Improving the In Vitro Culture of Geraldton Wax (*Chamelaucium uncinatum*)

B.P. Croxford, C. Newell, J.A. Considine, and G. Yan
Plant Sciences, The University of Western Australia, NEDLANDS WA 6907

Experiments were carried out to study the in vitro culture of *Chamelaucium uncinatum* hybrids. As the concentration of 6-benzylaminopurine (0 to 1.2 μM) in the medium increased, so did the number of lateral shoots (0 to 10.45). Rootstrike was shown to be significantly influenced by genotype and concentration of indole-3-butyric acid (0 to 10 μM) with 2.5 μM being optimal (93.3%). Transfer to Growool™ increased the survival rate of deflasked plantlets (97.1%) when compared to direct transfer to potting mix (51.4%).

INTRODUCTION

Geraldton wax (*Chamelaucium uncinatum* Schauer) is the most promising of the new floricultural crops selected from the Australian flora (Considine et al., 1994). The waxflower industry is mainly based on cultivars selected from wild and cloned via tip cuttings; however, continued growth will require improved second and third generation cultivars. Therefore, a joint breeding program between the University of Western Australia and Agriculture Western Australia was formed. Embryo rescue has been successfully employed to overcome problems associated with dormancy and postzygotic abortion in the breeding of this plant (Yan and Newell, pers. commun.).

Little is known about the in vitro propagation of this species, especially the highly variable hybrids from the breeding program, and problems were soon encountered with regard to in vitro rhizogenesis and transfer of plantlets to soil. This research aims to explore the use of cytokinin and auxin to optimise the growth and morphogenesis of in vitro Geraldton wax hybrids and to evaluate the use of Growool™ as an alternative substrate for deflasking.

MATERIALS AND METHODS

Three *C. uncinatum* hybrids were chosen at random for all experiments. Embryos were rescued and established in vitro and shoots formed were regularly subcultured to medium containing MS salts (Murashige and Skoog, 1962), with 0.3 μM 6-benzylaminopurine (BAP). The media used in all experiments contained 8 g litre^{-1} agar powder and 20 g litre^{-1} of sucrose. The pH was adjusted to 7 and 8 ml of the medium was dispensed into each 30-ml culture tube. All cultures were incubated in a culture room at 25±1C under fluorescent light providing a fluence rate of 30 μmol m^{-2} sec^{-1} for a 16 h photoperiod.

The effect of BAP concentration on multiplication rate was assessed using media as above but containing either 0, 0.15, 0.3, 0.6, or 1.2 μM BAP. One shoot tip 15 mm long was inserted approximately 3 mm into the medium per tube with ten replicates for each treatment/genotype combination. After 60 days in the culture room the cultures were measured and shoots divided into 3 size groups; <10 mm, 10 to 20 mm, and >20 mm.

The media used in rhizogenesis trials were as above except they contained half-

strength MS salts, no BAP and either 0, 1.25, 2.5, 5, or 10 µM indole-3-butyric acid (IBA). Cuttings 25 mm long had leaves from the lower three nodes removed and each microcutting was inserted approximately 5 mm deep in the medium per tube with ten replicates for each treatment/genotype combination. After 21 days in the culture room, the rooting percentage was measured.

To determine the carryover effect of BAP concentration used in the multiplication stage on rootstrike, shoots greater than 20 mm from the multiplication experiment were cut to 20 mm and transferred to media identical to that used in the rooting trial containing 2.5 µM IBA. Where available 10 replicates for each genotype/BAP pretreatment combination were used and rootstrike was assessed after 21 days in the culture room.

Seventy rooted shoots were used in a transfer trial. Half of the shoots from each genotype were transferred to individual pots (70 mm 5 50 mm) containing Waldecks™ potting mix and the other half were transferred to Growool™ cubes (25 mm 5 25 mm 5 40 mm) prepared by soaking in a half-strength solution of MS. Every attempt was made to ensure that similar shoots were used in both treatments. Pots and cubes were kept at high humidity and under 75% shade inside a glasshouse. Plantlets in soil were watered daily. Plantlets in Growool™ were watered with a half-strength MS solution (pH 7) so that cubes were always moist. Humidity was gradually reduced and the plastic covers were completely removed after 1 month.

RESULTS

The concentration of BAP significantly influenced shoot number ($p < 0.05$); however, genotype had no significant effect ($p > 0.05$). Although the total number of shoots produced increased as BAP concentration increased, this was mainly due to an increase in small lateral branches (Fig. 1). The number of shoots <10 mm and 10 to 20 mm is significantly greater at the higher concentrations of BAP when compared to the number of shoots >20 mm which remains relatively constant as BAP concentration increased.

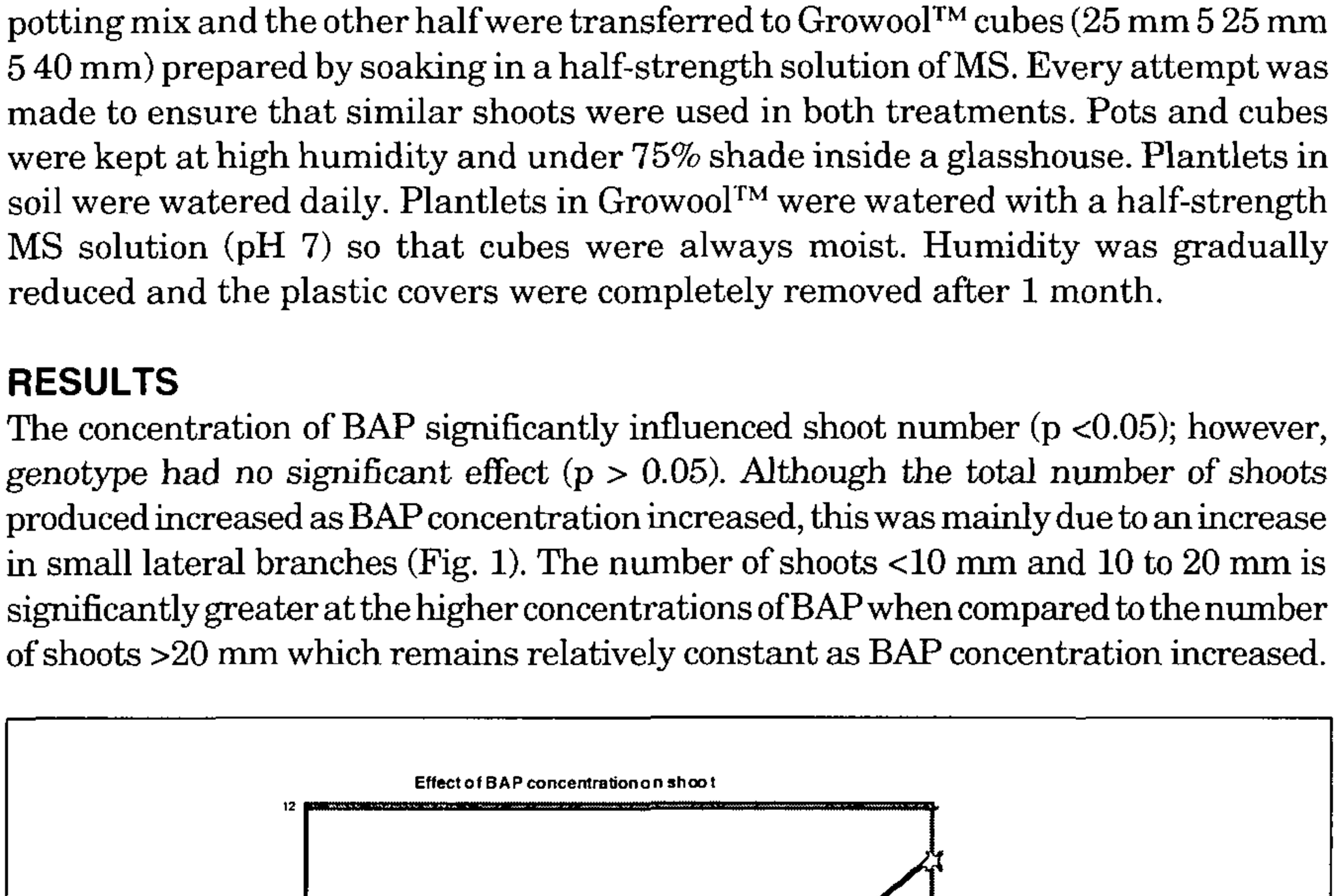

Figure 1. The effect of BAP on shoot number

In cultures where BAP was excluded no additional shoots were produced, the initial explant had grown less, greater than 10% of their leaves had senesced, and some roots formed which did not occur in any of the cultures containing BAP.

The percentage of shoots which formed roots was dependant on genotype and concentration of IBA (Table 1). As IBA concentration increases, rootstrike increases to a peak with IBA concentration at 2.5 μM and then decreases at higher levels up to 10 μM (Fig. 2).

Table 1. Rooting percentage of *Chamelaucium uncinatum* explants cultured for 21 days at various concentrations of IBA.

	IBA concentration (μM)				
	0	1.25	2.5	5	10
548/OP-1	40 a*	40 a	90 b	60 a	40 a
640/OP-3	70 a	90 ab	100 b	100 b	100 b
772/OP-1	70 ab	70 ab	90 bc	100 c	40 a

* Numbers followed by different letters are significantly different (p=0.05).

Pre-treatment with BAP had little effect on rootstrike percentage with few interesting differences between treatments and no general trend (Table 2).

More rooted plantlets survived the transfer to the greenhouse when Growool™ was used (97.1%) compared to direct transfer to potting mix (51.4%).

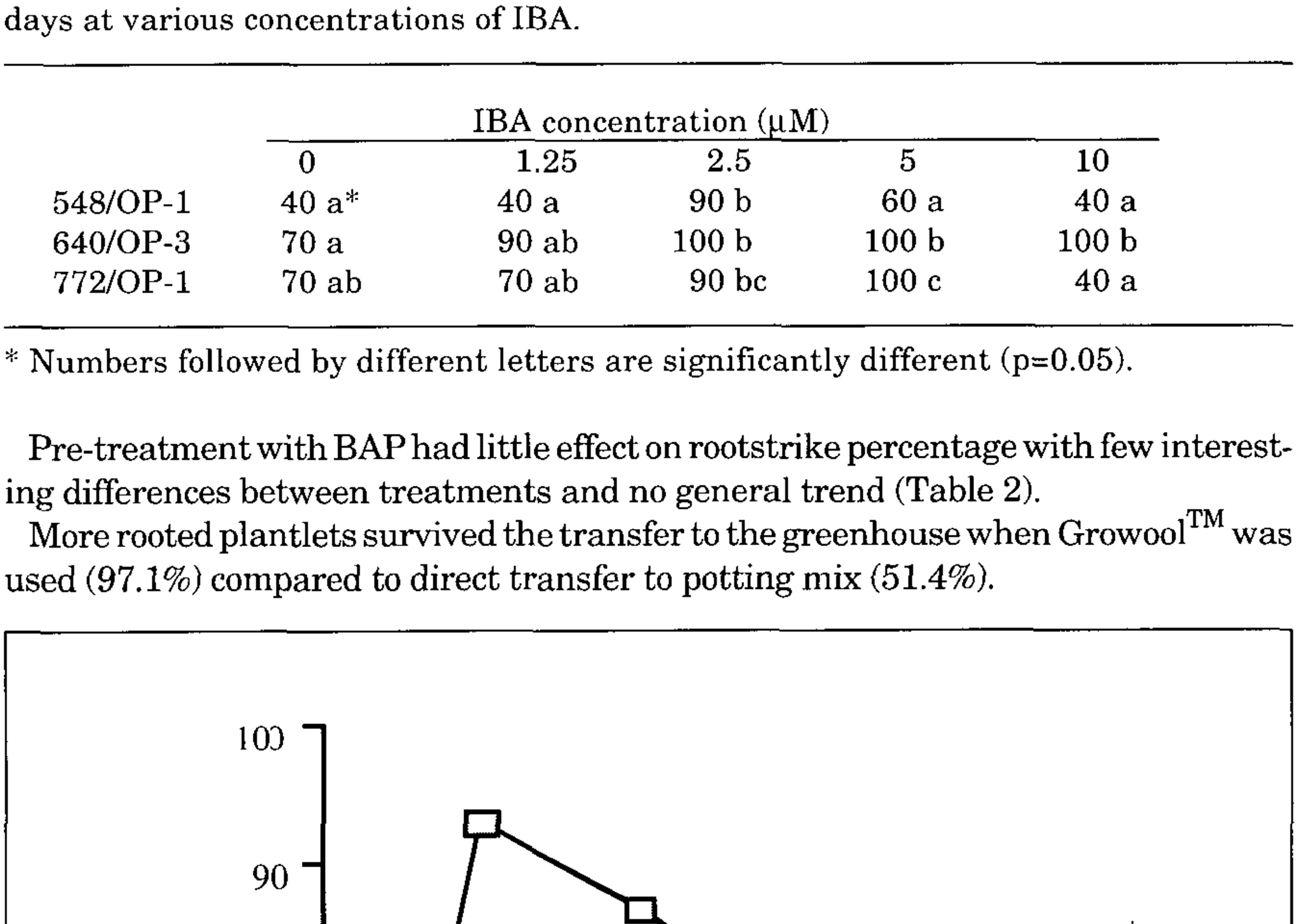

Figure 2. The effect of IBA (μM) on rooting.

Table 2. Rooting percentage of *Chamelaucium uncinatum* explants cultured for 21 days at 2.5 µM IBA following multiplication on different concentrations of BAP pretreatment.

		BAP pre-treatment (µM)				
	Genotype	0	0.15	0.3	0.6	1.2
% Rooting	1	66.7 a*	66.7 a	70 a	88.9 a	80 a
	2	25.0 a	85.7 b	80 b	75.0 b	80 b
	3	33.3 a	55.5 a	50 a	22.2 a	40 a

* Numbers followed by different letters are significantly different (p=0.05). .

DISCUSSION

The multiplication results are consistent with Speer (1993), and support a two-step multiplication stage where several small shoots are produced at high (1.2 µM) BAP that can be excised and transferred to low (0.15 µM) BAP medium and allowed to elongate without further branching and thus be suitable for rootstrike. Exogenous cytokinin was shown to be essential for growth and multiplication of these shoot tip cultures as root apices are the main centre for cytokinin production (Koda and Okazawa, 1980). Problems caused by insufficient cytokinin levels included poor shoot growth, inhibition of lateral bud break, and senescence. Rootstrike was completely inhibited by BAP.

No effect on rootstrike was observed in shoots cultured for 60 days at different concentrations of BAP up to 1.2 and then transferred to media containing IBA, suggesting little BAP is transferred to rooting stage or that BAP transferred is quickly degraded or used. The low rootstrike rates when no BAP was used in the multiplication stage is likely to be due to the poor health of cytokinin-deficient cuttings before being transferred to cytokinin-free rooting media. Surprisingly there was little genotypic difference in the response to applied BAP.

The percent of shoots that formed roots was shown to be influenced by both genotype and the concentration of IBA in the media. These results suggest that there is an optimal IBA concentration for rootstrike. This concentration is likely to be influenced by endogenous auxin levels and sensitivity to exogenous auxin. It is likely that individual genotypes differ in these aspects and hence optimal concentration for rootstrike.

Transfer to Growool™ was more successful than direct transfer to soil. Due to the high humidity and regular watering required for the survival of these plantlets, the oxygen content of potting mix is reduced and root growth suffers due to reduced respiration. Growool™, however, will maintain a percentage of air while providing easily available water (Donnan and Biggs, 1984).

LITERATURE CITED

Considine, J.A., Egerton-Warburton, L., Growns, D., and **A. Fuss.** 1994. Preliminary comparison of *Chamelaucium uncinatum* Schauer (Geraldton wax) genetic resources. Proc. 1994 National Workshop for Australian Native Flowers 10:17-20.

Donnan, R.S. and **A.G. Biggs.** 1984. Horticultural rockwool-Australian experiences. Proc 6th Intl. Congress on Soiless Culture PUDOC, Netherlands.

Koda, Y. and **Y. Okazawa.** 1980. Cytokinin production by asparagus shoot apex cultured in vitro. Physiol. Plant 49(2):193-197.

Murashige, T. and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15:473-479.

Speer, S.S. 1993. Micropropagation of some Myrtaceae species which show potential as 'new' ornamental plants. Aust. J. Exper. Agric. 33:385-391.