Propagation of Red Data Proteaceae Species of the Agulhas Plain©

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Propagation of Red Data Proteaceae on the Agulhas Plain remains limited. This study highlighted the threats of four *Leucadendron* species. Further emphasis is placed on the propagation of *Leucadendron laxum* which is harvested as a indigenous cut flower. The application of rooting hormone indoleacetic acid (IAA) in various rooting mediums in *L. laxum* was tested under greenhouse conditions and under shade house conditions. The treatments included: control, 500, 1000, 2000, and 4000 ppm, and four rooting mediums: (a) bark and polystyrene; (b) peat moss and polystyrene; (c) bark, river sand, and polystyrene; and (d) perlite and river sand. A randomised block design with three replicates was used. Compared with other media, 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. Evidence from this study was conclusive that the shade tunnel was more successful in rooting *L. laxum* than the environmentally controlled greenhouse environment.

INTRODUCTION

The Cape Floral Region (CFR) consists of 330 species of Proteaceae of which some are near extinction (Rebelo, 1995). The Agulhas Plain has the highest priority for conservation and the largest number of lowland threatened species in South Africa [United Nations Development Programme (UNDP), 2003]. The important vegetation type Elim Asteraceous Fynbos is highly threatened where many endemic Red Data Proteaceae species such as *Leucadendron elimense*, *L. laxum*, and *L. stelligerum* occur (UNDP, 2003; Mustard et al., 1997; Hilton-Taylor, 1996). *Leucadendron elimense* and *L. platyspermum* are both classified as vulnerable species (Protea Atlas, 2008). Two other endangered species *L. stelligerum* found in only three populations totalling 1000 plants and only 5,000 *L. laxum* plants remain in their natural habitat (Rebelo, 1995; Mustard et al., 1997). Most leucadendrons are considered speciality flowers due to their unique and naturally bright colours. These bright yellow flower colours and attractive cones are favoured for export market which have increased by 27% in the European winter period (Dodd and Bell, 1993; Coetzee and Littlejohn, 1994; Moody, 1995; Paterson-Jones, 2000; Berney, 2000; Jamieson, 2001; Rabie, 2002; Rabie, 2003).

Leucadendron laxum has the potential as a flowering pot plant as 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). *Leucadendron laxum,* is however a far more important component of the indigenous export cut flower industry (Dodd and Bell, 1993; Coetzee and Littlejohn, 1994; Moody, 1995; Paterson-Jones, 2000; Berney, 2000). Over-harvesting of species such as *L. laxum* from 436 ha of fynbos habitat has caused this species to be listed with Red Data status (Hilton-Taylor, 1996; Mustard et al., 1997; Robyn and Littlejohn, 2002). Sustainable cultivation of these plants should be encouraged as more than 80% are field harvested from low-income farm land where many habitats are destroyed (Dodd and Bell, 1993; Mustard et al., 1997; McVeigh, 1998; Paterson-Jones, 2000; Robyn and Littlejohn, 2002; Agulhas Biodiversity Initiative, 2008). More than 22% of the Agulhas Plain fertile soils are planted with cereals, vineyards, pastures, and cultivated flowers (Lombard et al., 1997; UNDP, 2003; Hughes, 2002). One of the largest threats for *L. laxum* wetland habitat is the expanding farm land especially for livestock (Mustard et al., 1997; Agulhas Biodiversity Initiative, 2008). The spread of alien invasive species has 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; Agulhas Biodiversity Initiative, 2008; Mustard et al., 1997). Seasonal wild veld fires during the dry summer months and irresponsible burning of the natural habitat remains a further threat to the loss in biodiversity of this area (Seydack et al., 2007). There have been few studies on the causes of destruction of Red Data species on the Agulhas Plain (Laubscher et al., 2009). As this area has the potential to become a major tourist destination (Cowling, 1993). More studies targeting these aspects are necessary to protect and propagate these threatened species as this region has the most distinctive, most diverse, and the highest density of endemic flora in the world (Jamieson, 2001; Robyn and Littlejohn, 2002; Cooper, 2003; Coetzee and Littlejohn, 1994).

The lack of studies on the vegetative propagation of threatened Proteaceae species such as *L. laxum* should increase the awareness and facilitate propagation and rehabilitation of species in their natural habitat to reduce the threat on the biodiversity (Wu et al., 2007a). Informal interviews with farmers indicated that a lack of propagation techniques for threatened species exists as only basic propagation skills are practiced (Laubscher et al., 2009). Certain plant species in the Proteaceae family remain difficult to root (Brown and Duncan, 2006), and some such as *L. elimense* subsp. *elimense,* do not propagate by vegetative means (Tansley and Brown, 2000). Vegetative propagation retain plant characteristics and is a faster technique to ensure high quality rooted plants which will flower a year earlier than seed-grown plants (Robyn and Littlejohn, 2002; Reinten et al., 2002; Brown and Duncan, 2006). Vegetative propagation of this species could also allow for replanting of threatened species after fires or grown for commercial cut flower plantations to supply the increase in demand for cut flower stems (Robyn and Littlejohn, 2002; Middlemann, 2004). Vegetative stem cuttings 150 mm in length for Proteaceae (South African Flower Export Council, 2002; Reinten et al., 2002) should be taken from semi-hardwood after shoot elongation (Aug-Nov) and rinsed and sprayed weekly with a fungicide (Ofori et al., 1996; Newton et al., 1992; Reinten et al., 2002; South African Flower Export Council, 2002).

Auxins have an important role in speeding up uniform rooting and increasing rooting percentages of cuttings (Hartmann et al., 2002; Fogaça and Fett-Neto, 2005). Some Proteaceae species are problematic to root, while others have shown positive responses to indole-3-butyric acid (IBA) auxin treatments (Hartmann et al., 2002; Laubscher and Ndakidemi, 2008a; 2008b). 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). Limited studies have been found which document the influence of different concentrations of auxin on rooting Proteaceae (Perez-Frances et al., 1995) especially the use of indole-3-acetic acid (IAA) auxin on the rooting of this plant family (Laubscher and Ndakidemi, 2008c). Indole-3-acetic acid that occurs naturally in the plants may not be adequate in enhancing auxin functions during rooting (Hartmann et al., 2002; Wu et al., 2007a). Indole-3-acetic acid, supplied to cuttings, has 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). 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 (Laubscher et al., 2009). Applications of IAA may induce economically viable results in Proteaceae by increasing rooting percentages. Information from such studies is likely to provide suitable auxin concentrations to help farmers and growers to successfully propagate and cultivate Red Data species such as *L. laxum*.

A number of studies have shown that the selection and combination of rooting medium components are important in the rooting success of plants (Laubscher, 1999; Leakey et al., 1990; Ofori et al., 1996; Hartmann et al., 1983). Rooting media for Proteaceae should be light with good drainage and also retain sufficient moisture (South African Flower Export Council, 2002). Adequate aeration and drainage 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 (Owings, 1996) and 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). A coarse grade peat moss will enhance the water-holding capacity of the medium (Hartmann et al., 2002). Polystyrene will improve aeration and coarse grade, washed river sand will improve the water drainage (Matkin, 2008). Testing the combinations of rooting components is necessary to ensure faster, improved root development (Brown and Duncan, 2006). Success in bark / polystyrene mediums has been reported in various Proteaceae species (Brown and Duncan, 2006; Reinten et al., 2002). 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). 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 (South African Flower Export Council, 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. 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; South African Flower Export Council, 2002). 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 *Phytophthora* fungal infection of roots (Reinten et al., 2002; South African Flower Export Council, 2002). Studies are needed to test the suitability of rooting mediums for Red Data species such as *L. laxum*.

Rooting environments for successful rooting should ensure 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 have not been well established although atmospheric air circulation has been identified as essential in rooting Proteaceae cuttings (Reinten et al., 2002; South African Flower Export Council, 2002). Apart from studies by Laubscher and Ndakidemi (2008c), propagation under shade-house conditions is not well documented. These cheaper structures could prove to be more economical for farmers in saving construction costs, maintenance, and electricity usage. Propagation structures for rooting indigenous plant species remains limited on the Agulhas Plain (Laubscher et al., 2009). The need exists for investigations suitable structures which will guarantee success in propagation of Red Data species such as *L. laxum* (Laubscher and Ndakidemi, 2008b; 2008c).

The aim of this study was to determine the rooting success of *L. laxum* in using different concentrations of IAA rooting hormones, different rooting mediums under controlled greenhouse environment with bottom heating, and under shade house conditions with no bottom heat.

MATERIALS AND METHODS

Experimental Setup and Design. The trials were conducted at the nursery of the Cape Peninsula University of Technology in Cape Town, South Africa, in 2006. Four growth mediums: (a) bark and polystyrene (1 : 1, v/v); (b) peat moss and polystyrene (1 : 1, v/v); (c) bark, river sand, and polystyrene (1 : 1 : 1, by vol.); and (d) perlite and river sand (1 : 1, v/v) were used. Four concentrations of IAA (0, 500, 1000, 2000, and 4000) were set up in a randomised complete block design with three replications each. The experiment was conducted in a environmentally controlled greenhouse covered in clear polycarbonate fitted with a shade screen (40%) with heated beds (20–25 °C) and an uncontrolled 40% white shade cloth tunnel structure (Brown and Duncan, 2006; Laubscher and Ndakidemi, 2008a; 2008b; 2008c).

Cutting material of *L. laxum* was collected from selected plant populations and rooted in plug trays. Terminal cuttings were rinsed in Benlate fungicide (10 $g{\cdot}L^{-1}$) before planting and sprayed weekly with a Captan (2 $g{\cdot}L^{-1}$) to prevent disease infection (Reinten et al., 2002; South African Flower Export Council, 2002). The IAA solution was prepared as described by Brown and Duncan (2006) and dissolved in 50% ethyl alcohol and made up in the appropriate concentration by adding distilled water. The basal end of cuttings were dipped for 5 sec in the IAA rooting hormone and planted afterwards (Reinten et al., 2002; Hartmann et al., 2002).

Data Collection and Analysis. Cuttings were evaluated on a weekly basis for a period of 8 weeks to monitor results of the IAA hormone treatment and the influence of the rooting medium on the rooting progress of *L. laxum*. The percentage of callused cuttings, rooted cuttings, and survival rates were recorded. Quality of growth was assessed on the numbers of roots, their length and the length of the shoots obtained. The 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 media 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 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of Different Growth Media on Callus Formation, Rooting, and Survival of *Leucadendron laxum* cuttings. The responses from cuttings being tested in various rooting media were positive. Overall the bark and polystyrene medium resulted in the highest callusing, rooting, and survival percentage (Table 1). The results suggest that the medium was highly suitable for the propagation of *L. laxum* owing to the moisture-holding and aerated properties, and hence suitable for the propagation of other Proteaceae species. A medium with good air : water ratio such as the bark / polystyrene medium is a key to successful rooting (Leakey et al., 1990).

Statistically the shade-house environment was the most successful in callus formation compared with the trials in the greenhouse. These results were followed by rooting and survival being both significant in the greenhouse and shade-house environments (Table 1). The lowest response in the perlite / river sand medium could be contributed to the possibility of a coarser aggregate with faster draining potential. The results indicated that higher survival and callusing occurred in both environments compared with the rooted cuttings. The rooting period of 8 weeks was too short and could be extended to increase survival and rooting percentages as some species that are slower to root (Brown and Duncan, 2006; Kibbler et al., 2004). Extending the rooting period should be considered in future studies for higher rooting percentages in *L. laxum*. Rooting facilities must be cost effective to make production economically viable for growers (Laubscher, 1999).

Effect of Different Growth Media on Shoot Growth, Root Length, and Number of Roots per Cutting of *Leucadendron laxum* Cuttings. Under shade-house conditions the shoot growth *L. laxum* was the most significant in the bark, river sand, and polystyrene medium. These results in the shoot growth were followed in the greenhouse in the bark and polystyrene medium. Root length in the shade house was significant with the longest root length (3 mm) in the peat and polystyrene medium compare to the greenhouse condition which were not significant (Table 2). The number of roots per cutting was significantly recorded in the greenhouse experiment compared to the shade-house experiment. Growth media may have profound influences on the rooting of cuttings in different plant species (Leakey et al., 1990; Ofori et al., 1996). From all the experiments in this study, the bark and polystyrene medium was significantly superior to all other media (Table 2). The overall results in this rooting medium may be attributed to good aeration characteristics and good water-holding capacity as these two characteristics are documented to positively favour the rooting physiology in different plant species (Grange and Loach, 1983; Newton et al., 1992). The addition of river sand to this medium is not in agreement with the bark / river sand / polystyrene medium recommended as suitable rooting medium for Proteaceae (Brown and Duncan, 2006; Reinten et al., 2002).

Table 1. Effect of different growth mediums on percentage callus formed, percentage of cuttings that rooted, and percentage survival rates of cuttings of *Leucadendron laxum*.

Treatment	Callused (%)		Rooted (%)		Survival (%)	
Main treatment	Green house	Shade house	Green house	Shade house	Green house	Shade house
Bark polystyrene	60	42	40	29	88	81
Peat polystyrene	35	31	10	31	66	79
Bark river sand poly	35	14	10	8	65	28
Perlite river sand	4.0	8	0.0	4	27	29
F Statistic	12.4***	5.3**	7.9***	18.2***	6.53***	37.4***

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.

Table 2. Effect of different growth mediums on shoot growth, root length and the number of roots per cutting of *Leucadendron laxum*.

Treatment	Shoot Growth		Root Length		Number of roots/cutting	
Main treatment	Green house	Shade house	Green house	Shade house	Green house	Shade house
Bark polystyrene	3.9	0.40	1.0	2.87	1.6	4.40
Peat polystyrene	1.1	0.53	4.1	3.00	0.7	3.73
Bark river sand poly	1.1	1.07	4.1	1.00	0.7	2.27
Perlite river sand	0.0	0.20	0.0	0.60	0.0	0.93
F Statistic	0.001**	3.53*	0.27 ns	9.22***	0.008**	6.66***

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.

The Effect of Indole-3-acetic Acid Concentrations on Callusing, Rooting, and Survival Rates of *Leucadendron laxum* Cuttings Under Greenhouse and Shade House Conditions. The results of the study showed that different IAA applications had significant results on callus formation in *L. laxum*. The highest percentage of callusing (45%) was recorded in cuttings supplied with IAA at 500 ppm (Fig. 1). These results were followed by the 2000 and 4000 ppm concentrations at 35%. Compared with the control, the 1000 ppm concentration had the lowest reading (Fig. 1). Callusing under greenhouse conditions was far more successful than in the shade house environment. The highest callusing in the shade house was measured in the 500 ppm (Fig. 1). The rooting results were slightly different.

Rooting under shade house conditions was more pronounced in the 500 ppm and 4000 ppm concentrations. The rooting percentages also showed a progressive increase from 1000–4000 ppm concentrations (Fig. 1). The highest rooting (25%) in the greenhouse was also recorded in the cuttings that received the 4000 ppm treatment (Fig. 1). The IAA treatments in both the greenhouse and shade house trials were high in percentages, but did not significantly affect the survival rate of the cuttings (Fig. 1). Results from the study showed that IAA supplied at different rates significantly enhanced different rooting and growth parameters of *L. laxum* (Fig. 1). Similar results were obtained in studies by Laubscher and Ndakidemi (2008b; 2008c). These results are also in agreement with other studies in which IAA stimulated rooting characteristics and growth in several plant species (Liu et al., 1996, 1998; De Klerk et al., 1997). The higher concentrations which resulted in rooting percentages indicate that IAA is limited in the plant tissues and hence its application in stimulating rooting in *L. laxum* cuttings. The study is also in agreement that IAA is a growth-promoting substance that stimulates the meristematic cell division and promotes rooting in cuttings (Rout, 2006). Several studies have reported correlations between higher IAA concentrations in plant tissues and rooting in different plant species (Hartmann et al., 2002; Baraldi et al., 1993; Dodd and Bell, 1993).

The environmentally controlled greenhouse with bottom heat and misting was conducive in enhancing rooting, growth, and survival of cuttings. The growth conditions of the environmentally controlled greenhouse are in agreement with studies in propagating other Proteaceae (Brown and Duncan, 2006; Reinten et al., 2002; Laubscher and Ndakidemi, 2008a), however this study showed that the highest rooting percentages were obtained under shade house conditions. These results are in agreement with results from other studies which documented the rooting of *L. laxum* under shade house conditions (Laubscher and Ndakidemi, 2008b; 2008c).

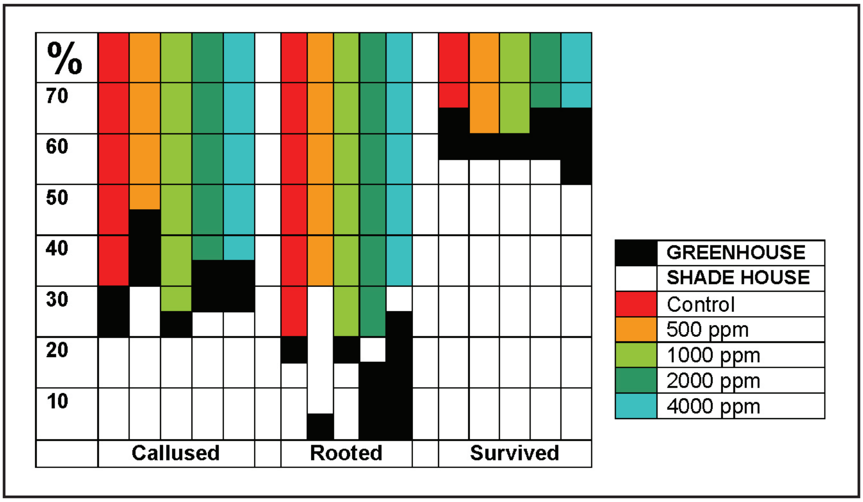

Figure 1. Growth parameters of percentage (%) callused, % rooted, and % survival rates of *Leucadendron laxum* cuttings treated with different indole-3-acetic acid hormone concentrations and rooted under greenhouse and shade house conditions.

CONCLUSION

To conclude, this study is in agreement with previous documented work that the bark and polystyrene medium contributed to the success in rooting Proteaceae. The use of IAA rooting hormone has also contributed to the root development in *L. laxum*. This study showed that Proteaceae species such as *L. laxum* remain threatened in their habitat, while little information on vegetative propagation of Red Data species of the Agulhas Plain has been documented. Developing improved propagation techniques for 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 be aimed at developing new propagation techniques using other growth hormones and environments to advance the propagation and replanting of threatened Proteaceae of the Agulhas Plain.

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