roots formed in *L. laxum* cuttings. These results are in accordance with the findings of Jones and Hatfield (1976), Zimmerman (1984) and Rout (2006) who proved that the accumulation of auxin in shoots was responsible for successive vegetative propagation of cuttings.

The cuttings of *L. laxum* performed differently when they were grown in different growth mediums and with different IBA concentrations (Table 3). Overall, and in comparison with other growth mediums tested, the bark and polystyrene medium was more effective in the stimulation of rooting, survival of cuttings, root length and number of roots formed (Table 3). This result was closely followed by the peat and polystyrene and the perlite and sand mediums (Table 3). The bark, polystyrene and sand medium produced less appreciable results compared with the other growth mediums tested (Table 3). Overall, the highest rooting and survival rate, observed in the bark and polystyrene medium, is an indication that the medium was highly advantageous for *L. laxum* propagation under shade house conditions. Similar results have been reported in other studies involving different plant species (Brown and Duncan, 2006; Reinten et al., 2002; SAFEC, 2002).

A significant interaction was observed between the different growth mediums used in this study, e.g., the effect of a) peat and polystyrene; b) bark and polystyrene; and c) bark, sand and polystyrene and the results of the IBA concentrations on the percentage of rooted cuttings, the percentage survival rate and the shoot growth and root lengths of cuttings (Figures 1-4). In most instances, the overall application of IBA used, in combination with growth mediums, induced more root and shoot formation. *L. laxum* is a member of the Proteaceae family which is documented as being very difficult to root (Wu et al., 2007a). In this study, the addition of IBA in most growth mediums stimulated root and shoot growth. It is well known that the exogenous supply of auxins in the Proteaceae family is a prerequisite for root

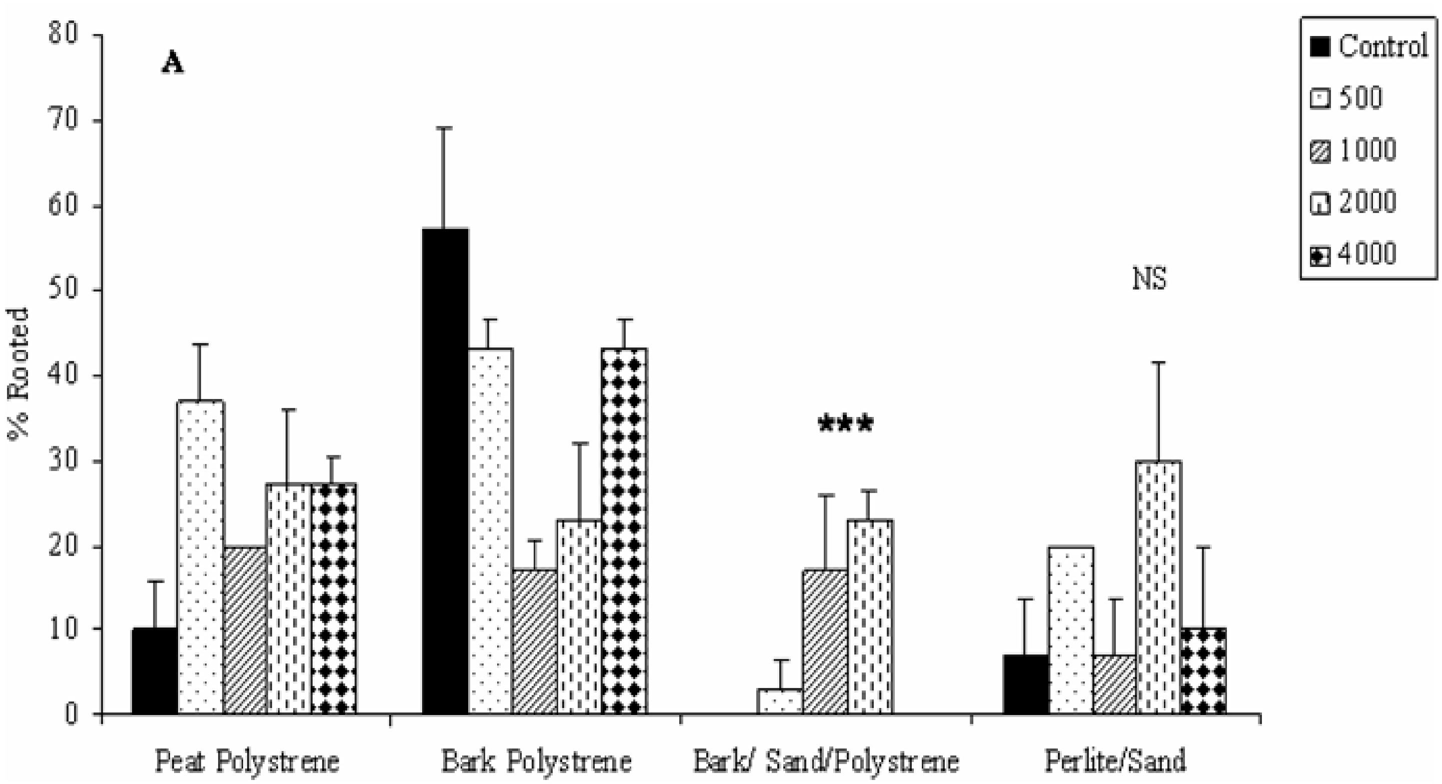

Figure 1. Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on % of rooted cuttings. ***= Significant at P < 0.001); NS = Not significant.

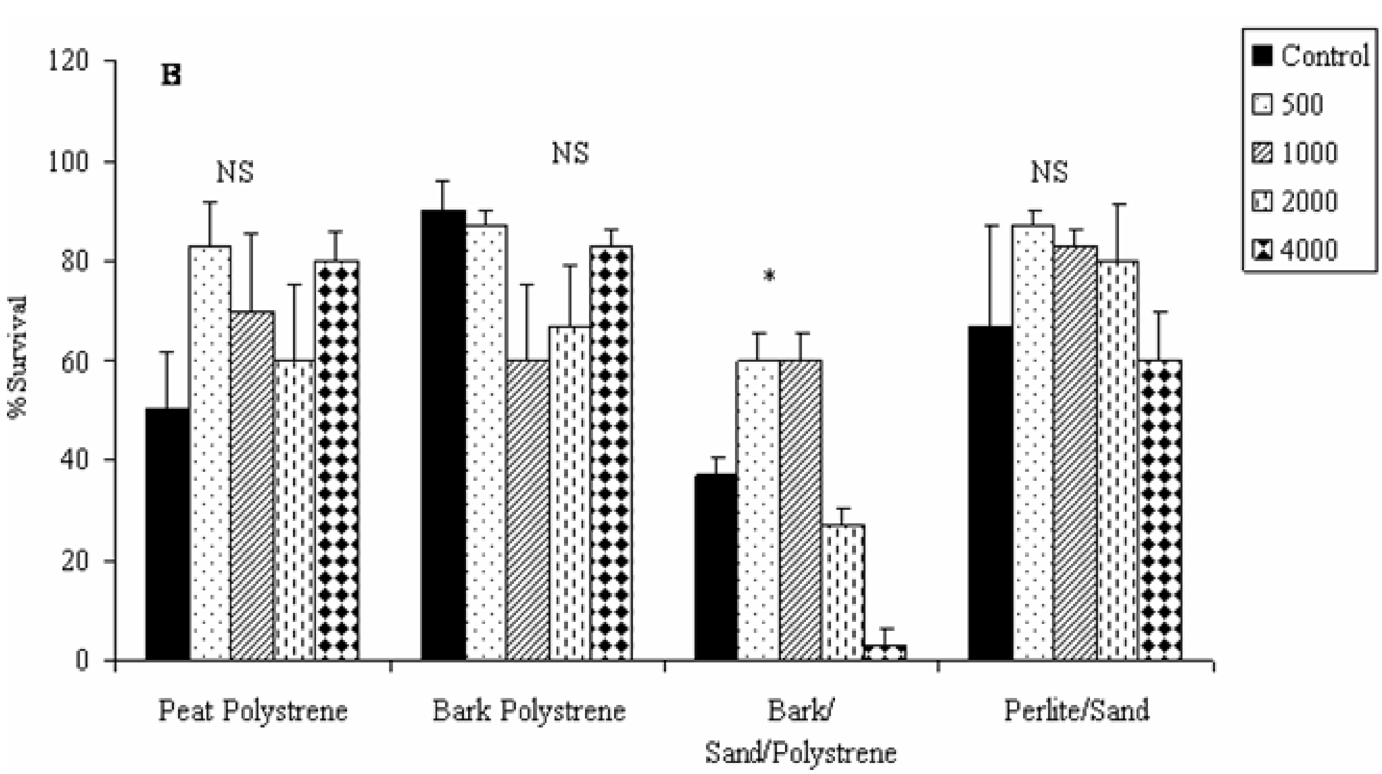

Figure 2. Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on % survival of cuttings. * = P $\leq$ 0.05; NS = Not significant.

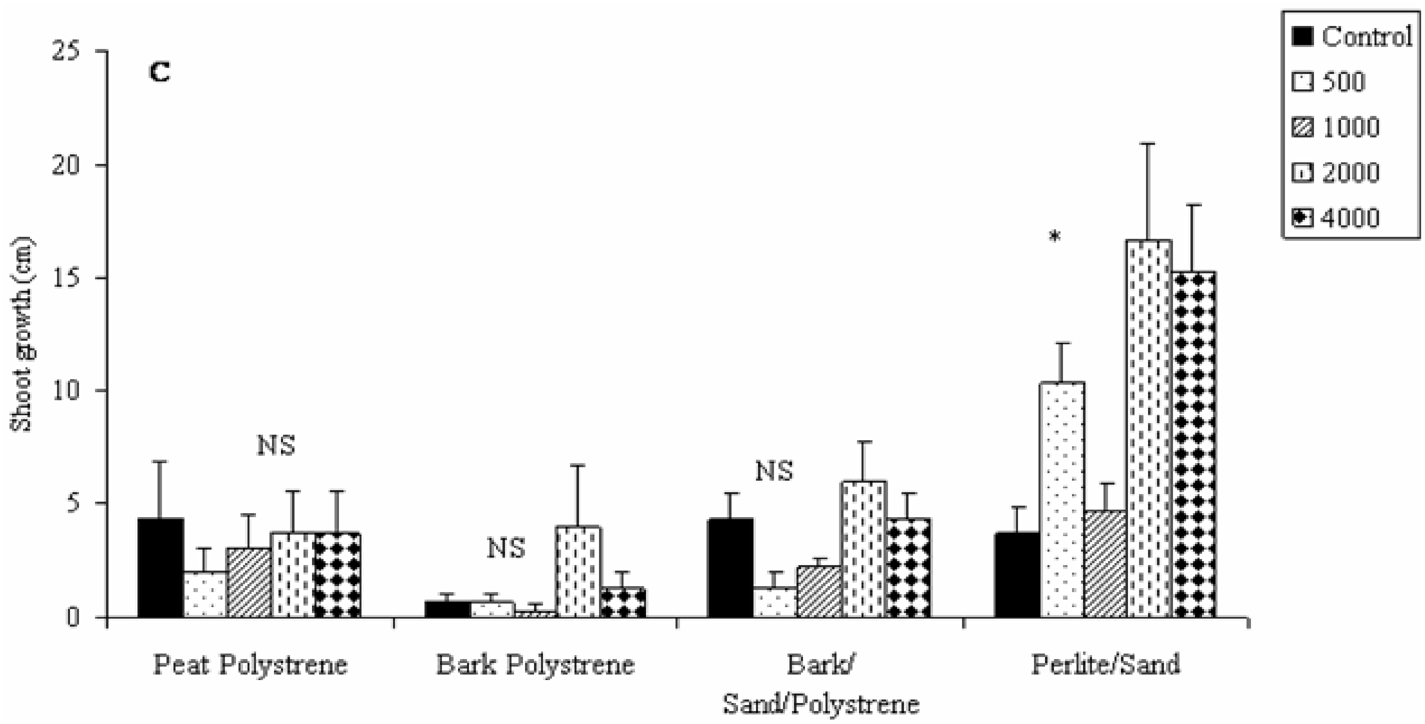

Figure 3. Interactive effects of peat, Bark, Bark/sand polystyrene and perlite/sand on shoot growth (cm). * = P $\leq$ 0.05; NS = Not Significant.

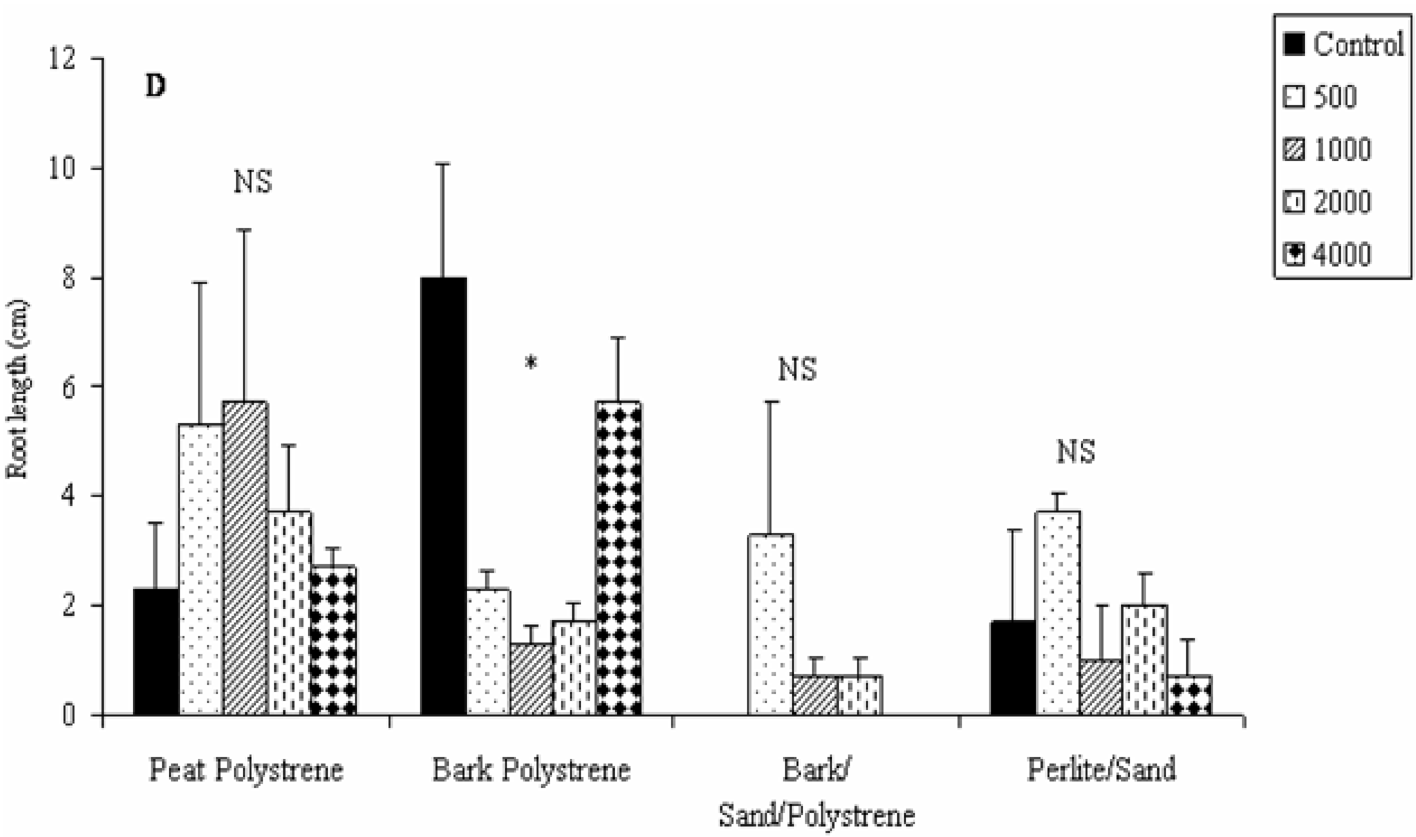

Figure 4. Interactive effects of peat, bark, bark/sand polystyrene and perlite/sand on root length (cm). * = P $\leq$ 0.05; NS = Not Significant.

induction in cuttings which are difficult to root (Wu et al., 2007b) such as the *L. laxum*. An exogenous supply of auxins has proved to stimulate root and shoot growth in stem cuttings grown in different rooting mediums (Wu et al., 2004; 2007a,b). In the bark polystyrene medium, significant decreases were reported in rooting characterristics by applying IBA (Figure 1 and 4). It is possible that the synergistic effects between IBA and the phenolic compounds in bark inhibited root formation in stem cuttings of *L. laxum*.

Conclusion

In conclusion, the results of this study have described the effects of different concentrations of the auxin (IBA) and different growth mediums on the rooting of *L. laxum* under shade house conditions. These results can provide growers with the opportunity to stimulate the rooting of *L. laxum* cuttings faster and at lower costs over a period of 8 - 12 weeks. This study also showed that the growth medium environment determines the survival rate success of cuttings, which in turn results in progressive rooting success. The use of IBA in the rooting of *L. laxum* was variable in different rooting mediums where moisture retention was important. Other endogenous factors in the growth medium of bark, warrants further investigation under different environmental conditions.

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REFERENCES

Berney J (2000). Combined research to benefit Protea growers. Aust. Hort. 98(2): 16-18.

Brown NAC, Kotze D, Botha P (1998). Grow Proteas. A Guide to the Propagation and Cultivation of Some South African Proteaceae. Kirstenbosch Gardening Series, National Botanical Institute, Cape Town, South Africa.

Brown N, Duncan G (2006). Manual for growing proteaceae in greenhouses Joseph Knight in 1809. www.protea.worldonline.co.za/growknight.htm, :14.

Coetzee JH, Littlejohn G (1994). Indigenous South African Flower Industry. Hortagrow. August 1994, pp. 9-11.

Cowling R, Richardson D (1995). Fynbos. South Africa's Unique Floral Kingdom. Fernwood Press, Cape Town, pp. 7-31.

Dodd J, Bell DT (1993). Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. Aust. J. of Bot. 18:281-293.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant Propagation Principles and Practices, Seventh Edition, Prentice Hall, New Jersey, pp. 367-374.

Hilton-Taylor C (1996). Red Data List of Southern African Plants. Strelitzia 4. National Botanical Institute, Pretoria.

Jones OP, Hatfield SGS (1976). Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds. J. Hort. Sci. 51:495-499.

Laubscher CP, Ndakidemi PA (2008). Rooting success using IBA auxin on endangered *Leucadendron laxum* (PROTEACEAE) in different rooting mediums. Afr. J. Biotechnol. 7(19) : 3437-3442.

Machrafi Y, Prevost D (2006). Toxicity of phenolic compounds extracted from bark residues of different ages. J. Chem. Ecol. 32:2595-2615.

Moody H (1995). Versatile Leucadendrons and Leucospermums. Aust. Hort. 5: 111-112.

Mustard P, Cowling R, Albertyn J (1997). Southern Overberg. South African Wild Flower Guide 8. National Botanical Institute, National Book Printers, South Africa.

Paterson-Jones C (2000). The Protea family of Southern Africa. Struik publishers, Cape Town, pp. 28-124.

Perez-Frances JF, Exposito A, Rodriguez JA (1995). Effect of different factors on i*n vitro* multiplication of *Leucadendron* 'Safari Sunset' (Proteaceae). Acta Hort. 387: 115-120.

Robyn A, Littlejohn G (2002). Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production. Elsenburg, South Africa, pp. 22-49.

Reinten E, Gertze S, Arends L (2002). Propagation methods. Cultivating fynbos for quality cut flower production. ARC training course manual. Elsenburg, South Africa, pp. 50-70.

Rice EL (1984). Allelopathy. Academic Press, Orlando, pp. 272-274.

Rout GR (2006). Effect of auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. Plant Growth Regul. 48:111-117.

SAFEC (2002). Preparing orchards for planting of Proteaceae. Certificate course in flower export readiness manual. South African Flower Export Council, South Africa.

Siqueria JO, Safir GR, Nair MG (1991). Stimulation of Vesicular-Arbuscular Mycorrhiza Formation and Growth of White Clover by Flavonoid Compounds. New Phytologist 118:87-93.

Steel RGD, Torrie JH (1980). Principles and procedures of statistics: A biometrical approach, Second Edition, McGraw Hill, New York.

Still SM, Dirr MA, Gartner, JB (1976). Phytotoxic effects of several bark extracts on mung bean and cucumber growth. J. Am. Soc. Hort. Sci. 101:34-37.

Volper R, Osswald, M, Elstner, EF (1995). Effects of cinnamic acid derivatives on indole acetic acid oxidation by peroxidase. Phytochem. 38:1-22.

Wu HC, du Toit, ES (2004). Reducing oxidative browning during in vitro establishment of *Protea cynaroides.* Scientia Hort. 100:355-358.

Wu HC, du Toit ES, Reinhardt CF. (2007a). Micrografting of *Protea cynaroides***.** Plant Cell Tiss. Cult. 89:23-28**.**

Wu HC, du Toit ES, Reinhardt CF, Rimando AM, van der Kooy F, Meyer JJM (2007b). The phenolic, 3,4-dihydroxybenzoic acid, is an endogenous regulator of rooting Protea cynaroides. Plant Growth Regul. 52:207-215.

Zimmerman RH (1984). Rooting apple cultivars in vitro: Interaction among light, temperature, phloroglucinol and auxin. Plant Cell Tiss. Cult. 3:01-311.

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Review

Conservation and propagation of endangered Proteaceae on the Agulhas plain for sustainable ecotourism development

Charles P. Laubscher[1], Patrick A. Ndakidemi[2]*, Mohammed S. Bayat[1] and André Slabbert[1]

[1]Faculty of Business, Cape Peninsula University of Technology, P. O. Box 652, Cape Town, 8000, South Africa.
[2]Faculty of Applied Sciences, Cape Peninsula University of Technology, P. O. Box 652, Cape Town, 8000, South Africa.

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Large parts of the Agulhas plain contribute to the commercial cut flower export industry. However, this industry is causing the destruction of many Proteaceae species both endemic and threatened. The Agulhas plain is a large region (34°50′00″S 20°00′09.15″E / 34.833333°S 20.0025417°E) where threatened red data species are not sufficiently valued as many landowners and local communities lack vigilance in the preservation of these species. Flowers are harvested from their natural habitat and trading permits are misused while the lack of authorities inspecting farms is clearly evident. Environmental threats include wine farming, alien invasive plants and wild fires. The occurrence of fires has become one of the biggest threats to the area. Involvement of stakeholders such as conservation, tourism and government departments are important for conservation to succeed. Education and training of farm workers remains undeveloped. Propagation techniques are limited as no red data species are propagated to increase an awareness of these species. There is a lack of guidance and available information in the conservation of the Agulhas plain while the ecotourism potential of the Cape Floral Kingdom (CFK) remains underdeveloped. Current practices which cause the destruction of red data species and the ecotourism potential on the Agulhas plain are assessed in this review.

Key words: Auxin, rooting mediums, cut flowers, flower potted plants, conservation, propagation, red data species sustainable tourism threatened species.

INTRODUCTION

In the Western Cape, the Cape Floral Kingdom (CFK) consists of 330 species of Proteaceae of which some are near extinction (Rebelo, 1995). The United Nations Development Programme (UNDP) views the Agulhas plain as area which has the highest priority for conservation and the largest number of lowland threatened species in South Africa (UNDP, 2003). The area is also known for important vegetation types such as Elim Asteraceous Fynbos, limestone proteoid, restioid fynbos and neutral sand proteoid vegetation types, all of which are highly threatened in the wild (UNDP, 2003). Elim Asteraceous Fynbos contains many endemic Proteaceae species such as *Leucadendron elimense*, *Leucadendron laxum*, *Leucadendron stelligerum* (Mustard et al., 1997). These plant species are classified as endangered plants on the red data List of South African Plants (Hilton-Taylor, 1996). The protea atlas (2008) classifies *L. elimense* and *Leucadendron platyspermum* as vulnerable species. *L. stelligerum* is found in only three populations, totalling 1000 plants in the Voëlvlei area (Rebelo, 1995). According to Mustard et al. (1997) only 5 000 *L. laxum* plants remain in their natural habitat.

The International Union for Conservation of Nature (IUCN) Red Data list of threatened species recorded more than 11 000 plant species which are threatened

*Corresponding author. E-mail: ndakidemip@cput.ac.za. Tel: +2721-460-3196. Fax: +2721-460-3193.

Abbreviations: ABI, Agulhas biodiversity Initiative; **C.A.P.E,** cape action plan for the environment; **CFK,** cape floral kingdom; **EuroGAP,** European good agricultural practice; **FVCT**, flower valley conservation trust; **IAA,** indole-3-acetic acid; **IBA,** indole-3-butyric acid; **IUCN,** international union for conservation of nature; **SAFEC,** south African flower export council; **ppm,** parts per million; **UNDP,** united nations development programme; **WCNCB,** western cape nature conservation board.

with extinction in South Africa (Jeffery, 2003). Leucadendrons on the Agulhas plain are seriously threatened by expanding farm land, uncontrolled veld fires, alien invasive plants and improper cut flower harvesting in the natural habitat (Mustard et al., 1997; Robyn and Littlejohn, 2002). Some species are naturally rare because of reproductive reasons (Paterson-Jones, 2000). Many plant populations will disappear if the destruction of these sensitive habitats continues. This would result in a loss of biodiversity and endemic species (Jamieson, 2001; Robyn and Littlejohn, 2002; Cooper, 2003) and ultimately may affect ecotourism activities in the Western Cape Region.

Agriculture and the keeping of livestock have been identified as the largest cause of habitat destruction (Lombard et al., 1997). On the Agulhas plain, 22.5% of the Asteraceous Fynbos and Renosterveld fertile soils are already covered in cereals, vineyards, pastures and cultivated flowers (UNDP, 2003). Studies on the vegetative propagation of some of the threatened Proteaceae species are lacking (Hartmann et al., 2002; Wu et al., 2007a). Their inception could increase awareness and facilitate rehabilitation of these species in their natural habitats. Future studies should also aim at providing information on the impact of current agricultural practices on sensitive habitats such as those involving Proteaceae and hence alleviating the threat on the biodiversity of these species on the Agulhas Plain.

PROMOTING ECOTOURISM THROUGH PROPER MAINTENANCE OF ENDANGERED PLANT SPECIES

The Agulhas plain has the potential to become a major tourist destination with tourists visiting these sites for its beautiful scenery and large diversity of plant species (Cowling, 1993). In the Western Cape, most of these plant species form part of the CFK which is documented as a world heritage site under exceptional habitat threat (Cowling and Richardson, 1995). Tourist visits to the Western Cape Province are expected to have grown with already 51% of international tourists visiting the area (ABI, 2008). The proximity of the Agulhas plain region to both the garden route and the Cape Peninsula also presents positive ecotourism potential (ABI, 2008). The region has been recognised as having the most distinctive, most diverse and the highest density of endemic flora in the world (Coetzee and Littlejohn, 1994), yet the ecotourism potential in this area remains undeveloped (Cowling and Richardson, 1995), and many parts of the land are threatened with the loss of endemic species which form part of tourist attractions.

Therefore, landowners should realise the value of conserving the remarkable flora of the Agulhas plain for future generations and the possible income benefit derived from ecotourism. This is supported by the fact that many farms on the AP are not sustainable for a variety of crops or livestock and would generate far more wealth in the development of ecotourism activities (Cowling, 1993; Paterson-Jones, 2000; Robyn and Littlejohn, 2002).

The Agulhas plain is a region where conservation, ecotourism and agriculture could work together to maintain a balance of protection, enjoyment and commercial gain of the habitat. An increase in ecotourism on the Agulhas plain is furthermore important in view of the role of extended conservation and job creation. More studies are needed to determine the effect that landowners, habitat destruction and the loss of red data species have on ecotourism development on the Agulhas plain.

Conserving endangered species through correct cut flower practices

The demand for new and interesting flowering plants around the world (Barzilay et al., 1992) has resulted in a strong interest in the diverse biodiversity of South African flora (Milandri et al., 2008). Most Proteaceae such as Leucadendrons are considered speciality flowers due to their unique and naturally bright colours. The export flowers are favoured for their bright yellow colour and attractive cones (Dodd and Bell, 1993; Coetzee and Littlejohn, 1994; Moody, 1995; Paterson-Jones, 2000; Berney, 2000; Jamieson, 2001). Export quantities for Leucadendron flowers increase by 27% in the European winter period (Rabie, 2002, 2003). Some species that are exported include *L. laxum, L. stelligerum* and *L. platyspermum* (Rabie, 2002). These popular flowers continue to be harvested for commercial gains (Robyn and Littlejohn, 2002) and sometimes through incorrect cut flower practices such as over-harvesting and incorrect pruning methods. A total of 80% of Leucadendrons are harvested from low income farm land (Dodd and Bell, 1993; Mustard et al., 1997; McVeigh, 1998; Paterson-Jones, 2000; Robyn and Littlejohn, 2002; ABI, 2008). Over-harvesting of one species such as *L. laxum* from 436 hectares of habitat has caused this species to be listed as endangered in the CFK (Hilton-Taylor, 1996; Mustard et al., 1997; Robyn and Littlejohn, 2002). Over-harvesting threatens many jobs of low-income people as natural habitats are depleted from a reserve of seed required for the next generation of species (Coetzee and Littlejohn, 1994). Because of the demand for *L. laxum*, more farmers should be encouraged to use sustainable cultivation and harvesting techniques, as field harvesting continues to destroy the habitats of many species.

It is therefore of great importance to investigate the harvesting practices of cut flower producers to determine at what level red data species are destroyed and which measures could be proposed for future conservation of the habitat.

Environmental to the conservation and propagation of endangered Proteaceae

The habitats of many indigenous species such as those involving Proteaceae have been replaced by commercial vineyards. The Agulhas plain produces high quality wines as the environmental conditions are conducive for vine growing (Hughes, 2002). These farming practices could pose further threats to the environment in which several members of Proteaceae family could struggle to survive. The impact of these new wine farms on the Agulhas plain species remains unknown and the effect of further expansion of this farming practice remain uncertain and needs to be validated.

The Agulhas plain consists of small pockets of broken up land where small scale dairy farming is practiced (Mustard et al., 1997). Close to half of the cattle farms on the Agulhas plain rely on natural habitat grazing, with cattle often grazing along wetlands (ABI, 2008). Many of these areas contain sensitive, endemic plant species, such as *L. laxum*. This type of grazing could further add pressure on the natural habitat of threatened species which could result in their extinction. The need for appropriate conservation measures by farmers is important to protect these sensitive habitats.

The spread of alien invasive species have been recorded as the second biggest threat to biodiversity on the Agulhas plain, with more than 40% of the area being infested and 14.7% of the natural habitat being already lost to alien species (Turpie, 2004; ABI, 2008) such as Mediterranean pine and Australian *acacia* plant species (Mustard et al., 1997). These species have changed the soil pH and nutrient levels (ABI, 2008). Soil types have also become affected by layers of pine needles and leaf mulch, which prevent the germination of natural vegetation species (UNDP, 2003). The removal of alien species has always been problematic and costly (Coetzee and Littlejohn, 1994; De Villiers, 2002). For sustainable conservation of endangered plant species, measures to eradicate alien invasive species are important in reclaiming natural habitats.

The Agulhas Plain is largely affected by seasonal wild veld fires during the dry summer months (Seydack et al., 2007). Unnecessary and irresponsible burning of the natural habitat is a further threat which reduces the biodiversity. Poor fire management control systems (ABI, 2008) along with irresponsible burning in the habitat are dually responsible for the loss of many plant populations. Burnt landscapes can take years to recover and result in a loss of revenue from tourist's visits to the area. This occurrence was seen during the 2006 fires on the Agulhas plain which destroyed ecotourist establishments and in excess of 40 thousand hectares of land. The result of this fire left many local people without employment for two to three years.

Such occurrences of unplanned burning can have a large negative impact on post-fire regeneration and cause the further extinction of species (Cowling and Richardson, 1995; Coetzee and Littlejohn, 1994). The impact of fire on the biodiversity of species is complex. For instance, fire can reduce the seed production output of species through damage caused by the heat of the burning plant material but also assist in germination of seed (Brown and Duncan, 2006).

On the Agulhas plain the existence of sensitive and threatened plant populations and their impeding environmental destruction has not been sufficiently publicised or brought to the attention of the landowners. The question should be asked whether threatened plant populations should not be made more visible by being marked by conservation authorities in their habitat, so service providers can be made aware of their locations? This suggestion would require concerted effort and co-operation between conservation authorities and municipal / provincial administration. The marking of threatened populations can also benefit tourists visiting the area. Ecotourism could provide nature lovers with the challenge to follow marked red data species trails to explore discover and enjoy these remarkable plant species in their natural habitat. To date there have been few studies on the causes of destruction of red data species on the Agulhas plain. More studies targeting these aspects are recommended.

Strategic propagation of threatened red data species

Plant species in nature will continue to survive provided conservation and horticultural efforts are made to maintain them (Jeffery, 2003). Informal interviews with farmers indicated that a lack of propagation techniques for threatened species exists. Many farmers practice basic propagation skills and some have developed and practise their own methods on an ad hoc basis. Many commercial growers are often reluctant to share techniques due to commercial competitiveness as their propagation information is not shared with other growers who may require the information (Laubscher, 1999).

On the Agulhas plain, propagation of red data species is mainly done by seeds. In this method, seed propagation can be natural whereby plants regenerate themselves in the natural habitat (Hartmann et al., 2002). Alternatively, seeds can also be used by flower producers to establish cut flower fields in their natural habitat (Robyn and Littlejohn, 2002). Unfortunately many seeds sown that have been introduced from other areas to an existing habitat have had a major impact on that environment. These foreign plant species, with new genes can change the biodiversity of an existing habitat irreversibly (Littlejohn, 2002) through genetic changes within species of that habitat.

Vegetative propagation techniques on the other hand would ensure faster rooting of higher quality plant products (Robyn and Littlejohn, 2002). Such plants also flower a year earlier than seed grown plants (Reinten et al., 2002) and have the potential to maintain the unique characteristics of the species (Brown and Duncan, 2006) and flower at predictable times (Reinten et al., 2002). An increase in the propagation of threatened species throug through vegetative means could benefit ecotourism where new plants could be replanted in the natural habitat where species are threatened, especially after fires (Robyn and Littlejohn, 2002). In addition, plants can also be grown for commercial plantations or for selling to other growers and farmers. The demand for Proteaceae such as *L. laxum* has increased to more than 23673 flower stems per annum (Middlemann, 2004). This growing market has created a further need for the improvement of propagation and cultivation techniques. Advanced techniques could also increase royalties and the sale of patented cultivars for future export purposes (Robyn and Littlejohn, 2002). Literature on propagation techniques of many species remains limited (Laubscher, 1999). Studies on the vegetative propagation of red data species are important to identify and expand the vegetative propagation possibilities of species such as *L. laxum* which has not been previously documented.

Auxin treatments in vegetative propagation of difficult to root red data species

Proteaceae species are generally propagated vegetatively from 150 mm stem cuttings (SAFEC, 2002; Reinten et al., 2002). Cuttings should be taken during dry weather from semi-hardwood stems after shoot elongation (Aug-Nov), rinsed with a fungicide before planting and sprayed weekly with a fungicide (Ofori et al., 1996; Newton et al., 1992; Reinten et al., 2002; SAFEC, 2002). Limited studies have been found on document that influences the different concentrations of auxin on rooting Proteaceae (Perez-Frances, 1995). Most members of this family are categorised as difficult to root plants and may require special auxin treatments and special environmental conditions to facilitate their propagation (Hartmann et al., 2002; Wu et al., 2007a). Auxins have proved to stimulate root and shoot growth in some difficult to root plant species (Baraldi et al., 1993; Wu and du Toit, 2004; Wu et al., 2007a, b).

Auxins play an important role in speeding up the percentage of uniform rooting and in increasing rooting percentages of cuttings (Dodd and Bell, 1993; Hartmann et al., 2002; Fogaça and Fett-Neto, 2005). It is uncertain what specific concentrations of Indole-3-butyric acid (IBA) or Indole-3-acetic acid (IAA) would be successful in rooting some Proteaceae species. An increase in root numbers using IBA has been reported in plants such as Olive, *Dorycnium* spp, *Shorea leprosula*, *Leucospermum patersonii,* and *Protea obtusifolia* (Wiesman and Lavee, 1995; Rodríguez Pérez, 1992; Aminah et al., 1995; Alegre et al., 1998). The auxin accumulation in shoots has been successful in vegetative propagation, however IBA applications could also inhibit rooting, or act together with other compounds to control plant functions such as root formation (Jones and Hatfield, 1976; Zimmerman, 1984; Volper et al., 1995; Hartmann et al., 2002; Reinten et al., 2002; Rout, 2006). Higher levels of auxin than those found in plant cells could result in cell death (Hartmann and Kester, 1983; Hartmann et al., 2002). IBA is one form of auxin that is effective in the rooting of a large number of plant species (Hartmann et al., 2002). In some woody species aryl esters and aryl amid of IBA are similar or more effective than acid formulations in root initiation (Hartmann et al., 2002).

Auxins such as IAA, supplied exogenously to cuttings, have been shown to promote rooting through meristematic cell division in many species (Liu et al., 1996; Liu et al., 1998; De Klerk et al., 1997; Robyn and Littlejohn, 2002; Hartmann et al., 2002; Rout, 2006). Natural occurring IAA in plants may not be adequate to enhance auxin functions (Hartmann et al., 2002:295). Applications of IAA may induce economically viable results in Proteaceae by increasing rooting percentages. The aryl esters of IAA are similar or more effective than acid formulations in root initiation. Several studies have reported correlations between higher IAA concentrations in plant tissues and rooting in different plant species (Hartmann et al., 2002). From the above background, it is clear that the need exists to test auxin application in propagating red data species by establishing the optimum level(s) of IAA necessary to stimulate root development. Information from such studies is likely to provide suitable auxin applications for individual plant species which are necessary to stimulate rooting. These methods are necessary to help farmers and growers to successfully propagate and cultivate red data species such as *L. laxum*.

Influence of rooting mediums in vegetative propagation of difficult to root red data species

A number of studies have shown that selecting the correct rooting medium for different plant species has a profound influence on the rooting of cuttings (Leakey et al., 1990; Ofori et al., 1996; Hartmann et al., 1983). Rooting mediums for Proteaceae should be light with good drainage and also retain sufficient moisture (SAFEC, 2002). Combinations of rooting components such as bark, peat, polystyrene and river sand should be tested to ensure faster, better quality root formation (Brown and Duncan, 2006). Success in bark / polystyrene mediums has been reported in various Proteaceae species (Brown and Duncan, 2006; Reinten et al., 2002). Milled pine bark is a medium widely used and must be of good quality (Owings, 1996). However, past studies have shown that pine bark is very rich in phenolic compounds, alkaloids and cyanogenic glycosides (Machrafi and Prevost, 2006) which can also have an inhibitory effect on the growth of plants (Still et al., 1976; Rice, 1984; Siqueria et al., 1991). Good quality shredded milled pine bark is recommended for pH control (Owings, 1996) or can be substituted with a coarse grade peat moss to enhance the water holding capacity of the growing medium (Lamb, 1972; Hartmann et al., 2002; Reinten et al., 2002). Matkin (2008) also suggested using a medium of pine bark / peat as a good combination for rooting. An average pH of 6.5-7 is recommended for rooting Proteaceae (SAFEC, 2002).

Aeration and good water holding capacity are necessary characteristics for a rooting medium to positively favour the rooting physiology in different plant species (Grange and Loach, 1983; Reinten et al., 2002; Hartmann et al., 2002; SAFEC, 2002). Good aeration is essential for some Proteaceae species which are slower to root and the cuttings therefore require a longer period in the medium which must be of good quality. Polystyrene improves aeration, whereas washed river sand provides coarseness and drainage (Hartmann et al., 2002). A medium with a good air to water ratio such as bark and polystyrene is key to successful rooting (Leakey et al., 1990; Matkin, 2008). Adequate drainage should be provided to prevent *Phytophtora* fungal infection of roots (Reinten et al., 2002; SAFEC, 2002). Studies are needed to test the suitability of rooting mediums for individual red data species. Information from such studies is likely to provide improved rooting mediums which will stimulate better rooting of difficult to root red data species such as those found on the Agulhas plain.

Selection of ideal rooting environments

An ideal rooting environment is necessary to provide optimum conditions for successful rooting, thereby ensuring the maximum quantity and quality of rooted plants. Bottom heat (20-25ºC) and misting are known to enhance rooting of Proteaceae cuttings (Brown and Duncan, 2006; Reinten et al., 2002). Propagation environments for many threatened Proteaceae species such as *L. laxum* have not been well established. Documentation exists on the rooting period for Proteaceae species which varies between 8 and 12 weeks. However, some species are slower to root and would benefit from extended rooting periods in order to increase rooting percentages (Brown and Duncan, 2006; Kibbler et al., 2004). Facilities to root plants must be cost effective to make production economically viable for growers with limited greenhouse facilities (Laubscher, 1999). Atmospheric air circulation is essential in rooting Proteaceae cuttings (Reinten et al., 2002; SAFEC, 2002). No information has been found on rooting these species under shade house conditions. Cheaper built structures could prove to be more economical for farmers as the cost of construction, maintenance and electricity usage could be reduced. More efficient environmental propagation structures would in turn add value to the rapid multiplication and hence the domestication of plant species which are listed as endangered on the Agulhas plain. Ecotourism could also benefit from guided tours of the propagation facilities on farms informing tourists how these interesting plants are grown. Most farmers have no propagation structures for rooting indigenous plant species. The need exist for the investtigation of suitable structures which will guarantee success in propagation of red data species. Information from such studies is likely to provide guidance on cost effecttive and simpler propagation structures compared with expensive propagation greenhouses.

Conservation strategies: Involvement of all stakeholders

The lack of focus on priorities and the failure to reduce the ongoing degradation of the biodiversity on the Agulhas plain remain causes for concern (Rebelo, 1997; Rouget et al., 2003). The success and development of ecotourism on the Agulhas plain should be based on well planned conservation strategies as this region is recognized as having a biodiversity that has globally significant and irreplaceable vulnerable plant species (UNDP, 2003). Any viable and successful conservation strategies should involve all stakeholders dealing with ecotourism and biodiversity on the Agulhas Plain. Many institutions, including conservation, tourism and government departments have become directly involved in the biodiversity of the CFK (Younge and Fowkes, 2003). For instance, strategies for the Cape Action Plan for the Environment (C.A.P.E.) were formulated to inform landowners of their responsibilities in managing the biodiversity of the region (Lochner et al., 2003). These include the establishment of private and public conservancies (Cooper, 2003). Further efforts to control flower harvesting are supported by Sappex, who encourages the European good agricultural practice (EuroGAP) code of practice with sustainable harvesting (Patterson, 2005). The Flower Valley Conservation Trust (FVCT) is a good example of practicing sustainable harvesting (Privet, 2002; ABI, 2008; Flower Valley, 2008). The Western Cape Nature Conservation Board (WCNCB) should play a major role in conserving the biodiversity and ecotourism on the Agulhas plain, yet informal interviews with farmers for this study revealed a negative attitude towards the WCNCB because of poor management. The WCNCB lacks the capacity to police and control flower harvesting and to protect endangered species (ABI, 2008).

Effective conservation strategies involving all stakeholders are likely to reduce illegal harvesting and lessen the depletion of vulnerable red data species in the wild. Other conservation efforts include the purchasing of land for conservation, the control of alien invasive species and private and government land owners recognizing the commercial potential for ecotourism (Paterson-Jones, 2000). Exploitation of the environment could be minimized if the successful development of ecotourism potential is regulated by the conservation of the natural habitat on the Agulhas plain by all stakeholders.

Conclusion

This review showed that threatened Proteaceae species such as *L. laxum* are continually being destroyed in the wild. The various factors responsible for the loss of important species which may attract tourists on the Agulhas plain remain uncertain. Further studies are necessary to investigate the management practices of landowners, such as wild flower harvesting, orchard plantings and the control of fire and alien plants to conserve the endangered plant species on the Agulhas plain. In the literature, there is little information on vegetative propagation of red data species. Developing improved propagation techniques of Proteaceae species such as the use of auxin applications, optimum rooting environment and mediums could help advance the growing and replacement of threatened species. Further studies should aim at developing new propagation techniques to advance the replanting of threatened species in the wild.

REFERENCES

ABI (2008). Agulhas Biodiversity Initiative research report. http://www.flowervalley.org.za/abi.html [5 November 2008].

Alegre J, Toledo JL, Martinez A, Mora O, De Andres EF (1998). Rooting ability of *Dorycnium* spp. Sci. Hort. 76: 123-129.

Aminah H, Dick JMc.P, Leakey RRB, Grace J, Smith RI (1995). Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. For. Ecol. Manag. 72: 199-206.

Baraldi R, Bertazza G, Predierii S, Bregoli A, Cohen JD (1993). Uptake and metabolism of Indole-3-butyric acid during the in vitro rooting phase in pear cultivar (*Pyrus comunis*). Acta. Hort. 329: 289-291.

Barzilay A, Ben-Jaacov J, Cohen A, Ion A, Halevy H (1992). Mini-gladiolus as a flowering pot plant. Sci. Hort. 49:117-124.

Berney J (2000). Combined research to benefit Protea growers. Aust. Hort. 98(2).

Brown N, Duncan G (2006). Manual for growing Proteaceae in greenhouses Joseph Knight in 1809 www.protea.worldonline.co.za/growknight.htm: 14.

Coetzee JH, Littlejohn G (1994). Indigenous South African Flower Industry. Hortagrow (8).

Cooper KH (2003). A future for SA environmental conservation. Park and Grounds. May 2003 pp. 50-52.

Cowling R (1993). Ecotourism - What is it and what can it mean for conservation. Veld and Flora 79(1): 3-5.

Cowling R, Richardson D (1995). Fynbos. South Africa's Unique Floral Kingdom. Fernwood Press, Cape Town.

De Klerk G, Ter Brugge J, Marinova S. (1997). Effectiveness of IAA, IBA and NAA during adventitious roots, new concepts, new possibilities. *In vitro* Cell. Dev. Biol. 35:188-189.

De Villiers T (2002). First Words: A farmer's viewpoint. Veld and Flora, 88(2): 42.

Dodd J, Bell DT (1993). Water relations of the canopy species in a Banksia woodland. Swan Coastal Plain, Western Australia. Aust. J. of Bot. 18:281-293.

Flower Valley (2008). Annual Report.

Fogaça CM, Fett-Neto AG (2005). Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrance. Plant Growth Regul. 45: 1-10.

Grange RI, Loach K (1983). The water economy of un-rooted leafy cuttings. J. Hort. Sci. 58 (10): 1-17.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant Propagation Principles and Practices. 7th Edition. Prentice Hall. New Jersey. pp. 367-374.

Hartmann HT, Kester DE. (1983). Plant propagation; principals and Practices. Prentice-Hall International, London.

Hilton-Taylor C (1996). Red Data List of Southern African Plants. Strelitzia 4. National Botanical Institute, Pretoria.

http://www.gefweb.org/Documents/Project_Proposal_for_Endorsm/South_Africa Agulhas Bio Initiative ABI.

http://www.givengain.com/cause_data/images/1866/FlowerValleyReport2008.pdf [9 February 2009].

Hughes C (2002). The potential impact of viticulture expansion on biodiversity in the Cape Floristic Region. MSc Thesis, University of Cape Town, Cape Town.

Jamieson HG (2001). *Leucadendron laxum* I. Williams. Kirstenbosch Botanic Garden, Cape Town, South Africa.

Jeffery R (2003). The challenges for the defence force. Equus 9(1): 8-9.

Jones OP, Hatfield SGS (1976). Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds. J. Hort. Sci. 51: 495-499.

Kibbler H, Johnston ME, Williams RR (2004). Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell: 2 Seasonal influences of temperature, rainfall, flowering and auxins on the the stock plant. Sci. Hort. 102: 343-358.

Lamb, JGD (1972). The use of Peat in Propagating Hardy Nursery Stock at Kinsealy. Acta Hort. 26: 125-128.

Laubscher CP (1999). Rooting techniques for selected tree species. M Thesis. Unpublished. Cape Technikon, Cape Town.

Leakey RRB, Mesén JF, Tchoundjeu Z, Longman KA, Dick, JMcP, Newton A, Martin A, Grace J, Monro RC, Muthoka PN (1990). Low technology techniques for the vegetative propagation of tropical trees. Commonwealth For. Rev. 69: 247-257.

Littlejohn G (2002). Legal Issues, Planning and Planting. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Agricultural Research Council, Elsenburg, South Africa. pp. 82-90.

Liu ZH, Hsiao IC, Pan YW (1996). Effect of naphthaleneacetic acid on endogenous indole-3-acetic acid, peroxidase and auxin oxidase in hypocotyls cuttings of soybean during root formation. Bot. Bull. Aca. Sin. 37: 247-257.

Liu ZH, Wang WC, Yen YS (1998). Effect of hormone treatment on root formation and endogenous indole-3-acetic acid and polyamine levels of *Glycine max* cultivated *in vitro*. Bot. Bull. Aca. Sin. 39:113-118.

Lochner P, Weaver A, Gerderblom C, Peart R, Sandwith T, Fowkes S (2003). Aligning the diverse: The development of a biodiversity conservation strategy for the Cape Floristic Region. Biol. Con. 112: 29-43.

Lombard A, Cowling RM, Pressey RL, Mustard PJ (1997). Reserve design on the Agulhas Plain, South Africa: a flexible tool for conservation in a species-rich and fragmented landscape. Cons. Biol. 11: 1101-1116.

Machrafi Y, Prevost D (2006). Toxicity of phenolic compounds extracted from bark residues of different ages. J. Chem. Ecol. 32: 2595-2615.

Matkin OA (2008). Perlite Vs. Polystyrene In Potting Mixes. The Schundler Company 150 Edison, NJ 08817 http://www.schundler.com/polystyrene.htm.

McVeigh S (1998). Fabulous fynbos a favourite for the small-scale farmer. Farmers Weekly 4: 30-32.

Middlemann M (2004). Fynbos industry statistics - Statistics appendix. Sappex News, p. 120.

Milandri S, Laubscher CP, Ndakidemi P (2008). Hydroponic culture of *Gladiolus tristis*: Application of paclobutrazol for flowering and height control. Afr. J. Biotechnol. 7(3): 239-243.

Moody H (1995). Versatile Leucadendrons and Leucospermums. Aust. Hort. 5: 111-112.

Mustard P, Cowling R, Albertyn J (1997). Southern Overberg. South African Wild Flower Guide 8. National Botanical Institute. National Book Printers. South Africa.

Newton AS, Mesén JF, Dick JMcP, Leakey RRB (1992). Low-technology propagation of tropical trees: rooting physiology and its practical implications. In: Mass production Technology for genetically Improved Fast Growing Forest Tree Species, Vol. 2 AFOCEL, Nangis, pp: 417-324.

Ofori DA, Newton AC, Leakey RRB, Grace J (1996). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentrations, leaf areas and rooting medium. For. Ecol. Manage. 84: 39-48.

Owings AD (1996). Variations in pH from different bark sources. Combined Proceedings International Plant Propagators' Society 46: 699.

Paterson-Jones C (2000). The Protea family of Southern Africa. Struik Publishers. Cape Town.

Patterson T (2005). Sappex develops a code of practice. Talking Flowers Africa. J. for Floric. SA. July 2005.

Perez-Frances JF, Exposito A, Rodriguez JA (1995). Effect of different factors on in vitro multiplication of *Leucadendron* 'Safari Sunset' (Proteaceae). Acta Hort. 387: 115-120.
Privet S (2002). Putting fynbos to work on the Agulhas Plain. Veld & Flora 88(2): 50-51.
Protea A (2008). Current Rarity Status of the Proteaceae 1990. http://protea.worldonline.co.za/redstatus2.htm.
Rabie M (2002). Export statistics for season June 2001 to July 2002. (sappex@hermanus.co.za).
Rabie M (2003). Research/ Navorsing – "Think Tank / Dinkskrum". (sappex@hermanus.co.za).
Rebelo AG (1995). *Proteas.* A Field Guide to the Proteas of Southern Africa. Cape Town: Fernwood Press.
Rebelo AG (1997). Conservation. In Cowling, R.M. Richardson, D.M. & Pierce SM (eds). Vegetation of Southern Africa. Cambridge: Cambridge University Press: pp. 571-590.
Reinten E, Gertze S, Arends L (2002). Propagation methods. Cultivating fynbos for quality cut flower production. Unpublished ARC training course manual. Elsenburg, South Africa pp. 50-70.
Rice EL (1984). Allelopathy. Academic Press, Orlando, pp. 272-274.
Robyn A, Littlejohn G (2002). Proteas and Cape Greens: products marketed and production methods. Cultivating fynbos for quality cut flower production, pp. 22-48.
Rodríguez Pérez JA (1992). Propagation by leaf bud cuttings of *Leucadendron* 'Safari Sunset', *Leucospermum cordifolium*, *Leucospermum patersonii* and *Protea obtusifolia*. Acta. Hort. 316: 35-46.
Rouget M, Richardson DM, Cowling RM, Lloyd JW, Lombard AT (2003). Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region. Biol. Cons. 112: 63-95.
Rout GR (2006). Effect of auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. Plant Growth Regul. 48: 111-117.
SAFEC (2002). South African Flower Export Council: Preparing orchards for planting of PROTEACEAE. Certificate course in flower export readiness. Unpublished booklet. South Africa.
Seydack AHW, Bekker SJ, Marshall AH (2007). Shrubland fire regime scenarios in the Swartberg Mountain Range, South Africa: implications for fire management. Int. J. Wildland Fire 16: 81-95.
Still SM, Dirr MA, Gartner, JB (1976). Phytotoxic effects of several bark extracts on mung bean and cucumber growth. J. Am. Soc. Hort. Sci. 101: 34-37.
Turpie J (2004). The role of resource economics in the control of invasive alien plants in South Africa. SA. J. Sci. 100: 87-93.
UNDP (2003). United Nations Development Programme. Agulhas Biodiversity Initiative. Global Environmental Facility Project document: Cape Action Plan for the Environment.
Volper R, Osswald M, Elstner EF (1995). Effects of cinnamic acid derivatives on indole acetic acid oxidation by peroxidase. Phytochem. 38: 1-22.
Wiesman Z, Lavee S (1995). Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. Sci. Hort. 62: 189-198.
Wu HC, du Toit ES (2004). Reducing oxidative browning during in vitro establishment of *Protea cynaroides*. Scientia Hort. 100: 355-358.
Wu HC, du Toit ES, Reinhardt CF, Rimando AM, van der Kooy F, Meyer JJM (2007b). The phenolic, 3,4-dihydroxybenzoic acid, is an endogenous regulator of rooting Protea cynaroides. Plant Growth Regul. 52: 207-215.
Wu HC, du Toit ES, Reinhardt CF. (2007a). Micrografting of *Protea cynaroides***.** Plant Cell Tiss. Cult. 89: 23-28.
Younge A, Fowkes S (2003). The Cape Action Plan for the Environment: overview of an eco-regional planning process. Biol. Cons. 112: 15-28.