Effects of auxin treatment, cutting type and air and root zone temperatures on cutting propagation of eastern species of *Conospermum* and *Persoonia* with potential horticultural value

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Summary

Lack of knowledge of the propagation requirements of many Australian native plants has limited their development as cut flowers or ornamental species. The propagation requirements of *Conospermum mitchellii* were examined, focussing on the effects of auxin treatment, air and root zone temperatures and cutting type (softwood, semi-hardwood or hardwood) on the percentage rooting and death of stem cuttings. Anatomical studies were carried out to determine whether the stem anatomy of *C. mitchellii* influences rooting ability. Preliminary investigations were made of the effects of auxin treatment and air temperature on the rooting and death of *Conospermum patens* and *Persoonia pinifolia*. Indole-butyric acid (IBA) was found to be the most effective auxin in stimulating rooting of cuttings of all three species, while naphthalene acetic acid (NAA) had an adverse effect on cutting survival of the two *Conospermum* species. Softwood cuttings gave the highest rooting percentage, and the use of root zone heating was found to be beneficial for propagation of *C. mitchellii*.

Introduction

Amongst Australia's vast array of flowering plants, relatively few species are currently exploited for commercial floricultural and horticultural purposes. Waxflowers, *Banksia* and *Thryptomene* alone accounted for over 70% of Australia's cultivated area for wildflower production in 1993 (Karingal Consultants, 1994). Hundreds of species with aesthetic value and unknown economic value are not grown commercially because they are difficult to propagate, and harvesting them from naturally occurring stands is currently more financially viable than investing in research into their propagation requirements.

Wild vegetation is still a major source of Australian floricultural products. In 1993, 26.4 million stems were harvested from naturally occurring stands in Western Australia, having a 'farm gate equivalent' value of approximately A$3.7 million (Karingal Consultants, 1994). The inconsistent supply and poorer quality of bush harvested flowers and foliage are major hindrances to the international competitiveness of the Australian wildflower industry, as the demand for native Australian flowers increases. In addition, the pressure of continued harvesting on wild stands may eventually lead to the decline of some species, such as the geographically restricted *Conospermum densiflorum* (Burgmann & Hopper, 1982). Bush harvesting has been found to result in the spread of the root rot *Phytophthora cinnamomi* (Wills & Keighery, 1994), and provides opportunities for weed invasion.

The deleterious effects of bush harvesting, and doubt over its long term sustainability point to the need for species of actual or potential commercial value to be artificially propagated. Two such genera which have proven difficult to propagate are *Conospermum* and *Persoonia*.

Conospermum, or smokebush, is a predominantly Western Australian genus which contains many floriculturally important species that are in high demand both locally and overseas. The small white, cream or blue flowers are used as 'filler' flowers in floral arrangements. The supply of smokebush to the cut flower industry depends almost entirely on wild sources. Half a million stems were harvested in 1992/1993, with a 'farm gate equivalent' value of around A$26,000 (Karingal Consultants, 1994). The very small number of smokebushes in commercial cultivation reflects their recalcitrance to conventional propagation methods. *Conospermum* apparently sets low numbers of viable seed (Wrigley & Fagg, 1989; Tan & de Vos, 1994). Most Western Australian species have proved very difficult to propagate vegetatively, due to the lack of available cutting material, although Seaton and Webb (1996) reported some success with *C. floribundum*, *C. incurvum* and *C. triplinervium*. Research on *in vitro* germination of *C. triplinervium* seeds by Tan and de Vos (1994) indicated that germination of fresh fruits is prevented by the impervious fruit wall. *Conospermum* has also been micropropagated (Seaton & Webb, 1996), but further research is required for the technique to be commercially viable.

Conospermum mitchellii (Victorian smokebush) and *C. patens* (Slender smokebush) are eastern Australian species of smokebush which are not currently commercially exploited. *C. mitchellii*, possessing terminal corymbs of small cream flowers, may have potential use as a cut flower (T. Slater, pers. comm). *C. patens* is similar to *C. mitchellii*, except that its flowers are tinged with lilac blue. Eastern species may also have value either as ornamental garden species or as rootstocks for the valuable western species. Vegetative propagation from tip cuttings of these two *Conospermum* species has not been reported to date. The abundance of leafy stems makes them more amenable to cutting propagation than many western species.

The genus *Persoonia*, the geebungs, contains many species with potential for development as ornamental crops. Displaying yellow flowers and bright green foliage in many cases, geebungs are attractive garden and landscape plants. The striking yellow racemes and pine-like foliage of *Persoonia pinifolia* make it potentially valuable as a cut flower and an ornamental. The foliage of species such as *P. longifolia* and *P. virgata* are currently bush harvested for use in floristry. *P. longifolia* was the sixth most heavily exploited species in Western Australia in 1993, with almost 800,000 stems harvested from the wild (Karingal Consultants, 1994). *Persoonia* is rarely seen in cultivation, due to difficulties in propagation. Research to date has shown that intact *Persoonia* seeds exhibit very low germination rates, possibly due to restriction of embryo growth by the woody fruit wall (Ketelhohn *et al.*, 1994, 1995). Strike rates of tip cuttings are usually extremely low, in the order of 20% for *P. pinifolia* and 40% for *P. chamaepitys* (Ellyard, 1981). Ketelhohn (1996) reported strike rates for *P. virgata* which varied from 0 to 90%, depending on genotype, highlighting the need for careful selection of cutting material.

Of the many important factors in cutting propagation, such as growth regulator treatment, cutting type and condition of the stockplant, optimal temperatures for propagation vary enormously between species. Hartmann & Kester (1975) reported that most temperate species require temperatures of between 21°C and 27°C with night temperatures of about 15°C for rooting of cuttings. Dawson and King (1994) noted that high root zone temperature relative to air temperature improved rooting percentages of Australian plants, with minimal rooting occurring at root zone temperatures of less than 26°C at an air temperature of 20°C.

The stem anatomy of a species may also affect the ease of rooting of cuttings. Several studies have indicated that sclerenchyma tissue in the phloem of some woody plants may act as a mechanical barrier to root emergence in cuttings (Beakbane, 1961, 1969; Goodin, 1965; Mahlstede & Watson, 1952). These studies found that the ease of rooting of several species was correlated with the degree of sclerification of stem tissue. Increased sclerification as primary tissues mature may explain the decrease in rooting ability observed in many species as juvenile tissue (softwood) matures into adult tissue (hardwood) (Gardner, 1929). In contrast, a study of Australian plants by Williams *et al.* (1986) indicated that suberisation of stem tissues, rather than sclerification, is associated with low rooting percentages of cuttings.

The aims of this study were:

1) to identify a reliable method for propagating *Conospermum mitchellii* from tip cuttings, examining:
a. growth regulator requirements;
b. air and root zone temperature requirements;
c. the effect of cutting type (softwood, semi-hardwood or hardwood);

2) to investigate rooting of hardwood cuttings of *C. mitchellii*, *C. patens* and *Persoonia pinifolia* without the use of root zone heating;

3) to examine the stem anatomy of *C. mitchellii* in relation to:
a. the tissue of origin of root formation;
b. features of the stem tissue which may act as possible mechanical barriers to root emergence.

Materials and Methods

1. Collection and preparation of cuttings

Cutting material was taken from the most recent vegetative tips of parent plants as follows:

Species	Location	Type of cutting	Time of collection
Conospermum mitchellii	No. 2 Road, Anglesea	Softwood Semi-hardwood Hardwood	late November 1996 mid March 1997 mid June 1996, late May 1997
Persoonia pinifolia	Monash University, Clayton	Hardwood	early June 1996
Conospermum patens	near Rocklands Reservoir, west of Grampians National Park	Hardwood	mid June 1996

The softwood, semi-hardwood and hardwood experiments on *C. mitchellii* conducted between November 1996 and May 1997 will be referred to as the "*C. mitchellii* cutting experiment series". The three hardwood cutting experiments conducted in June 1996 on *C. mitchellii*, *C. patens* and *P. pinifolia* will be referred to as the "June 1996 hardwood cutting experiments".

All plant material was stored overnight at 4°C before preparation of cuttings. Tip cuttings were trimmed to approximately 8 cm in length and leaves were removed from the basal half of the stem prior to treatment with auxin solution for approximately 40 seconds. For the June 1996 hardwood cutting experiments, the basal half of the stem was immersed in undiluted Tween 20 (a wetting agent) (Sigma Chemical Co., St. Louis, MO, USA) for approximately 20 seconds immediately before immersion in auxin solution. Application of a wetting agent was intended to enhance auxin uptake. The pure auxins used were indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and α-naphthalene-acetic acid (NAA) (all from Sigma Chemical Co., St. Louis, MO, USA), each dissolved in 50% ethanol. Two commercial hormone preparations were tested: hormone preparation A (gel) (Growth Technology, South Fremantle, WA) and hormone preparation B (liquid) (Rye Pharmaceuticals, Ultimo, NSW) (see Appendix for constituents). Twenty cuttings per treatment were treated as shown below.

2. Auxin treatments

C. mitchellii cutting experiment series

Each cutting type (softwood, semi-hardwood and hardwood) was treated with the following solutions:

Water
Undiluted commercial hormone preparation A (active constituent 3000 ppm IBA)
5000 ppm IBA
5000 ppm IAA
5000 ppm NAA
3500 ppm IBA and 1500 ppm IAA
3500 ppm IBA and 1500 ppm NAA
3500 ppm IAA and 1500 ppm NAA

3500 ppm IBA, 750 ppm IAA and 750 ppm NAA
The semi-hardwood experiment contained the following additional treatments:

3000 ppm IBA
2.5% (v/v) commercial hormone preparation B (active constituents 1600 ppm IBA and NAA)
1600 ppm IBA and 1600 ppm NAA (equivalent concentrations of active constituents in hormone preparation B)

The hardwood cutting experiment contained the following additional treatments:

3000 ppm IBA
2.5% (v/v) commercial hormone preparation B (active constituents 1600 ppm IBA and NAA)
1600 ppm IBA and 1600 ppm NAA (equivalent concentrations of active constituents in hormone preparation B)
Undiluted commercial hormone preparation B (active constituents 1600 ppm IBA and NAA)

June 1996 hardwood cutting experiments

Water
Commercial hormone preparation A (active constituent 3000 ppm IBA)
5000 ppm IBA (no Tween 20)
5000 ppm IBA
5000 ppm IAA
5000 ppm NAA
*2500 ppm IBA and IAA
*2500 ppm IBA and NAA
*2500 ppm IAA and NAA
*2500 ppm IBA, IAA and NAA

* *C. mitchellii* was treated with 5000 ppm of each of these auxin combinations.

3. Incubation of cuttings

Individual cuttings were placed into 2" forestry tubes containing a steam-sterilised mixture of equal proportions of coarse sand, perlite (Laparte Group Australia Ltd., Jandakot, WA) and peat (Southland Peat, New Zealand). The tubes were placed into environmentally controlled glasshouses (ECGs) which enabled average air temperature to be controlled.

For the *C. mitchellii* cutting experiment series, tubes were placed in heated trays filled with vermiculite (Neuchatel Pty. Ltd.), which enabled control of the average root zone temperature of cuttings. An automatic misting system in each tray maintained the relative humidity at close to 100%. The heated trays were covered with polythene humidity frames, which had the effect of slightly increasing the air temperature inside the frames relative to the ambient air temperature in each ECG cell. Cuttings were watered approximately every five days and rooting response was assessed after 8 weeks.

For the June 1996 experiments, the tubes, contained in polystyrene foam boxes, were placed inside polythene humidity frames with capillary matting underneath. These experiments did not use root zone heating. Cuttings were watered approximately every second day and rooting response was assessed after 12 weeks.

4. Temperature treatments

C. mitchellii cutting experiment series

Softwood cuttings

Each treatment was incubated under each of the following four day/night temperature regimes which had been set in four ECG cells: 20°C/16°C, 22°C/17°C, 25°C/ 20°C and 28°C/ 23°C. The heated trays were set at a temperature of 28°C.

Semi-hardwood and hardwood cuttings

Each treatment was incubated at two root zone temperatures in each of three day/night temperature regimes. Two heated trays were set at temperatures of 28°C and 32°C in each of three ECG cells, which had day/night air temperature regimes set at 20°C/16°C, 25°C/ 20°C and 28°C/ 23°C.

June 1996 hardwood cutting experiments

Each auxin treatment was incubated under the following four day/night temperature regimes: 20°C/16°C, 22°C/17°C, 25°C/ 20°C and 28°C/ 23° C.

5. Anatomy of *C. mitchellii* stems

Segments approximately 2 mm long were sampled from the base of *C. mitchellii* stem cuttings, including those with roots. The segments were fixed in 2.5% glutaraldehyde in 0.02M phosphate buffer for 24 hours, dehydrated using a series of aqueous ethanol solutions of increasing concentration, and then vacuum infiltrated in 100% LR white resin for two half days. Specimens were embedded in gelatin capsules at 70°C.

Transverse sections of the stem bases (approximately 4µm thick) were made using an ultramicrotome (Reichert-Jung, Austria) and stained with toluidine blue or Sudan IV.

6. Statistical analysis

Analysis of variance was performed on the results for percentage of both rooted cuttings and dead cuttings for each auxin and temperature treatment, according to cutting type (softwood, semi-hardwood or hardwood) using SYSTAT™ statistical software. The effect of cutting type on percentage rooting and death of cuttings was analysed for the *C. mitchellii* cutting experiment series. The effect of auxin and temperature treatment on the average percentage rooting and death across all cutting types for *C. mitchellii* was also analysed. Pairwise comparisons were tested for significance using Tukey's test.

Results

1. Influence of auxin treatment on percentage rooting and death of cuttings

C. mitchellii cutting experiment series

Mean values for percentage of rooted and dead cuttings of *C. mitchellii* according to auxin treatment are shown in Figure 1 (Table 1). Each result is the average of all three cutting types, and all temperature treatments. Hormone preparation A gave the highest mean rooting percentage (61%) and 5000 ppm NAA gave the lowest mean rooting percentage (3%). The highest mean percentage of cutting deaths occurred in the IBA and NAA treatment, while the lowest occurred in the water treatment. The type of auxin treatment used on cuttings had an overall significant effect ($p = 0.000$) on rooting and death of all cutting types except death of softwood cuttings.

Figure 4 (Table 4) compares the percentage rooting for cuttings of each of the three cutting types, softwood, semi-hardwood and hardwood, averaged across temperature treatments. The highest rooting percentage (87.5%) was obtained by treating softwood cuttings with hormone preparation A. This treatment was also the best for hardwood cuttings, but not semi-hardwood cuttings. Treatment with NAA gave no higher than 3.3% rooting for any cutting type. In most cases, treatments containing NAA gave rooting percentages which were lower than the control treatment, water.

The percentage of dead *C. mitchellii* cuttings for each cutting type after 8 weeks is shown in Figure 5 and Table 5. The highest percentage of deaths for semi-hardwood (77.5%) and hardwood cuttings (96%) occurred in the 5000 ppm NAA treatment. For softwood cuttings, NAA resulted in significantly fewer deaths than for the other cutting types. The IBA and IAA treatment resulted in the most deaths for softwood cuttings, but this result was not significantly different to any other treatment at the 0.05 level of significance.

June 1996 hardwood cutting experiments

Figures 6, 7 and 8 display the results for percentage of rooted and dead cuttings for each auxin treatment for each of the three species tested. Results are averaged across all temperature treatments. All species showed relatively low rooting compared to the results for softwood cuttings of *C. mitchellii*. Auxin treatment had a significant effect on rooting and death for both *Conospermum* species ($p = 0.000$), but was not significant for rooting and death of *P. pinifolia* hardwood cuttings.

The maximum percentage rooting (40%) occurred in cuttings of *C. patens* treated with hormone preparation A (Figure 7, Table 7). In most cases, the percentage of dead cuttings was greater than that of rooted cuttings. For *P. pinifolia*, the treatment giving maximal rooting was 5000 ppm IBA without prior treatment with Tween 20 (Figure 6, Table 6), while the water treatment gave the lowest rooting percentage. In contrast, water treatments gave moderate to high rooting percentages in both *Conospermum* species (Figures 7 and 8, Tables 7 and 8). For both of the *Conospermum* species, treatment with hormone preparation A gave the highest rooting percentages, while NAA gave the lowest rooting percentages. For *C. mitchellii*, the result for hormone preparation A (35%) represented a significant improvement in rooting over all of the other treatments (Table 8). As seen in the *C. mitchellii* series, treatments containing NAA generally caused the most deaths in the *Conospermum* species, but not in *P. pinifolia*.

Table 1.

Auxin treatment	mean % of rooted cuttings (averaged across all *C. mitchellii* cutting types and temperature treatments)	mean % of dead cuttings (averaged across all *C. mitchellii* cutting types and temperature treatments)
NAA	2.78 +/- 4.41[a]	53.89 +/- 41.97[bc]
IBA + NAA	12.78 +/- 13.94[ab]	61.11 +/- 29.34[c]
IAA + NAA	18.33 +/- 17.85[ab]	39.44 +/- 29.63[abc]
IBA, IAA + NAA	18.33 +/- 16.58[ab]	52.22 +/- 34.47[bc]
water	26.67 +/- 23.85[b]	10.56 +/- 14.46[a]
IBA + IAA	32.78 +/- 23.99[bc]	30.56 +/- 24.55[abc]
IAA	34.44 +/- 17.58[bc]	17.22 +/- 19.54[ab]
IBA	52.22 +/- 13.72[cd]	27.78 +/- 20.33[abc]
hormone preparation A	60.56 +/- 25.91[d]	18.33 +/- 11.18[ab]
grand mean	**28.77 +/- 12.54**	**34.57 +/- 14.28**

Table 2.

Air temperature (degrees C)	mean % of rooted cuttings (averaged across all *C. mitchellii* cutting types and auxin treatments)	mean % of dead cuttings (averaged across all *C. mitchellii* cutting types and auxin treatments)
20	29.81 +/- 22.21[a]	23.52 +/- 28.45[a]
25	26.48 +/- 25.71[a]	39.26 +/- 30.63[ab]
28	30.00 +/- 27.46[a]	40.93 +/- 30.70[b]

Table 3.

Cutting type	mean % of rooted cuttings (averaged across all *C. mitchellii* auxin and temperature treatments)	mean % of dead cuttings (averaged across all *C. mitchellii* auxin and temperature treatments)
Softwood	42.64 +/- 25.07[a]	30.97 +/- 12.23[a]
Semi-hardwood	19.58 +/- 13.24[b]	42.43 +/- 23.77[a]
Hardwood	17.69 +/- 17.51[b]	40.64 +/- 31.08[a]

Values followed by a different letter differ significantly at the 0.05 significance level

Figure 1. Mean percentage of rooted and dead cuttings of *C. mitchellii* versus auxin treatment

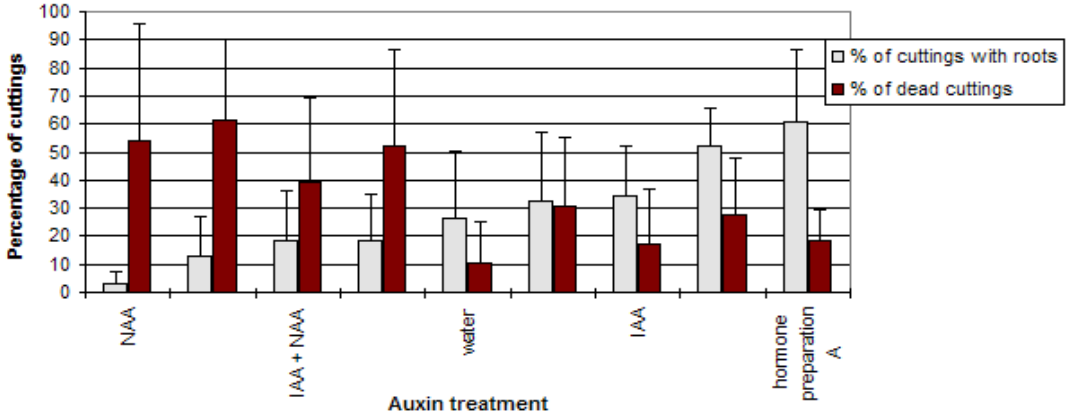


Figure 2. Mean percentage of rooted and dead cuttings of *C. mitchellii* versus air temperature treatment

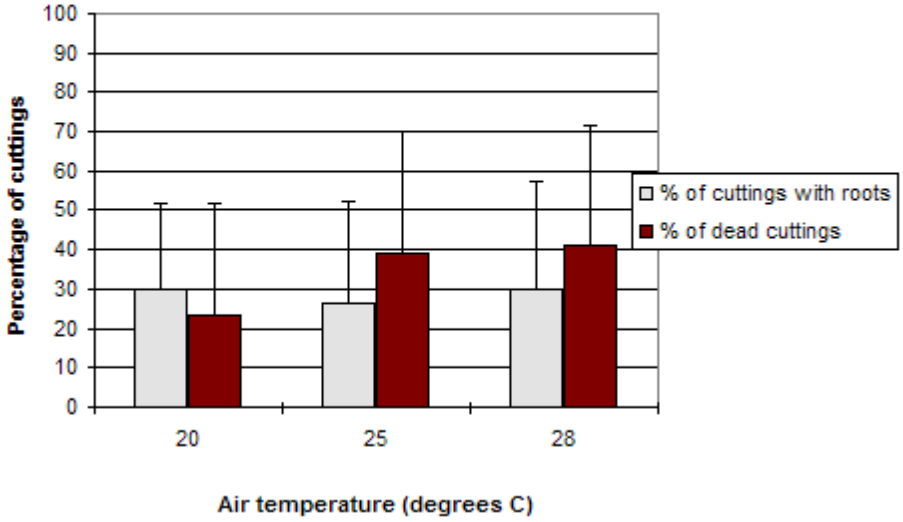


Figure 3. Mean percentage of rooted and dead cuttings of *C. mitchellii* versus cutting type

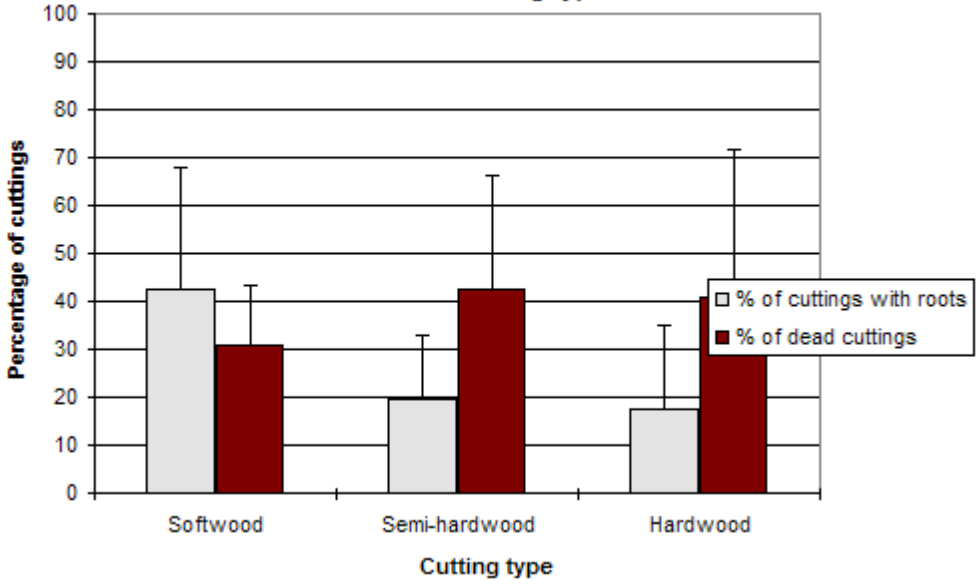


Table 4.

Auxin treatment	Percentage of *C. mitchellii* cuttings with roots after 8 weeks			
	Softwood	Semi-hardwood cuttings	Hardwood cuttings	Mean across all cutting types
NAA	2.50 +/- 5.00[a]	3.33 +/- 4.08[a]	0.83 +/- 10.33[a]	2.78 +/- 4.41[a]
2.5% (v/v) hormone prep B	–	9.17 +/- 8.61[ab]	19.17 +/- 14.29[abc]	–
hormone prep B equivalent	–	9.17 +/- 9.70[ab]	3.33 +/- 5.16[ab]	–
hormone prep B	–	–	10.83 +/- 5.85[ab]	–
IBA + NAA	20.00 +/- 16.83[ab]	10.00 +/- 7.07[ab]	1.67 +/- 2.58[a]	12.78 +/- 13.94[ab]
IBA, IAA + NAA	35.00 +/- 7.07[b]	11.67 +/- 8.76[ab]	3.33 +/- 4.08[ab]	18.33 +/- 16.58[ab]
IAA + NAA	26.25 +/- 20.56[ab]	14.17 +/- 13.57[ab]	3.33 +/- 5.16[ab]	18.33 +/- 17.85[ab]
water	48.75 +/- 14.36[b]	14.17 +/- 14.63[ab]	8.33 +/- 10.33[ab]	26.67 +/- 23.85[b]
IAA	48.75 +/- 20.16[b]	25.83 +/- 14.97[bc]	25 +/- 18.97[bc]	34.44 +/- 17.58[bc]
IBA + IAA	52.50 +/- 15.55[b]	25.83 +/- 11.58[b]	21.67 +/- 17.22[abc]	32.78 +/- 23.99[bc]
hormone prep A	87.50 +/- 6.45[c]	25.83 +/- 21.31[bc]	55.83 +/- 10.68[d]	60.56 +/-25.91[d]
hormone prep A equivalent	–	39.17 +/- 13.57[bc]	40 +/- 17.61[cd]	–
IBA	62.50 +/- 8.66[bc]	46.67 +/- 18.89[bc]	36.67 +/- 18.35[cd]	52.22 +/- 13.72[cd]

Table 5.

Auxin treatment	Percentage of dead *C. mitchellii* cuttings after 8 weeks			
	Softwood	Semi-hardwood cuttings	Hardwood cuttings	Mean across all cutting types
NAA	15.00 +/- 17.80[a]	77.50 +/- 28.06[d]	95.83 +/- 3.76[d]	53.89 +/- 41.97[bc]
2.5% (v/v) hormone prep B	–	42.50 +/- 31.90[abcd]	5.83 +/- 5.85[a]	–
hormone prep B equivalent	–	55.83 +/- 36.39[bcd]	78.33 +/- 20.41[cd]	–
hormone prep B	–	–	38.33 +/- 30.44[ab]	–
IBA + NAA	42.50 +/- 14.43[a]	71.67 +/- 27.69[d]	78.33 +/- 19.92[cd]	61.11 +/- 293.34[c]
IBA, IAA + NAA	40.00 +/- 14.72[a]	69.17 +/- 35.70[cd]	65.00 +/- 33.91[bcd]	52.22 +/- 34.47[bc]
IAA + NAA	33.75 +/- 23.94[a]	57.50 +/- 35.46[bcd]	60.00 +/- 24.29[bc]	39.44 +/- 29.63[abc]
water	21.25 +/- 16.52[a]	8.33 +/- 8.16[a]	8.33 +/- 8.76[a]	10.56 +/- 14.46[a]
IAA	37.50 +/- 15.55[a]	12.50 +/- 10.84[a]	14.17 +/- 19.08[a]	17.22 +/- 19.54[ab]
IBA + IAA	47.50 +/- 27.23[a]	32.50 +/- 28.77[ab]	17.50 +/- 16.96[a]	30.56 +/- 24.55[abc]
hormone prep A	13.75 +/- 6.29[a]	29.17 +/- 16.56[ab]	10.83 +/- 8.61[a]	18.33 +/- 11.18[ab]
hormone prep A equivalent	–	17.50 +/-12.55[a]	24.17 +/- 30.07[a]	–
IBA	27.50 +/- 14.43[a]	35.00 +/- 23.66[abc]	31.67 +/- 24.01[ab]	27.78 +/- 20.33[abc]

Values followed by a different letter differ significantly at the 0.05 significance level.

Figure 4. Percentage of *C. mitchellii* cuttings with roots after 8 weeks

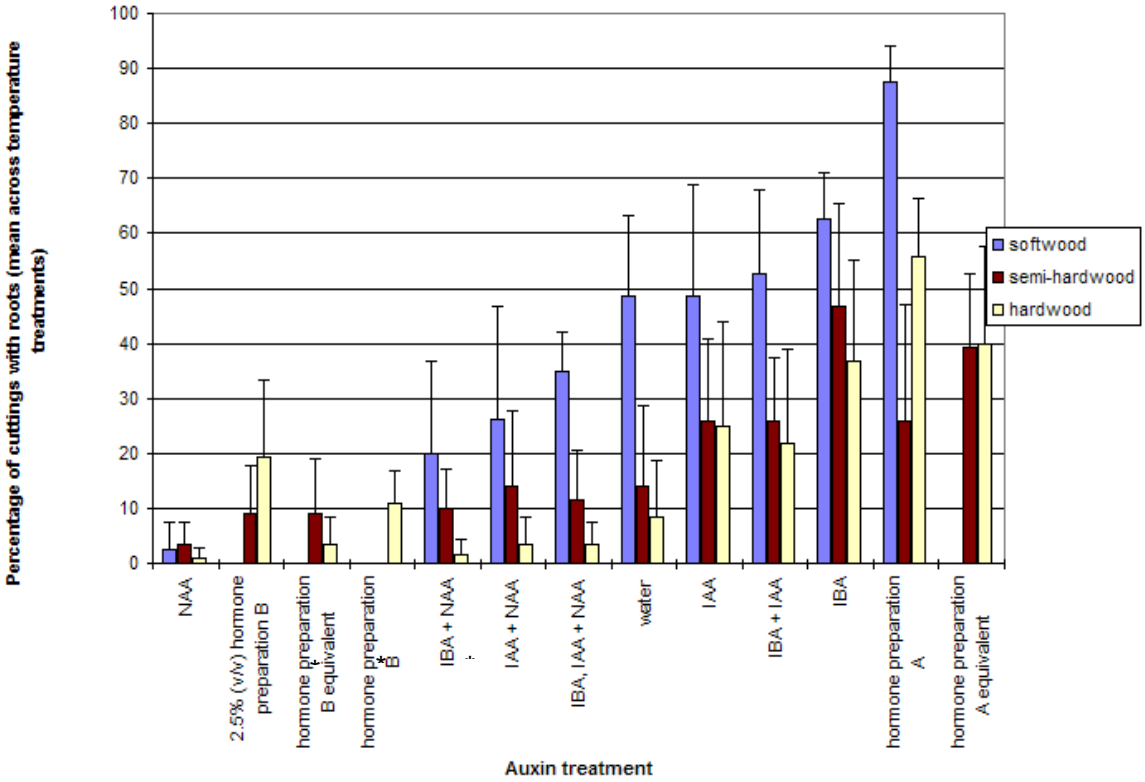
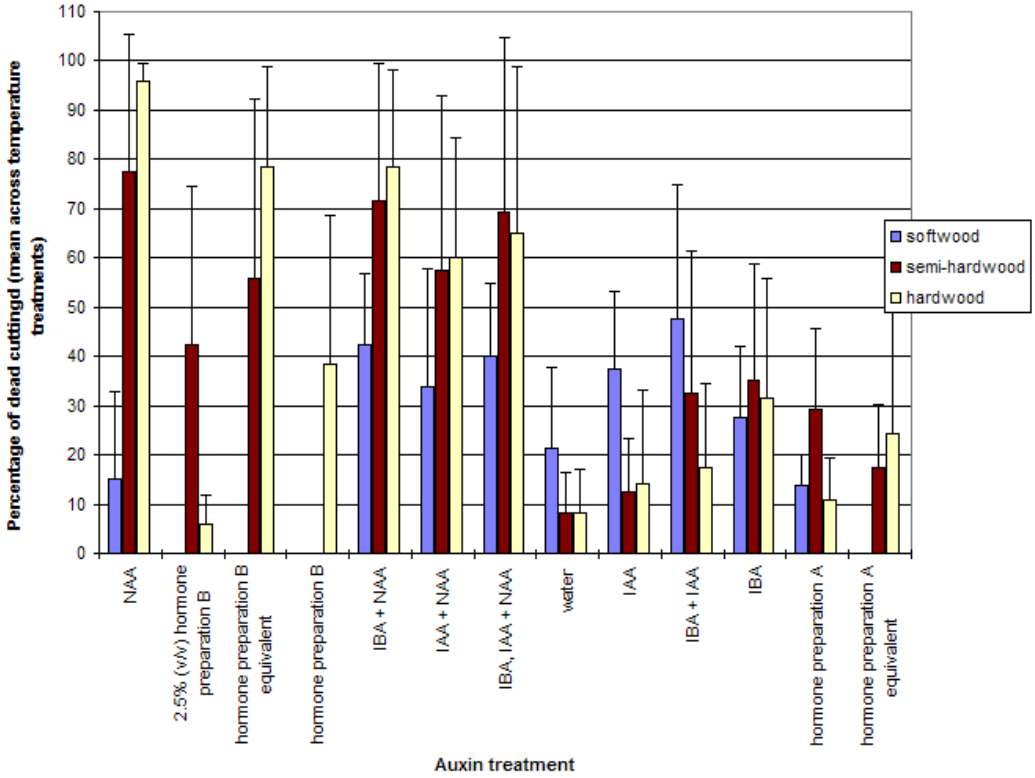


Figure 5. Percentage of dead *C. mitchellii* cuttings after 8 weeks



Missing data points indicate that this auxin treatment was not applied to the cutting type represented.

Table 6.

P. pinifolia hardwood cuttings		
Auxin treatment	% cuttings with roots	% dead cuttings
water	2.50 +/- 2.89[a]	21.25 +/- 24.62[a]
IBA	2.50 +/- 2.89[a]	2.50 +/- 2.89[a]
IBA + IAA	5.00 +/- 4.08[a]	17.50 +/- 25.98[a]
IAA	12.50 +/- 9.57[a]	13.75 +/- 13.77[a]
IBA + NAA	15.00 +/- 4.08[a]	16.25 +/- 13.15[a]
IBA, IAA + NAA	16.25 +/- 14.36[a]	18.75 +/- 21.75[a]
NAA	17.50 +/- 23.63[a]	35.00 +/- 31.09[a]
hormone preparation A	20.00 +/- 21.21[a]	3.75 +/- 4.79[a]
IAA + NAA	22.50 +/- 28.72[a]	27.50 +/- 39.26[a]
IBA (no Tween 20)	35.00 +/- 26.46[a]	3.75 +/- 2.50[a]

Table 7.

C. patens hardwood cuttings		
Auxin treatment	% cuttings with roots	% dead cuttings
NAA	12.50 +/- 11.90[a]	60.00 +/- 30.28[b]
IAA	12.50 +/- 6.45[a]	32.50 +/- 25.33[ab]
IBA + NAA	16.25 +/- 11.09[ab]	50.00 +/- 31.89[ab]
IBA + IAA	17.50 +/- 11.90[ab]	32.50 +/- 15.55[ab]
IBA	18.75 +/- 8.54[ab]	32.50 +/- 9.57[ab]
IBA (no Tween 20)	28.75 +/- 13.77[ab]	33.75 +/- 20.97[ab]
IBA, IAA + NAA	30.00 +/- 9.13[ab]	31.25 +/- 24.96[ab]
IAA + NAA	32.50 +/- 15.55[ab]	46.25 +/- 13.77[ab]
water	36.25 +/- 6.29[b]	17.50 +/- 15.55[a]
hormone preparation A	40.00 +/- 7.07[b]	22.50 +/- 16.58[ab]

Table 8.

C. mitchellii hardwood cuttings		
Auxin treatment	% cuttings with roots	% dead cuttings
NAA	2.50 +/- 2.89[a]	67.50 +/- 46.28[a]
IAA	2.50 +/- 2.89[a]	68.75 +/- 23.94[a]
IAA + NAA	5.00 +/- 4.08[a]	80.00 +/- 18.26[a]
IBA, IAA + NAA	5.00 +/- 7.07[a]	23.75 +/- 47.50[a]
water	7.50 +/- 9.57[a]	47.50 +/- 28.72[a]
IBA + IAA	8.75 +/- 4.79[a]	48.75 +/- 21.75[a]
IBA + NAA	12.50 +/- 10.41[a]	52.38 +/- 14.93[a]
IBA	12.50 +/- 6.45[a]	45.00 +/- 27.39[a]
IBA (no Tween 20)	13.75 +/- 4.79[a]	38.75 +/- 8.54[a]
hormone preparation A	35.00 +/- 8.16[b]	27.50 +/- 24.66[a]

Values followed by a different letter differ significantly at the 0.05 level.

Figure 6. *P. pinifolia* hardwood cuttings

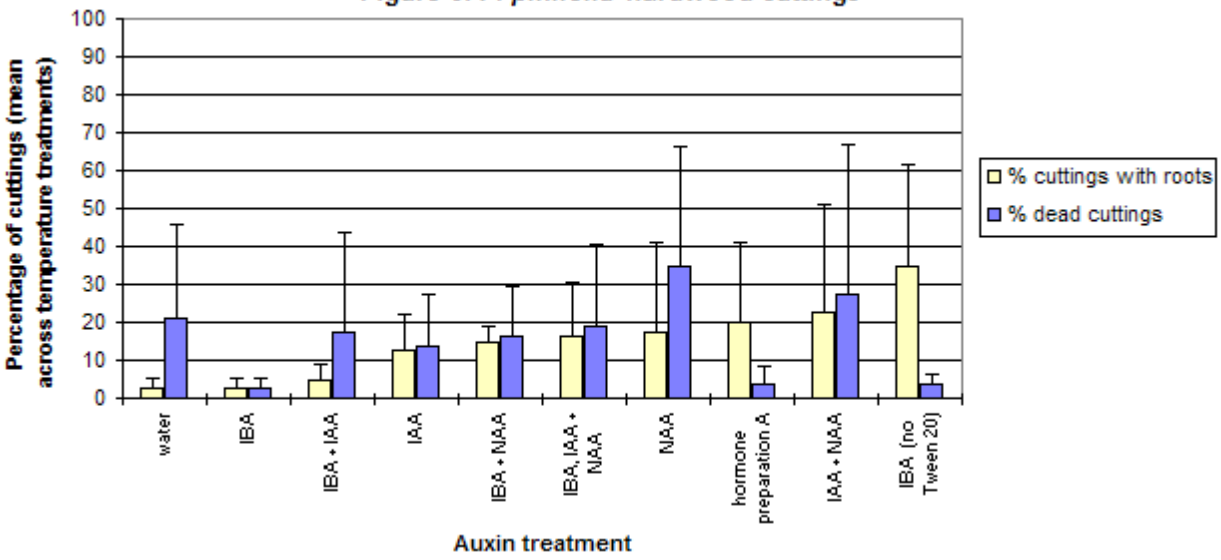


Figure 7. *C. patens* hardwood cuttings

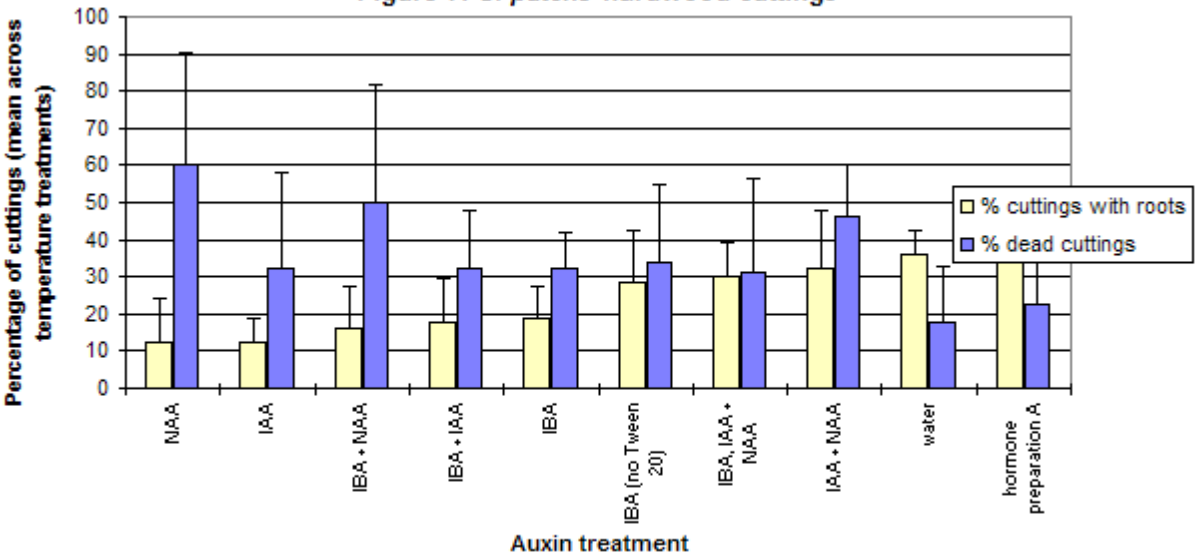
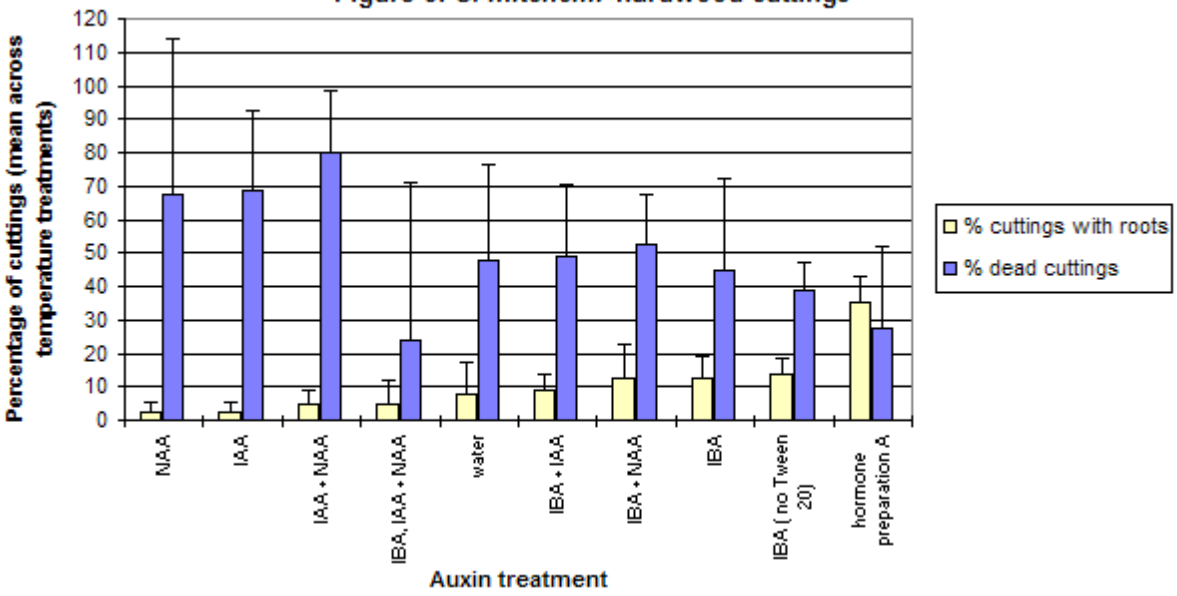


Figure 8. *C. mitchellii* hardwood cuttings



2. Influence of air and root zone temperatures

***C. mitchellii* cutting experiment series**

The air temperatures tested in this study did not have a significant overall effect on rooting of *C. mitchellii* cuttings (Figure 2, Table 2), although significantly more deaths occurred at an air temperature of 28°C than at 20°C. However, the combinations of air and root zone temperatures did significantly affect rooting and death of semi-hardwood cuttings and death of hardwood cuttings (Tables 9 and 10).

Figure 9 shows the rooting percentage for each cutting type according to temperature treatment, averaged across auxin treatments. Softwood cuttings, for which only a root zone temperature of 28°C was tested, show a general trend of increased rooting with increasing air temperature, however, none of the results differed significantly at the 0.05 significance level. Semi-hardwood cuttings gave lower rooting percentages when incubated at a root zone temperature of 32°C, particularly at air temperatures of 20°C and 25°C, than at a root zone temperature of 28°C. Hardwood cuttings showed no significant variation in rooting percentages.

Cutting death was generally higher in the 32°C than the 28°C root zone temperature (Figure 10). Softwood material showed a trend of decreasing cutting death as air temperature increased, although this was not significant. Semi-hardwood cuttings sustained most deaths at the higher air temperatures in the 28°C root zone treatments, and more deaths at a root zone temperature of 32°C than at 28°C. Hardwood cuttings showed a trend of increasing cutting death with increasing air temperature.

June 1996 hardwood cutting experiments

For *P. pinifolia*, air temperature had a significant effect on both rooting and death of cuttings. For the *Conospermum* species, air temperature significantly affected death but not rooting of cuttings. For *P. pinifolia* and *C. patens*, the highest rooting percentages were obtained at the highest air temperature (28°C) (Figures 11 and 12, Tables 11 and 12). In contrast, the highest rooting percentage for *C. mitchellii* (12.5%) was obtained at an air temperature of 20°C, and the lowest at 25°C (Figure 13, Table 13), although these results did not differ significantly at the 0.05 significance level. In general, cutting death increased with air temperature. Of the three species tested, *C. mitchellii* had the highest overall percentage of cutting deaths, while *P. pinifolia* had the lowest.

3. Influence of cutting type

Softwood cuttings of *C. mitchellii* gave a significantly higher average rooting percentage (43%) than semi-hardwood or hardwood cuttings (20% and 18% respectively) (Figure 3, Table 3). Cutting death did not differ significantly according to cutting type.

4. Anatomy of *C. mitchellii* stems

Figure 14 shows root development on a typical *C. mitchellii* cutting treated with 5000 ppm IBA after approximately four weeks. Most roots developed in the lowermost half centimetre of stem cuttings. The degree of branching and overall development of roots varied between treatments.

Figure 15 shows a transverse section of an emerging root. The root appears to have originated in the region of the phloem tissue, possibly in the cambium.

Figures 16 and 17 show transverse sections of *C. mitchellii* semi-hardwood stems stained with toluidine blue. The stem in Figure 16 is at an earlier stage of development than that in Figure 17, as can be seen by the discrete vascular bundles. In the older stem (Figure 17), the vascular tissue is continuous and there is a greater degree of lignification of xylem tissue. Sclerenchyma sheaths are observable in both stem sections, but appear to be thinner and more dispersed in the older section. Staining with Sudan IV did not indicate the presence of suberised tissue.

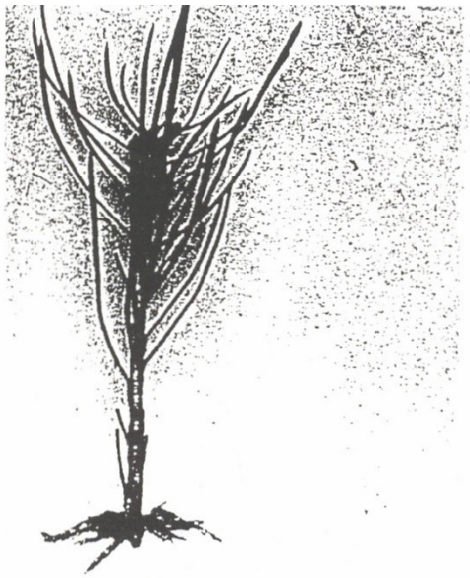


Figure 14. Rooted cutting x0.64

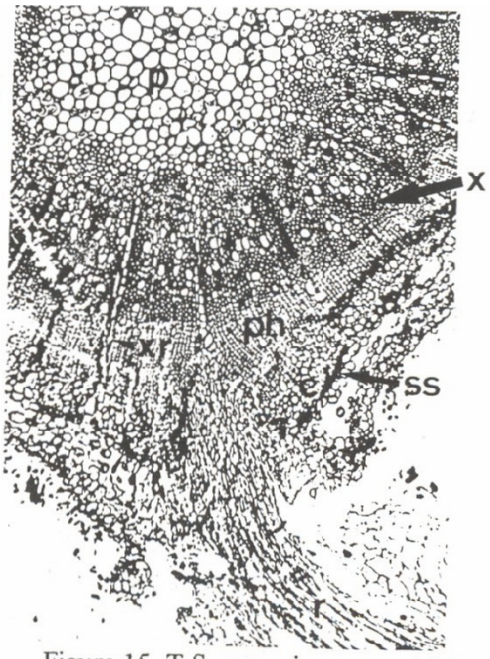


Figure 15. T.S. emerging root x12.8

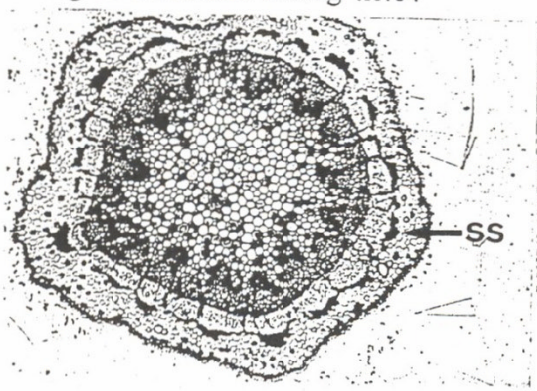


Figure 16. T.S. young stem x10

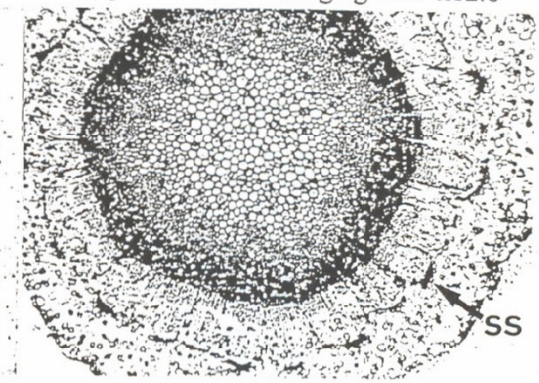


Figure 17. T.S. old stem x10

p: pith; x: xylem; xr: xylem ray; ph: phloem; c: cortex; ss: sclerenchyma sheath; r: root

Table 9.

Temperature (degrees C)		Percentage of *C. mitchellii* cuttings with roots after 8 weeks			
Root zone	Air zone	Softwood cuttings	Semi-hardwood cuttings	Hardwood cuttings	Mean across all cutting age types
28	20	38.33 +/- 26.69[a]	27.92 +/- 15.29[d]	24.23 +/- 19.46[a]	29.81 +/- 22.52[a]
28	22	40.00 +/- 30.10[a]	-	-	-
28	25	40.00 +/- 30.82[a]	22.92 +/- 17.51[bcd]	16.54 +/- 25.11[a]	26.48 +/- 25.71[a]
28	28	52.22 +/- 21.52[a]	24.58 +/- 21.89[cd]	13.46 +/- 21.83[a]	30.00 +/- 27.46[a]
32	20	-	10.83 +/- 14.90[ab]	23.46 +/- 20.04[a]	-
32	25	-	9.17 +/- 8.21[a]	12.69 +/- 16.53[a]	-
32	28	-	22.08 +/- 18.88[abcd]	15.77 +/- 17.66[a]	-

Table 10.

Temperature (degrees C)		Percentage of dead *C. mitchellii* cuttings after 8 weeks			
Root zone	Air zone	Softwood cuttings	Semi-hardwood cuttings	Hardwood cuttings	Mean across all cutting age types
28	20	35.56 +/- 27.32[a]	6.67 +/- 8.62[a]	25.77 +/- 33.16[a]	23.52 +/- 28.45[a]
28	22	35.00 +/- 19.20[a]	-	–	-
28	25	26.67 +/- 16.01[a]	48.33 +/- 30.18[bc]	43.08 +/- 38.87[abc]	39.26 +/- 30.63[ab]
28	28	26.67 +/- 14.14[a]	34.17 +/- 29.68[b]	56.92 +/- 36.77[c]	40.93 +/- 30.70[b]
32	20	-	57.08 +/- 36.27[c]	26.15 +/- 26.70[ab]	-
32	25	-	62.08 +/- 26.84[c]	44.62 +/- 40.49[abc]	-
32	28	-	46.25 +/- 33.79[bc]	47.31 +/- 32.83[c]	-

Values followed by a different letter differ significantly at the 0.05 significance level.

Figure 9. Percentage of *C. mitchellii* cuttings with roots after 8 weeks

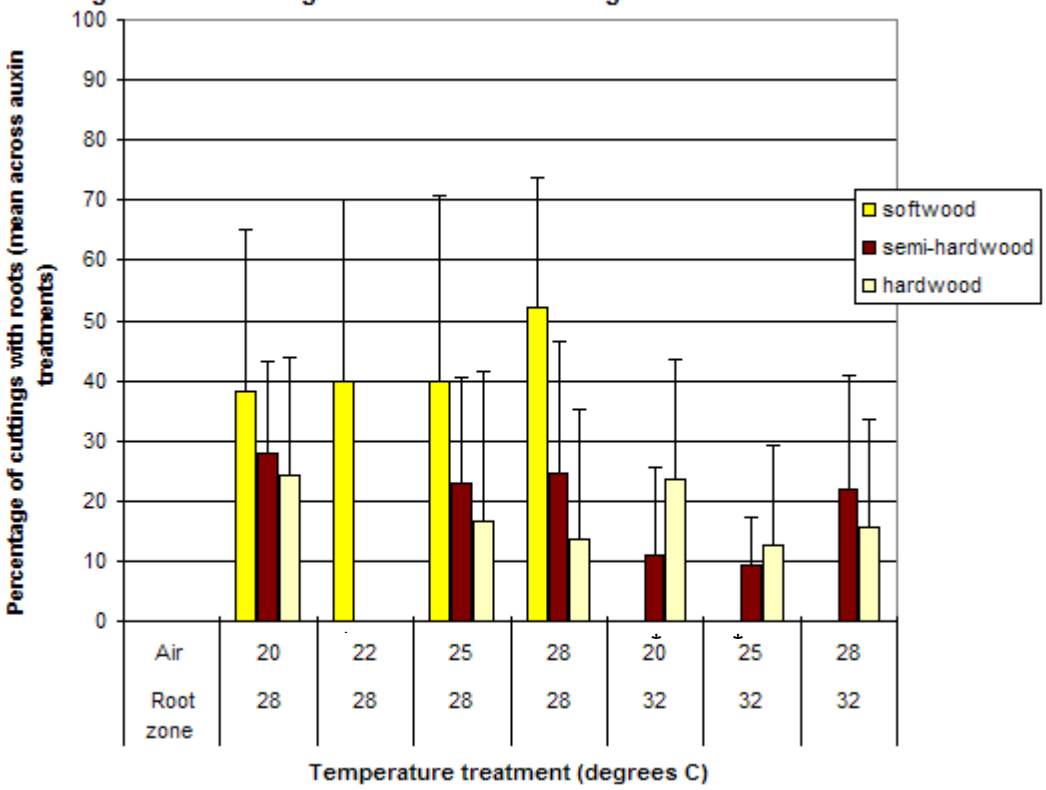
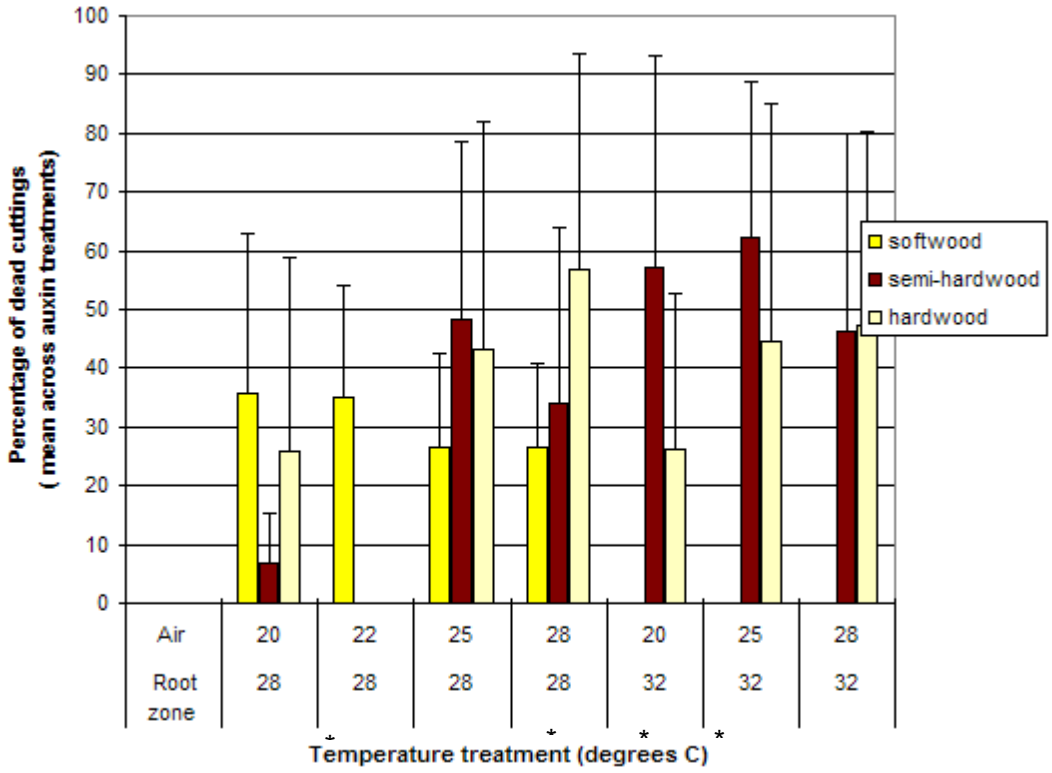


Figure 10. Percentage of dead *C. mitchellii* cuttings after 8 weeks



Missing data points indicate that this temperature treatment was not applied to the cutting type represented

Table 11.

P. pinifolia hardwood cuttings		
Air temperature (degrees C)	% cuttings with roots	% dead cuttings
20	18.50 +/- 20.01[ab]	3.50 +/- 6.69[a]
22	10.50 +/- 8.96[ab]	4.00 +/- 5.16[a]
25	5.50 +/- 5.99[a]	27.50 +/- 24.18[b]
28	25.00 +/- 24.49[b]	29.00 +/- 26.12[b]

Table 12.

C. patens hardwood cuttings		
Air temperature (degrees C)	% cuttings with roots	% dead cuttings
20	21.00 +/- 11.97[a]	22.50 +/- 14.95[a]
22	24.00 +/- 14.30[a]	22.50 +/- 11.12[a]
25	21.00 +/- 9.37[a]	47.50 +/- 19.18[b]
28	32.00 +/- 16.19[a]	51.00 +/- 26.33[b]

Table 13.

C. mitchellii hardwood cuttings		
Air temperature (degrees C)	% cuttings with roots	% dead cuttings
20	12.50 +/- 13.79[a]	37.50 +/- 26.90[a]
22	12.00 +/- 10.06[a]	42.00 +/- 32.16[a]
25	8.00 +/- 11.60[a]	68.00 +/- 32.16[a]
28	9.50 +/- 8.32[a]	62.00 +/- 28.21[a]

Values followed by a different letter differ significantly at the 0.05 level.

Figure 11. *P. pinifolia* hardwood cuttings

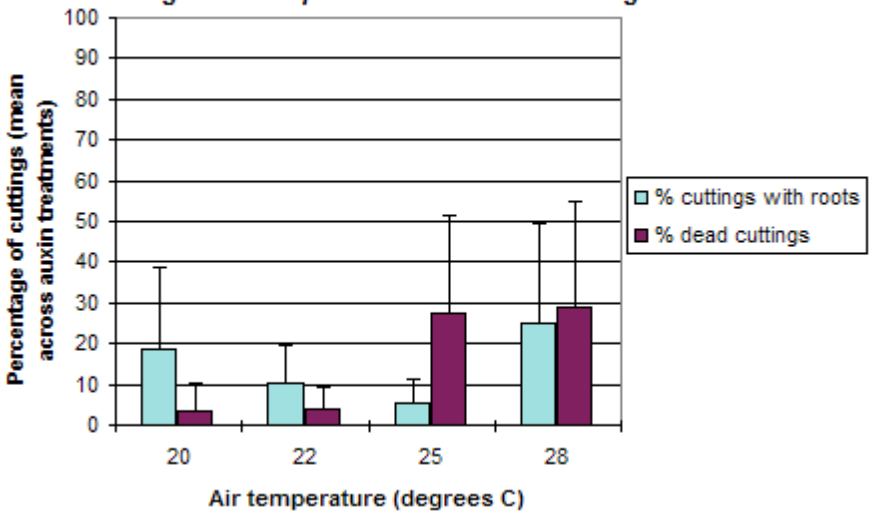


Figure 12. *C. patens* hardwood cuttings

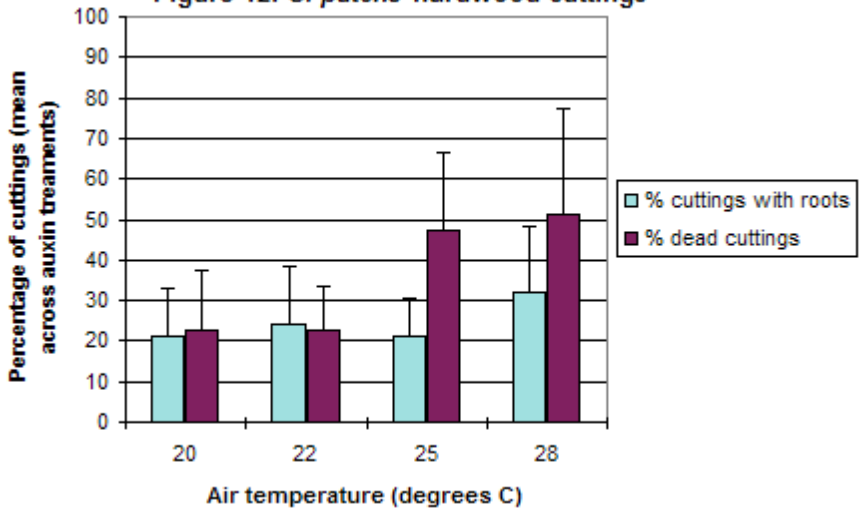
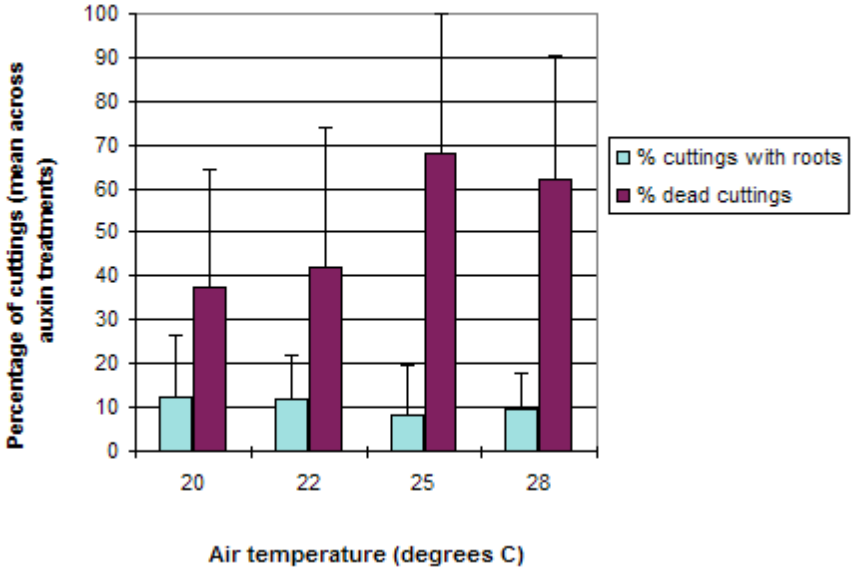


Figure 13. *C. mitchellii* hardwood cuttings



Discussion

Auxin effects on rooting and death of cuttings

The results of this study indicate that for stem cuttings of *Conospermum mitchellii*, IBA at a concentration of 3000 ppm applied as a gel formulation gave the highest mean rooting percentage (Figure 1). This relatively high rooting percentage (61%) and low percentage of cutting death (18%) may be due to the fact that this preparation contained mineral nutrients (see Appendix) which may have enhanced cutting survival as well as stimulating root growth. In particular, boron, in combination with IBA, has been shown to improve rooting percentage in English holly (Weiser & Blaney, 1960). In addition, the gel formulation may have improved auxin uptake as it adheres to the cutting more effectively than liquid formulations.

Treatments containing NAA had an adverse effect on rooting and resulted in a significantly higher percentage of cutting deaths than other auxin treatments. Softwood cuttings treated with NAA, however, were far less susceptible to death, compared to semi-hardwood and hardwood cuttings. The low rooting percentages obtained in treatments of *C. mitchellii* cuttings containing both IBA and NAA (Figure 1) may be due to the stimulatory effect of IBA being antagonised by the presence of NAA. The high percentage of cutting death in treatments containing NAA may be attributable to accelerated senescence resulting from the production of ethylene caused by auxin (Zimmerman & Wilcoxon, 1935). Although hormone preparation B contained 1600 ppm IBA, its stimulatory effect on rooting may have been antagonised by the presence of 1600 ppm NAA. Treatment of cuttings with a diluted solution of hormone preparation B (2.5% (v/v)) gave the same result as that obtained with an auxin solution of equivalent auxin concentration to the undiluted hormone preparation (1600 ppm IBA and NAA). The concentration of both auxins in the diluted commercial preparation (40 ppm IBA and NAA) may have been too low to be effective in stimulating root formation, while the concentration of NAA in the undiluted commercial preparation was high enough to be antagonistic to root formation.

Hardwood cuttings of *C. patens* showed similar results to those for *C. mitchellii* (Figure 7), except that some NAA combinations were not as inhibitory to rooting. Further experiments with softwood and semi-hardwood cuttings of this species would give these results greater definition. The results for both of these *Conospermum* species compare favourably with those of Seaton and Webb (1996). They obtained rooting percentages ranging from 20-50% for *C. floribundum* to greater than 50% for *C. triplinervium* using 3000 ppm IBA, applied as a powder or gel to semi-hardwood cuttings.

For hardwood cuttings of *Persoonia pinifolia*, a 5000 ppm solution of IBA was also the most effective auxin, resulting in 35% rooting (in the absence of Tween 20) (Figure 6). This result for *P. pinifolia* contrasts with an earlier study by Ellyard (1977) on rooting of *P. pinifolia*, in which it was found that auxin mixtures were superior to IBA alone. Cuttings taken in February treated with a solution containing 1000 ppm IBA and 200 ppm NAA gave 8% rooting compared to 0% rooting for 1200 ppm IBA alone. Clearly, IBA concentrations in the order of 3000-5000 ppm, as used in the present study, are more effective than lower concentrations. In contrast to results for the two *Conospermum* species, treatments containing NAA did not appear to be as inhibitory to rooting of *P. pinifolia* hardwood cuttings. 65% of cuttings formed roots when treated with a combination of IAA and NAA and incubated at 20°C (data not shown). Further experiments with soft and semi-hardwood cuttings of *P. pinifolia* would confirm the effectiveness of NAA in stimulating rooting of *Persoonia*.

Air and root zone temperature effects on rooting and death of cuttings

The air temperatures tested in this study had no significant effect on rooting percentages of *C. mitchellii* and *C. patens*, although cutting death was significantly higher at the higher temperatures (Figures 2 and 12). The general trend of increased cutting death with increasing air temperature observed across all the species tested may be a result of the accelerated senescence occurring at higher temperatures. However, for *P. pinifolia*, an almost fivefold increase in rooting was observed at an air temperature of 28°C relative to 25°C (Figure 11, Table 11). For all species in the June 1996 hardwood cutting experiments, 25°C gave the lowest rooting percentage. This result may indicate that either this air temperature is unsuitable or that some factor unique to this treatment was inhibiting rooting.

Effects of root zone heating are apparent when comparing results for rooting of *C. mitchellii* hardwood cuttings in the 1996 and 1997 experiments (Figure 4 and Figure 8). Without root zone heating, a mean maximum rooting percentage of 35% was obtained, while the mean maximum obtained with root zone heating was 56%. Clearly, use of root zone heating is beneficial. However, a root zone temperature of 32°C seemed to be less effective than 28°C (Figure 9) for semi-hardwood and hardwood cuttings, although this result was not significant. Lower rooting percentages observed at the higher root zone temperature may have resulted from air temperatures in these treatments being raised to levels which may have adversely affected rooting. Dykeman (1976) observed that optimal temperatures for root initiation differed from optimal temperatures for root development, which occurs after root emergence. For *Chrysanthemum* and *Forsythia*, a temperature of 30°C resulted in more rapid root initiation, while root development was promoted at a temperature of 25°C. Decreased respiration at the lower temperature may have allowed greater photosynthate to be allocated to root development. Thus, although more roots may have been initiated in *C. mitchellii* cuttings incubated at the higher temperature, root development may have been inhibited.

Effect of cutting type on rooting and death

Collection of *C. mitchellii* cuttings in November (spring), when vegetative growth was relatively soft, gave significantly higher rooting percentages compared to other times of the year (Figure 3, Table 3). Cutting death was also lowest for these cuttings. More detailed research would define the time period over which cuttings remain soft. For *P. pinifolia* and *C. patens*, further data is required to determine the best time for collection of cuttings, however there are indications that softwood cuttings of *P. pinifolia* may give higher rooting percentages than hardwood cuttings (Ellyard, 1977).

Results for *C. mitchellii* are in accordance with seasonal rooting patterns found in many genera, e.g. *Syringa* (Schmidt, 1978), *Picea* (Tognoni *et al.*, 1977) and *Populus* (Smith & Wareing, 1972). *Cotinus coggygria*, a woody shrub, roots well in spring, but not at all later in the season (Kelley & Forret, 1977). Gradual decline in rooting ability during the growing season was found to correlate well with a decline in endogenous auxin levels in the rooting zone of the stem (Blakesley, 1991). Other explanations for loss of rooting ability over time include increasing production of rooting inhibitors such as those related to phloroglucinol (Paton *et al.*, 1970), decreased production of phenolic compounds, which are thought to act as auxin cofactors during the rooting process (Girouard, 1969), or gradual formation of anatomical barriers to root emergence (Beakbane, 1961, 1969). Stem anatomy of *C. mitchellii* is discussed in the following section. Further information on the mechanism of change in rooting ability of *C. mitchellii* cutting types would be gained from biochemical analyses of the concentrations of compounds such as auxins and phenolics in cuttings over time.

Anatomy of *C. mitchellii* stems in relation to rooting ability

Roots appeared to be initiated in the phloem tissue, possibly at the ends of xylem rays, which occur between adjacent vascular bundles (Figure 15). Thus, the heavy lignification of the xylem tissue internal to the phloem would not present a barrier to root emergence. Suberin was absent in sections examined, indicating that suberised tissue does not present a mechanical barrier to root emergence in *C. mitchellii*. Sclerenchyma sheaths were observable in *C. mitchellii* semi-hardwood stems at two slightly different stages of development (Figures 16 and 17). Contrary to the frequent observation of greater sclerification in older stems, the sclerenchyma sheaths were thinner and more dispersed in the older stem. Roots appear to emerge from between adjacent vascular bundles. Thus the presence of sclerenchyma does not appear to be inhibiting the emergence of roots through the phloem and outer layers of bark. Further data is required on the presence of sclerenchyma tissue in softwood and hardwood cuttings. However, since the rooting percentage obtained for hardwood cuttings was very similar to that for semi-hardwood, the degree of sclerification of hardwood cuttings is not expected to influence rooting. Anatomical studies of *C. mitchellii* to date do not support observations of others that increased sclerification of older stems inhibits rooting (Beakbane, 1961, 1969; Goodin, 1965; Mahlstede & Watson, 1952). However, these results support the conclusion of Sachs *et al.* (1964) that there is no simple relationship between ease of rooting and the density or continuity of a ring of sclerenchyma seen in transverse stem sections.

Conclusion

Softwood stem cuttings of *C. mitchellii* treated with a gel containing 3000 ppm IBA gave an average rooting percentage of 87.5%. An air temperature of 28°C with a root zone temperature of 28°C was found to be most effective for softwood cuttings. Stem anatomy did not appear to influence rooting. Preliminary experiments indicated that maximal rooting for hardwood cuttings of *C. patens* is obtained by treating cuttings with a gel containing 3000 ppm IBA. Hardwood cuttings of *P. pinifolia* rooted best when treated with a solution containing 5000 ppm IBA dissolved in 50% ethanol. Investigation of the rooting response of softwood material of these two species as well as the application of root zone heating may lead to significant improvements in the rooting percentages obtained for hardwood cuttings.

From this study, the recommended propagation requirements for optimal rooting of *C. mitchellii* cuttings are as follows:

1. The use of an IBA-based auxin treatment in a gel formulation, containing mineral nutrients but not containing NAA;

2. Application of root zone heating at a temperature no greater than 28°C;

3. The use of softwood cutting material in preference to hardwood material.

Propagation of *Conospermum mitchellii* appears to be commercially viable. Further research into its cultivation requirements, cut flower characteristics and potential market demand will enable it to be utilised as a floricultural or horticultural crop. *Conospermum patens* and *Persoonia pinifolia* may have similar commercial prospects, but there is a need for further research into their propagation requirements.

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