

Full Length Research Paper

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

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Accepted 15 September, 2008

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

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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 18-25°C 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 Polystyrene				Bark Polystyrene			
	% 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 $P \leq 0.05$, 0.01, ns = not significant respectively. Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$.

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 Polystyrene, and Perlite and River Sand medium.

	Bark, River Sand and Polystyrene						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 $P \leq 0.05$, 0.01, 0.001, ns = not significant respectively. Means followed by the same letter(s) are not significantly different from each other at $P \leq 0.05$.

rene (Tables 1-2). 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 capa-

ity 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 Polystyrene	22	±13.2a	24	±3.2b	69	±5.6a	3.3	±0.73b	3.9	±0.84a	5.9	±0.98a
Bark Polystyrene	29	±12.5a	37	±4.7a	77	±4.7a	1.4	±0.59b	3.8	±0.81a	5.9	±0.98a
Bark/Sand /Polystyrene	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 $P \leq 0.05$.

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 suc-

cessive 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

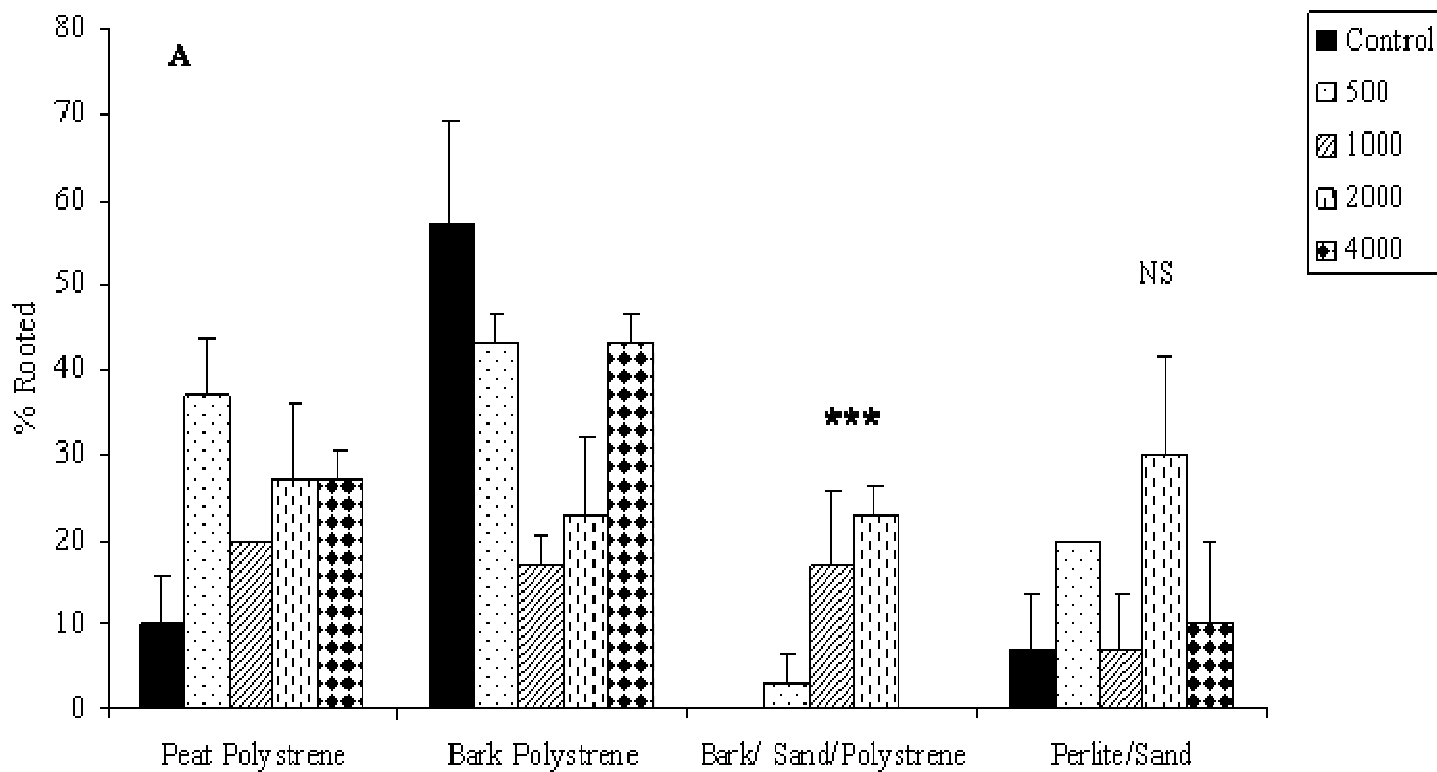


Figure 1. Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on % of rooted cuttings. ***= Significant at $P < 0.001$; NS = Not significant.

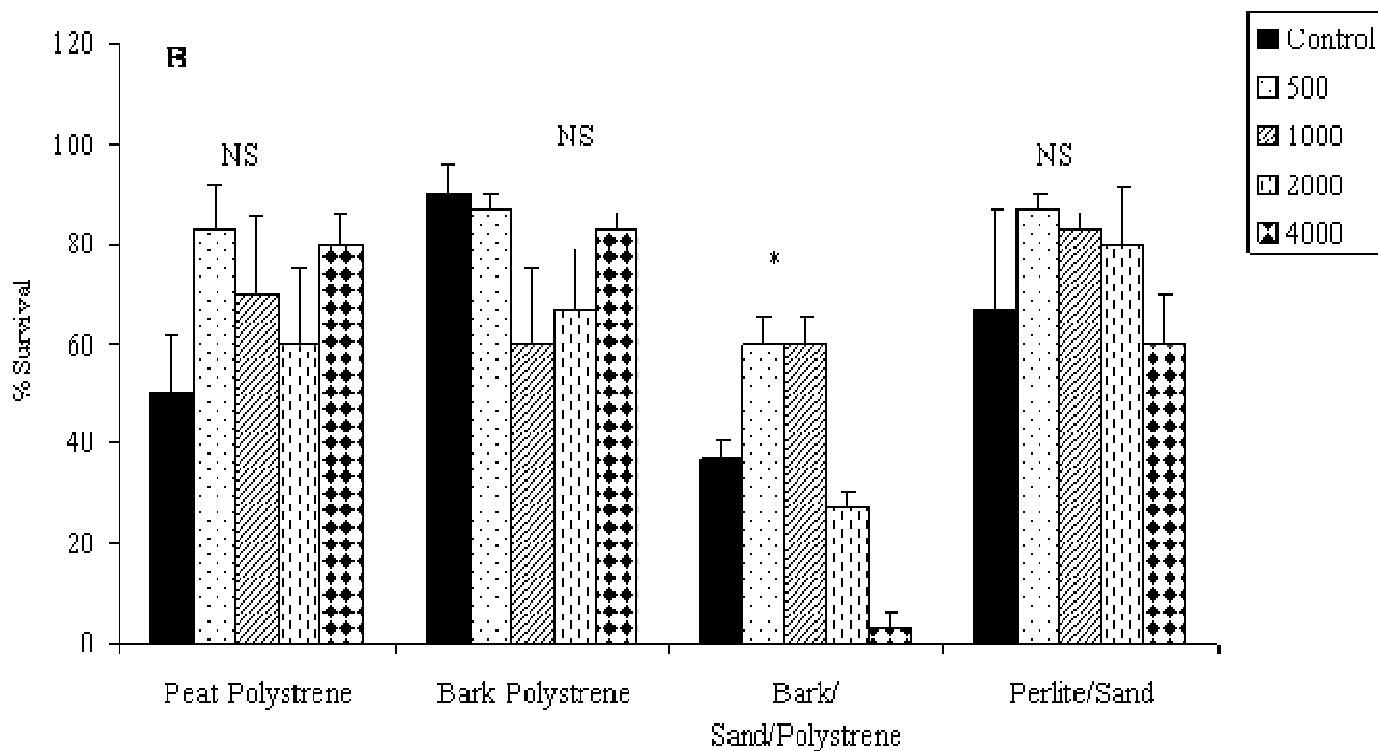


Figure 2. Interactive effects of Peat, Bark, Bark/sand Polystyrene and Perlite/sand on % survival of cuttings. * = $P \leq 0.05$; NS = Not significant.

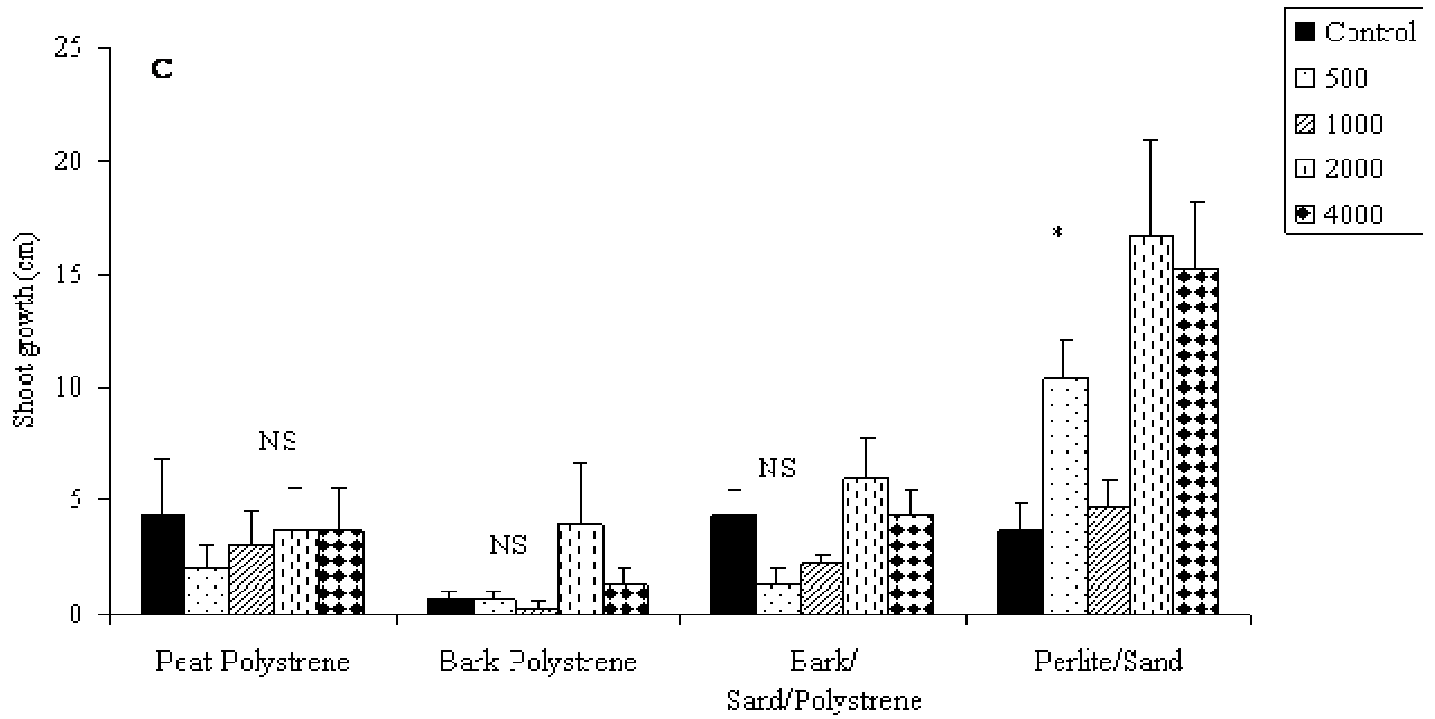


Figure 3. Interactive effects of peat, Bark, Bark/sand polystyrene and perlite/sand on shoot growth (cm). * = $P \leq 0.05$; NS = Not Significant.

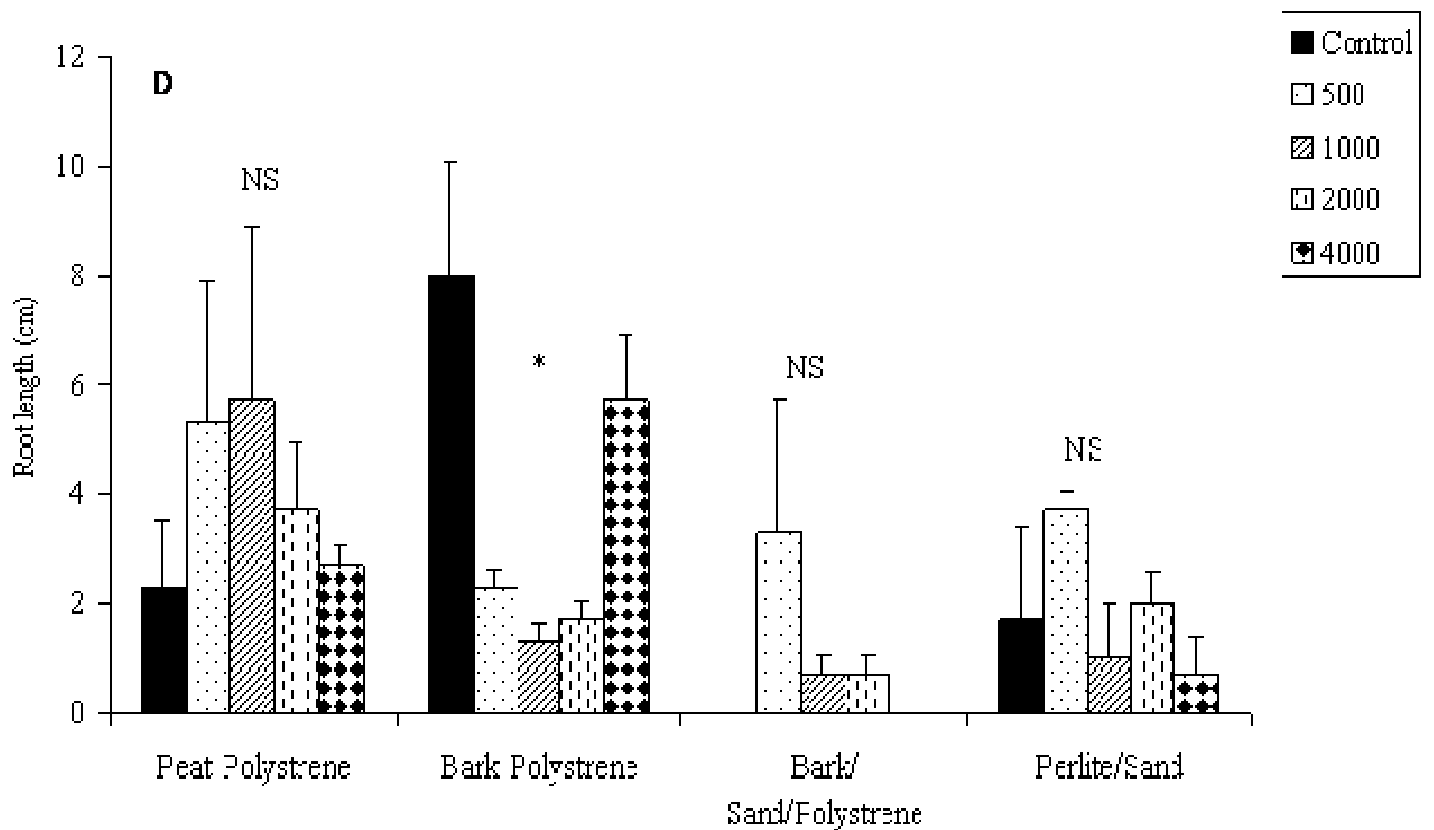


Figure 4. Interactive effects of peat, bark, bark/sand polystyrene and perlite/sand on root length (cm). * = $P \leq 0.05$; NS = Not Significant.

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 characteristics 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.

ACKNOWLEDGEMENTS

Appreciation goes to the following: Ms A Jephson for critical reading of the script. The Department of Horticultural Sciences, Cape Peninsula University of Technology for the use of the nursery facilities and providing materials for the study. Assegai Bosch Farm on the Agulhas Plain for donating *L. laxum* cutting material.

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