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& DEVELOPMENT CORPORATION

Double Pink Waxflower

Propagation Development

A report for the Rural Industries Research and Development Corporation

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Foreword

Geraldton wax has been cultivated as a garden ornamental for many decades, and more recently cultivated for large scale floriculture.

Early commercial plantings concentrated on traditional plants with colours of purple, pink and white. The past decade has seen the development and introduction of many new varieties having differing form and colour.

A unique new variety of wax bearing pink double flowers is being developed for release to the industry.

This project deals with difficulties in the propagation of this variety. Overcoming difficulties in propagation will make available to industry and subsequently the general public, a unique new variety adding to the range of waxflower varieties in commercial production.

This project was funded from RIRDC Core Funds which are provided by the Federal Government.

This report, a new addition to RIRDC's diverse range of over 600 research publications, forms part of the Wildflowers and Native Plants R&D program, which aims to improve the profitability, productivity and sustainability of the Australian Wildflower and Native Plant Industry.

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Managing Director
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Objectives

To investigate the propagation techniques of the new double pink flowering waxflower with a view to improving the success rate during the propagation phase.

An improvement in the propagation success rate will make available a new and unique waxflower for the wildflower and native plant component of the floriculture industry.

Background

Geraldton wax contributes a large proportion of the Australian wildflowers and native plants segment of the export and local cut flower industry. Early plantings concentrated on a few cultivars with limited range in flower colour and flowering time, which imposed constraints upon growers in terms of their continuity of supply to the market.

Additional varieties have now been selected and developed which provide a broader range in the size and colour of the flowers as well as an extended flowering period. This new and unique double pink flowering variety will provide additional impetus to the waxflower market.

All waxflower plants originate from seedlings or cuttings taken from seedling plants. Each plant shows some variation in its genetic makeup and physiology impacting upon the vigour of the plant, the size and colour of the flowers and the period of time over which it flowers. Environmental factors such as day length and temperature also make some contribution.

These individual plants also present degrees of difficulty in their propagation. Many are easy to propagate and others very difficult. The double flowering variety falls into the class of being difficult to propagate.

Propagation involves the selection of appropriate plant material, treating it with fungicide and plant growth substances, use of suitable growing media and subsequent location in the environmental conditions will enhance plant growth. The success rate depends upon the best combination of factors for that particular clone.

Outcome

The project has enabled improved techniques to be applied to the propagation of waxflowers. This has made it possible to increase the yield of the more difficult to propagate clones of waxflower. When compared to other varieties the yield for the double pink clone was extremely low but when a different protocol is employed the yield increases considerably.

The double pink flowering waxflower is now to be released to industry for production by selected growers. Since it is a later flowering unique variety, more continuity of supply will assist the waxflower growers and flower markets.

1. General Introduction

1.1 Introduction

Waxflowers now form the largest component of the fresh cut flower export market for Australian flowers.

The term waxflower has been adopted by the local and export fresh cut flower industry to refer to the flowers belonging to the genus *Chamelaucium*. This genus is unique to the south west of Western Australia. The major proportion of the waxflowers cultivated can be attributed to various cultivars of the plant commonly called Geraldton Wax, or *Chamelaucium uncinatum*.

Prior to the 1960's waxflowers formed part of ornamental and landscape plantings. Fresh flowers were sourced from natural stands and plants in gardens. Several selected clones having purple, pink and white flowers had been cultivated. This continued with very few additional varieties for many years.

The now popular variety *Chamelaucium uncinatum* 'Purple Pride' introduced by George Lullfitz in the early 1970's has now become one of the industry standards.

1.2 Commercial Waxflower Production

The commercial production of waxflowers for the fresh cut flower market began in the late 1970's when several large areas were planted. Flowers are exported to Europe, Japan and North America. The varieties included the purple flowered 'Purple Pride' and white flowered 'Alba'. A pink medium flowering variety called 'Mullering Brook' was also introduced.

Other species of waxflower such as *C. ciliatum*, *C. axillare*, *C. megalopetalum* and *C. floriferum* were also grown in smaller quantities.

Production areas were located in different areas of Western Australia and other locations in Australia.

Waxflowers are now grown commercially in many overseas regions such as Israel, South Africa, South America and Europe.

1.3 New Varieties of Waxflower

The increasing demand for fresh cut flowers for the export market has also led to demand for continuity of supply over longer periods of the year. This has been satisfied to some extent by having production areas in different locations in hemisphere and latitude.

The introduction of new varieties has been performed by a very few astute persons who are familiar with the locations and potential of individual plants. These plants have been selected carefully for their desirable characteristics from plants in natural populations and seedlings in cultivated gardens.

Introduction of the Plant Variety Rights [PVR] and subsequently Plant Breeders Rights [PBR] legislation in the 1980's provided some incentive [mostly financial] for those who selected and were engaged in the breeding of plants to develop new varieties.

Deliberate breeding programs have commenced in an attempt to develop new varieties with desirable characteristics. The direction in which these programs move will probably be directed towards the following:

- selection of plants more suitable for particular environmental conditions

- hybridisation [inter specific and inter generic]
- potted plants

1.4 Propagation of Waxflower

The propagation of waxflowers is mostly by cuttings. However, as breeding programs develop other techniques such as tissue culture and grafting will become involved.

The objective of this project is to examine ways of increasing the success rate in the propagation of the new waxflower variety having double pink flowers.

It has proved to be extremely difficult to propagate in comparison to other common clones, which vary in their ease of propagation.

Figure 1 Close up photograph of the double pink flowering variety of *Chamelaucium uncinatum.*

Figure 2 Photograph of the double pink flowering variety of *Chamelaucium uncinatum* showing a single stem.

2. METHODOLOGY

2.1 Introduction

The selection of plant material for propagation is usually made from plants that display particular desirable characteristics. These may be related to flower colour and size, time of flowering, vase life, the habit of the plant or adaptability to certain environmental and cultural conditions.

Most trees and shrubs reproduce naturally from seed. The flowers are mostly cross-pollinated and the plants are heterozygous, therefore, genetic variability is most likely to be observed in the offspring (Hartmann and Kester, 1983, p87). Individual seedlings and hence mature plants from a population of plants will possess genetic differences expressed themselves in various ways.

Waxflower plants can be propagated using seed, however, this leads to variations in the offspring. This technique is therefore not used to propagate selected clones but limited to plant breeding and situations where deliberate production of new varieties is the desired outcome.

To produce plants having identical characteristics to the parent plant, vegetative propagation must be used. This usually involves specific techniques such as cuttings, grafting or tissue culture.

Since each individual seedling is to some extent genetically different it has the potential to influence plant growth when the plant is propagated vegetatively. It is generally known by experienced propagators that individual plants of a particular variety reveal degrees of difficulty in the production of roots on cuttings. Therefore, if possible, one should select the seedling that propagates more easily. For general propagation of a species this is the desirable method. When a seedling, which has unique characteristics, is selected from the population this method is not possible. It may be easy to propagate or be extremely difficult. An appropriate protocol must then be developed to propagate the unique plant.

The age of the plant from which the vegetative material is taken also has an influence upon the ease of propagation. Generally, the more juvenile the plant the easier it is to reproduce. If the selected plant is many years old it could be very difficult to propagate. There have been some instances where plants produced by tissue culture through several generations become somewhat easier to propagate, perhaps indicating that some juvenility has been developed in the plant explants whilst in culture.

This suggests that several methods should be investigated to enable the double flowering pink waxflower to be successfully propagated.

2.2 Cuttings

Propagation of plants by cuttings is the most commonly used technique used to reproduce particular cloned varieties of plants. This is because it is easier and more economical.

Almost all waxflower plants are propagated using stem cuttings. Some cultivars such as 'Purple Pride' produce roots readily at percentages from sixty to eighty percent but others such as the double pink have percentages much less than ten percent.

Factors which contribute to cuttings producing roots include the type of cutting material selected, the treatment provided to the cuttings and the environmental conditions into which they are located.

The object of the project was to investigate ways of increasing the percentage of cuttings which produce roots.

The method adopted was to select various cutting material from terminal shoots in active growth, side shoots in active growth and a larger more complete semi-mature shoot. This type of material is normally used on various other cultivars at different times during the year.

Two different media were selected, peat and perlite and a sand/sawdust/pinebark based mix. Both included slow release fertiliser incorporated into the mix. The sand/sawdust/pinebark based mix was pasteurised at 60 °C for half an hour using aerated steam.

Each type of cutting was treated with fungicide and plant growth substance. Eight different treatments were selected based upon powdered and liquid formulations normally employed by different propagators.

Table 1 Treatments applied to cuttings for each type of cutting and media selected.

1	Cutting powder 8000 ppm IBA The end of each cutting is dipped into the powder
2	Cutting powder 16 000 ppm IBA The end of each cutting is dipped into the powder
3	Easy Root liquid 1.6 g L^{-1} IBA + 1.6 g L^{-1} NAA [25 mL of stock solution diluted to 1 L with water] The ends of the cuttings were dipped into the diluted solution for 15 s
4	Easy Root liquid 1.6 g L^{-1} IBA + 1.6 g L^{-1} NAA [10 mL of stock solution diluted to 4 L with water] The whole cuttings were dunked into the diluted solution for 15 minutes
5	Easy Root liquid 1.6 g L^{-1} IBA + 1.6 g L^{-1} NAA [10 mL of stock solution diluted to 4 L with water] The whole cuttings were dunked into the diluted solution for 15 minutes followed by dipping into cutting powder 8000 ppm IBA
6	IBA solution 2000 ppm [2 L of 2000 ppm IBA diluted to 10 L with water] The ends of the cuttings stand in this solution for 2 hours
7	IBA solution 2000 ppm [2 L of 2000 ppm IBA diluted to 10 L with water] The ends of the cuttings stand in this solution for 4 hours
8	Easy Root liquid 1.6 g L^{-1} IBA + 1.6 g L^{-1} NAA [10 mL of stock solution diluted to 4 L with water] The whole branch from which the cuttings are to be taken was stood in the diluted solution for 4 hours Cuttings were prepared and dipped into Previcur, followed by dipping into cutting powder 8000 ppm IBA

The cuttings were placed into individual cells in propagation trays and located in a poly tunnel on mesh benches. The cuttings were watered in with Previcur fungicide solution and followed by application of fungicide at weekly intervals alternately with Benlate, Previcur and Fongarid.

Watering is provided by overhead high pressure mist sprays operated by a time clock. Temperatures in the tunnel during the trial were 25 - 30 °C during the day and 15 - 18 °C during the night.

Each treatment involved 96 cuttings for each type of cutting material and media. This process was repeated twice, thus making a total of 288 cuttings for each cutting type and media. When the cuttings had begun to produce roots, the trays were relocated into a shadehouse (70% shadecloth) on mesh benches and watered by overhead mini sprinklers.

2.3 Grafting

Grafting is a technique often used in ornamental horticulture to propagate plants that cannot be produced easily from cuttings or they do not survive successfully on their own roots due to unfavourable environmental conditions.

In the case of *Chamelaucium uncinatum* it should be possible to graft the difficult to root clone on a stock of the same species that makes roots more readily. This would enable plants with the desirable characteristics to be produced. If the success rate in propagating plants by cuttings is reasonably high, say 50%, then it becomes uneconomical to graft *Chamelaucium uncinatum*.

Another reason for grafting of *Chamelaucium uncinatum* would be to identify a compatible rootstock tolerant to normally unsuitable environmental conditions such as soil pH or soil type. There is potential to investigate different rootstocks suitable for *Chamelaucium sp*. The genus *Leptospermum* may present some possibilities, particularly for heavier soil types. If a dwarfing rootstock was identified then it may be possible to develop grafted waxflower plants suitable for pot culture and traditional ornamental and landscape gardens.

Adequate time was not available to pursue this potential technique of propagation for waxflowers.

2.4 Tissue Culture

This propagation technique involves the production of plants from small pieces of plant material grown under aseptic conditions in a controlled environment.

Tissue culture was chosen as a possible technique to assist in the propagation of this difficult wax. Generally, plants that are very difficult to propagate by cuttings are also difficult by tissue culture. Despite this it is desirable to propagate using tissue culture in an attempt to induce juvenility in the plant material.

Cuttings taken from some other plant varieties that have been produced by tissue culture produce roots more readily than cuttings from plants not grown by tissue culture (Personal observation). Similar results have been observed in grevillea species by other propagators using tissue culture.

Preparation of Explants

Plant material was selected from mature plants of the double pink flowering wax and transported to the laboratory for preparation.

Terminal shoots approximately 30 mm long were removed and placed into purified water for washing to partially remove any surface contamination in the form of dust, fungi and bacteria.

Next, the shoots were trimmed slightly and placed in 2% sodium hypochlorite for a period of 15 minutes with regular agitation (The bleach solution was prepared from commercial product 'White King' 4% sodium hypochlorite by dilution). The bleach was removed from the shoots by rinsing three times in sterilised purified water.

The shoots were trimmed (both leaves and stem) to approximately 10 mm before transferring to polycarbonate tubes containing the culture media.

Culture Media

The culture media used for the establishment of the explants was the traditional Murashige and Skoog formulation (MS). The pH was adjusted to 6.0, solidified with 8 g L^{-1} agar, then autoclaved for 20 minutes at 120 °C.

Establishment and multiplication

After four weeks those explants not contaminated were transferred to new media containing the cytokinin benzyladenine (BA) at a concentration of 0.1 mg L^{-1}. The explants were cultured in a growth room at 20 - 25 °C under white light with a 16 hour photoperiod.

Explants were subcultured and transferred to fresh media at four weekly intervals. Growth is primarily lengthwise and the multiplication rate is low and very slow. Those explants taken from the lower stem portion have a much lower survival rate than the shoots.

Further multiplication will be undertaken until sufficient explants are available to conduct further trials. Trials with differing MS strengths, cytokinins, and auxins will continue to determine the most suitable multiplication media and rooting media.

The project has reached this stage of propagation of the double wax in vitro.

Figure 3 Photograph of the double pink flowering variety of *Chamelaucium uncinatum* showing shoots growing in tissue culture.

3. RESULTS

3.1 Data

After all trays of plants had been in the shadehouse for six weeks the number of cuttings that had produced roots were recorded. These are shown in Table 2.

Table 2 Total number of cuttings with roots for each treatment, type of cutting and media

Total cuttings with roots [out of a total of 288]						
CUTTING TREATMENT	**TERMINAL SHOOT**		**SIDE SHOOT**		**SEMIMATURE SHOOT**	
	PeatPerlite	S/SD/PB	PeatPerlite	S/SD/PB	PeatPerlite	S/SD/PB
1	24	24	33	28	23	20
2	32	33	36	33	31	30
3	47	38	63	59	47	45
4	61	46	75	64	59	49
5	61	58	100	87	72	62
6	34	27	49	38	37	25
7	33	28	51	42	38	31
8	36	35	56	47	46	38

For comparison purposes these numbers are also shown as percentages in Table 3.

Table 3 Percentage of cuttings with roots for each treatment, type of cutting and media

Percentage cuttings with roots						
CUTTING TREATMENT	**TERMINAL SHOOT**		**SIDE SHOOT**		**SEMIMATURE SHOOT**	
	PeatPerlite	S/SD/PB	PeatPerlite	S/SD/PB	PeatPerlite	S/SD/PB
1	8.3	8.3	11.5	9.7	8.0	6.9
2	11.1	11.5	12.5	11.5	10.8	10.4
3	16.3	13.2	21.9	20.5	16.3	15.6
4	21.2	16.0	26.0	22.2	20.5	17.0
5	21.2	20.1	34.7	30.2	25.0	21.5
6	11.8	9.4	17.0	13.2	12.8	8.7
7	11.5	9.7	17.7	14.6	13.2	10.8
8	12.5	12.2	19.4	16.3	16.0	13.2

3.2 Graphical Representation of Data

The data are graphed in Figure 1 to Figure 5 to illustrate the percentage of cuttings with roots for differing treatments and propagation media

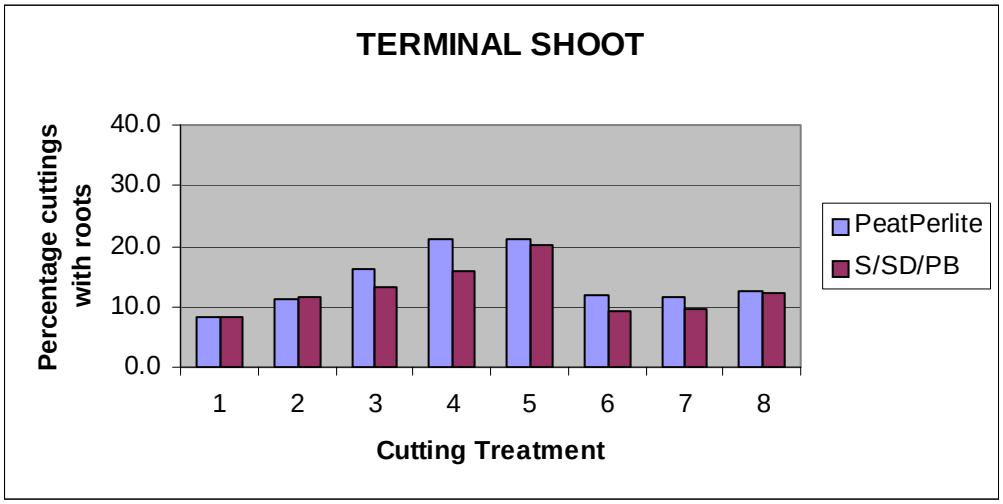

Figure 4 Percentage of terminal shoot cuttings with roots using various cutting treatments and for two different media

Figure 5 Percentage of side shoot cuttings with roots using various cutting treatments and for two different media

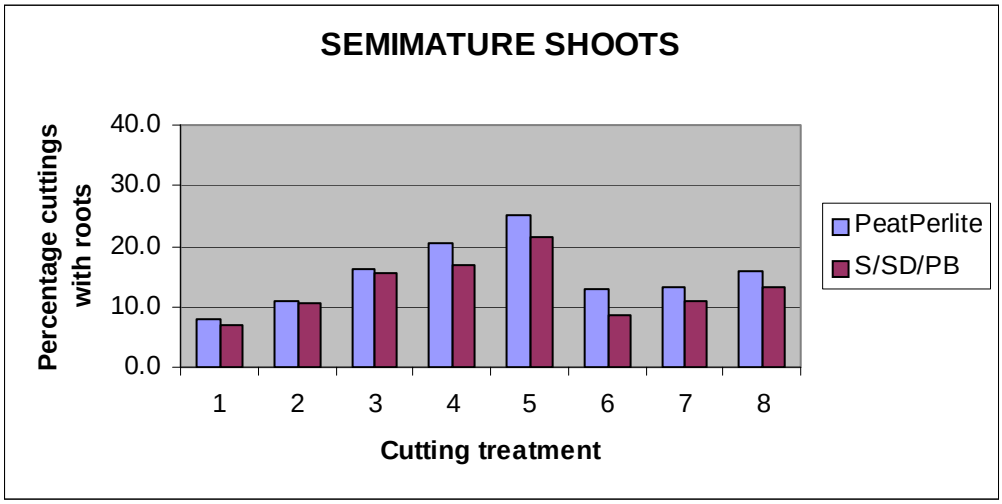

Figure 6 Percentage of semimature shoot cuttings with roots using various cutting treatments and for two different media

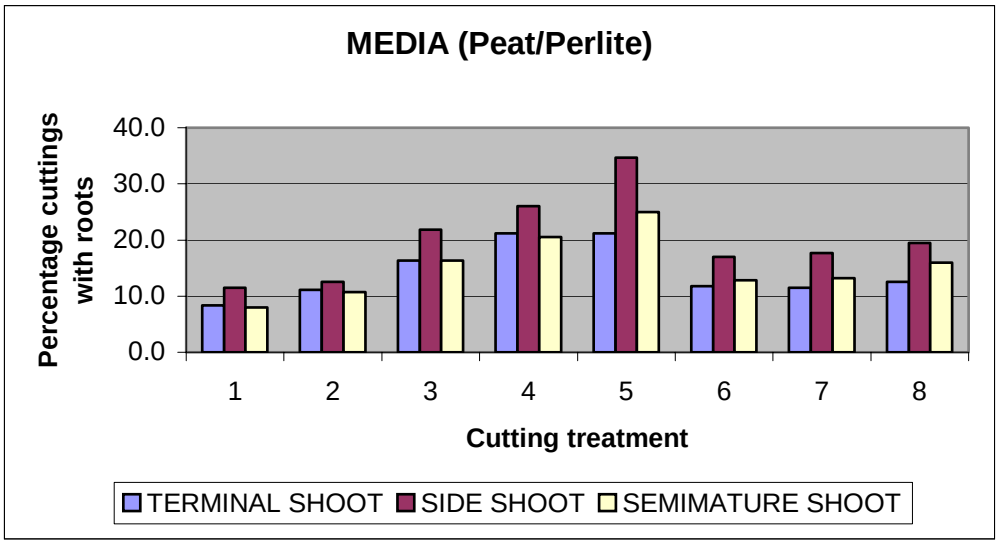

Figure 7 Percentage of each type of cutting with roots using various cutting treatments and for the Peat/Perlite media

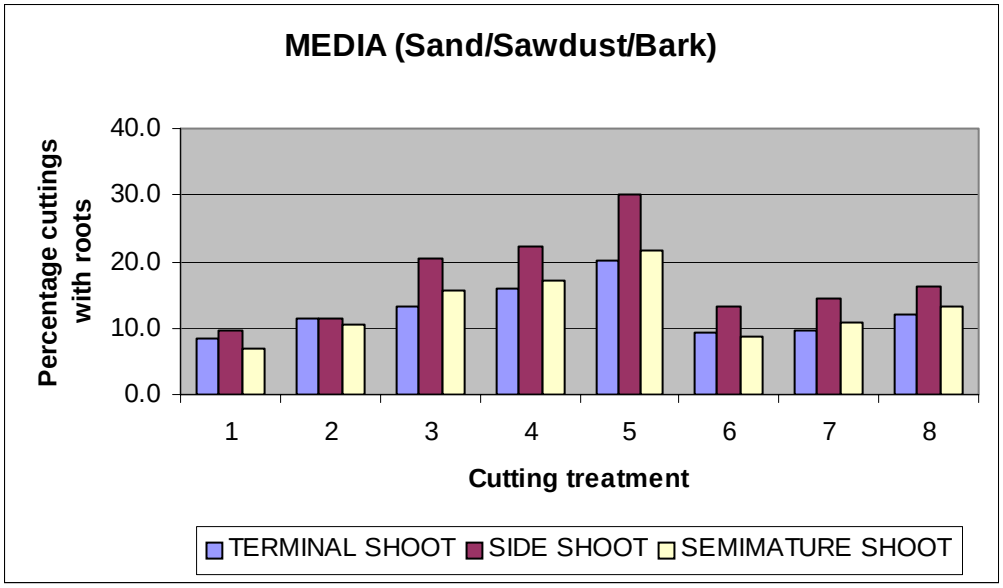

Figure 8 Percentage of each type of cutting with roots using various cutting treatments and for the Sand/Sawdust/Bark media

4. DISCUSSION AND CONCLUSION

4.1 Effect of media

Examination of the data as represented in Figure 4, Figure 5 and Figure 6 illustrates a marginal difference in the effect of the media on cuttings that produce roots. The media containing a mixture of perlite and peat appears to produce more success in all cutting treatments.

For the terminal shoots, the effects of different media are small for treatments 1, 2, 5 and 8, but larger for treatments 3, 4, 6 and 7. The side shoots produce a larger difference in all treatments with variations from 1% for treatment 2 up to 4.7% for treatment 5. Similarly, for the semimature shoots, very little difference for treatments 1, 2 and 3 but greater variation for treatments 4, 5and 6.

These results would therefore suggest that the perlite/peat media leads to a greater success rate, particularly for treatments 4, 5 and 6.

4.2 Type of cuttings

The type of cutting selected for propagation produces quite a variation in the success rate. Terminal shoots in active growth varied from 8.3% to 21.2% success, side shoots in active growth 9.7% to 34.7%, and semmimature shoots 6.9% to 25%. The range depending upon the treatments applied to the cuttings.

It is clear from Figure 7 that greatest success is obtained using side shoot cuttings for all treatments. The semimature cuttings produce better results than terminal shoots

4.3 Cutting Treatments

When propagating by cuttings it is usual practice to apply root-promoting substance of some kind to assist in root initiation and development. Traditionally, use has been made of a commercially available powder based formulation of talc incorporating IBA (Indole Butyric Acid). Many native plants are propagated using this material either in strength 8000 ppm IBA or 16000 ppm IBA. Generally, success depends on the plant species, condition of the cutting material and environmental conditions under which the cuttings are located following planting.

While this trial was being conducted a batch of *Chamelaucium uncinatum* 'Purple Pride' was also propagated. The success rate for this batch overall was 73% [349 out of 480] using treatment 1 [cutting powder 8000 ppm IBA]. Previous batches of the double pink flowering variety yielded percentages less than 10%.

This trial again yielded similar results using the same treatments. In an attempt to obtain higher yields, other cutting treatments were selected. These were based upon similar treatments applied to other recalcitrant plant varieties.

Treatments 1 and 2 involve the normal process of preparing the cuttings and dipping them into the powder formulation 8000 ppm IBA and 16000 ppm IBA, followed by insertion into the media. Results were similar to those obtained previously with little variation within the type of cutting.

Treatments 6 and 7 involved an IBA solution containing 400 ppm IBA. Cuttings were prepared then dipped for 2 hours and 4 hours prior to placing into the media. Results show an increase of 1-2% for terminal and semimature shoots, but approximately 5% for side shoots. Explanation for these increases could be due to a more effective contact of IBA with the plant tissue using the liquid preparation.

Treatments 3 and 4 involve a liquid preparation involving a mixture of IBA and NAA (Naphthalene Acetic Acid). The first contained 40 ppm IBA and 40 ppm NAA solution, the ends of the cuttings were dipped for 15 seconds prior to inserting into the media. The second involved 4 ppm IBA and 4 ppm NAA solution, but this time the cuttings were immersed into the solution for 15 minutes then inserted into the media. Results indicate a general increase in strike rate, side shoots now up to 22% for treatment 3 and 26% for treatment 4. The increase would not appear to be attributable to IBA levels but perhaps the NAA and again the more effective contact of auxin with plant material.

Treatment 5 was the same as treatment 4 but in addition, the cuttings were dipped into 8000 ppm IBA powder formulation. Results indicate the best strike rate, up to 34% for side shoots.

Treatment 8 involved the whole piece of plant material from which the cuttings were to be taken being dipped into a 4 ppm IBA and 4 ppm NAA for 4 hours. The cuttings were then prepared, dipped in Previcur followed by dipping into powder formulation containing 8000 ppm IBA. The results this time were lower than for treatments 3 and 4.

Overall it would appear that treatment 5 produces greatest strike rates. The reason why this has occurred is not readily apparent. The liquid formulation containing auxins appear to produce greater strike rates than those having powder formulations. Those containing NAA appear to contribute to a greater strike rate.

4.4 Conclusions

This trial was conducted in an attempt to develop a propagation protocol that produced an increased strike rate therefore enabling plants to be available for release to commercial growers.

The outcomes of this trial show that the best method appears to be by cuttings. Greatest percentage strike rate was obtained using side cuttings in active growth, and treating them with liquid formulations containing auxins in combination with the powdered formulation. Further increase in strike rate could be expected by use of bottom heating, but it was not available for this project.

The outcomes from tissue culture are not yet available due to the very slow rate of growth and multiplication in vitro. This will continue to be pursued until plants are produced.

Grafting was not pursued due to the slower rate of production and additional costs that make this method of propagation uneconomical. It should be possible to graft this variety to a suitable rootstock more suited to a particular location. This technique will be followed up as time permits.

A more comprehensive trial needs to be performed in order to determine the effects of specific variables upon the successful production of this variety.

5. REFERENCES

Hartmann, H.T., Kester, D.E., (1983). Plant Propagation Principles and Practices. 4th Ed., Prentice-Hall, Sydney. p87