Simple Methods of Micropropagation

V. J. Hartney and J.G.P. Svensson
CSIRO Division of Forestry, Banks Street, Yarralumla ACT 2600

Many Australian tree species can be readily micropropagated using simple techniques and low-cost facilities. This enables the advantages of micropropagation to be integrated into propagation programs. Advantages may include very high multiplication rates, freedom from pests and pathogens, cheap and reliable transport across quarantine barriers, reliable inoculation with symbiotic microorganisms, and long-term storage of clones *in vitro*. The simple methods of micropropagation described in this paper are worth testing for other species. They can be readily integrated into most existing nursery operations.

INTRODUCTION

Micropropagation has a number of advantages over propagation of plants by conventional cuttings. These include:

- Higher multiplication rates per unit area.
- The ability to easily maintain cultures, once decontaminated, free of pests and pathogens for long periods.
- Cheap transport across quarantine barriers or between nurseries for growing-on of plants close to markets.
- The ability to inoculate plants with specific mycorrhizal fungi or other symbiotic microorganisms which are beneficial to survival and growth after planting.
- The ability to manipulate many more factors in the medium and the environment than is practicable with cuttings.

In Australia, and elsewhere, many commercial micropropagation laboratories exist as specialized facilities with a high capital cost, supplying micropropagated plants to other nurseries for growing-on and marketing.

Plant propagators are very innovative and test numerous propagation techniques to produce plants cheaply. However, micropropagation has been considered to require special skills and expensive facilities, and the technique is not generally used (even on a small scale) by most commercial nurseries. This situation has arisen because in many cases there are poor links between micropropagation researchers and the plant propagation industry. However, some researchers are notable exceptions to this rule (de Fossard and Bourne, 1977).

In addition, the application of micropropagation in the plant propagation industry is hindered by researchers who make unrealistic claims about multiplication rates and the performance of clones over seedlings (Australian Science Technology Newsletter, 1990).

This paper discusses how you can use *microrpopagation techniques* in your nursery with your plants, and minimal additional facilities. Propagation managers can then assess multiplication rates and other critical factors first-hand, in order to make decisions on whether to use specialized facilities or laboratories for large-scale micropropagation. Most plants that can be readily propagated by cuttings can be readily micropropagated.

SIMPLE METHODS

Micropropagation involves four basic steps:

1) Shoot surface sterilization and establishment.

2) Shoot multiplication by repreated subculturing of shoots onto new medium. Cultures can be maintained for long periods of time (years) in this way.

3) Selected shoots are rooted *in vitro* on a rooting medium or set as microcuttings using conventional procedures.

4) Hardening-off and growing-on of rooted plants.

These techniques, and several others, are covered in standard texts such as Plant Propagation by Tissue Culture (George and Sherrington, 1984) and Plant Propagation - principles and practices (Hartmann and Kester, 1983). In principle, micropropagation is similar to propagation by stem cuttings from a block of clonal mother plants. With micropropagation, shoot cultures represent the block of clonal mother plants which produce shoots for rooting *in vitro* or for setting as microcuttings.

1) **Surface sterilization.** Shoots can be readily sterilized in dilute solutions of domestic bleach (about 0.5% chlorine). About 10 to 15 minutes is enough time for most species but you will have to experiment. Shots are then rinsed once or twice in sterile water.

2) **Media formulations.** Many species can be propagated on just a few basic media (George et al., 1987; Hartney and Svensson, 1990) which are readily available as pre-packed mixtures from commercial suppliers (Flow, Sigma, Gibco). Some of these pre-packed formulations contain organic growth factors and plant hormones or these can be added separately if you wish to conduct your own experiments.

In our laboratory we have been able to propagate all of the species listed in Table 1 on only two basic media (Hartney and Svensson, 1990). Many of these species were previously very difficult to micropropagate.

3) **Containers.** Take-away food containers, petri dishes and a wide range of plastic and glass containers are suitable. Aluminium foil and some plastics can substitute for lids of containers.

4) **Growth environments.** We have grown cultures on window ledges, in shade houses (about 80% shade) and on shelves illuminated by one or two fluorescent tubes. Care must be taken not to grow cultures under very high light levels as this will cause the plants to overheat (because of the greenhouse effect in miniature); 10% to 20% full sunlight is ample. Accurate temperature control is not essential for many species.

5) **Laboratory equipment.** Expensive laboratory items are not essential. Transfer chambers for subculturing shoots from one medium to another under sterile conditions can be made out of fish-aquariums with plastic covers and sleeves (Figure 1). Such still-air chambers can be readily sterilized by spraying a dilute chlorine solution (about 1% chlorine) over the work area. Domestic pressure cookers can replace autoclaves for sterilization of media, and simple pH meters or indicator solutions are adequate for small-scale operations.

6) **Environments for rooting and growing-on of plants.** Many species develop roots when they are set as microcuttings in standard misting beds, fog houses or plastic enclosures as used with traditional cuttings.

Table 1. Number of clones of various species micropropagated under various conditions.

Species	Under commercial conditions	Under research conditions	Presently recalcitrant	Rooted ex vitro*
Acacia auriculiformis	4	3	1	
A. maconochieana	1			
A. mangium	6	5		
A. stenophylla	2			
Allocasuarina verticilliata	1			y
Casuarina glauca (= *C. obesa*)			1	
Chrysanthemum cinerariifolium (= Tanacetum cinerariifolium)	2			y
Eucalyptus aggregata	1			
E. andrewsii subsp. *campanulata*			1	
E. annulata	1			
E. blakelyi		2	1	
E. camaldulensis	42	11		y
E. curtisii	1			
E. deglupta	1			y
E. desmondensis	1			
E. diptera	1		1	
E. ficifolia		1		
E. grandis	4	3		
E. gillii	1			
E. globulus subsp. *bicostata*		1	1	
E. globulus subsp. *globulus*	1	6	2	y
E. macrandra	1			
E. marginata	6			
E. melliodora	1			
E. nitens		20	2	
E. nitens x *E. globulus*	2	1		
E. ochrophloia		1	1	
E. ovata		1	1	
E. pileata	1			
E. rudis	1			
E. tereticornis	1			y
E. viridis	1			
E. wandoo		4	5	
E. yarraensis		1		y
Flindersia brayleyana		2		
Melaleuca alternifolia	7			y
M. bracteata	4	1		y
M. cajuputi		1		
M. decora			1	
M. eleuterostachya			1	
M. glomerata	1	2		
M. halmaturorum	2	2	1	
M. lanceolata	1	3	1	
M. lateriflora subsp. *lateriflora*	4	1		
M. quinquenervia			1	
M. thyoides	3	2		
Pinus radiata		20		y
Populus deltoides		2		y
P. deltoides x *P. nigra*		3		y
Simmondsia chinensis		2		
Toona australis		1		
TOTALS	**105**	**103**	**22**	

* = successfully rooted outside of culture

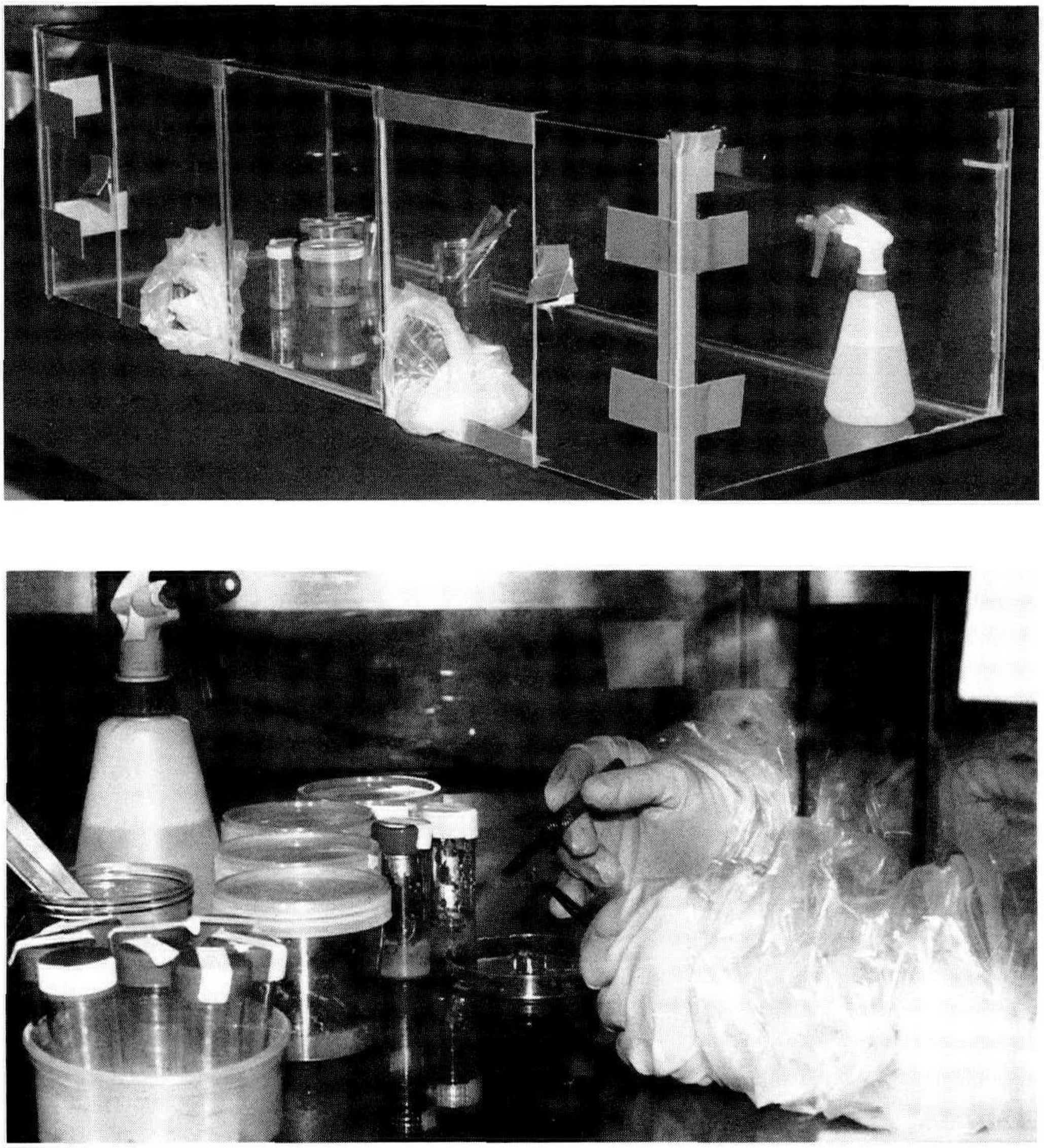

Figure 1. A transfer chamber made from a modified aquarium.

However, micropropagated plants and shoots in culture are growing under high-humidity conditions and they are much more sensitive than standard cuttings to water stress. Shoots or rooted plants in culture can be hardened by allowing water to evaporate from the container. This is simply achieved by covering the container with plastic food wrapping (e.g. 'Glad Wrap', 'Seal Wrap') as many of these are permeable to water vapour (Figure 2). In addition, growing cultures at a higher light intensity prior to hardening-off is often an advantage.

Plants can be grown-on in bean-sprout containers with standard potting mixtures (Figure 3). These containers enable adequate shoot and root growth prior to placing plants in standard nursery trays.

Figure 2. Containers covered with plastic film to enable hardening-off of rooted plants.

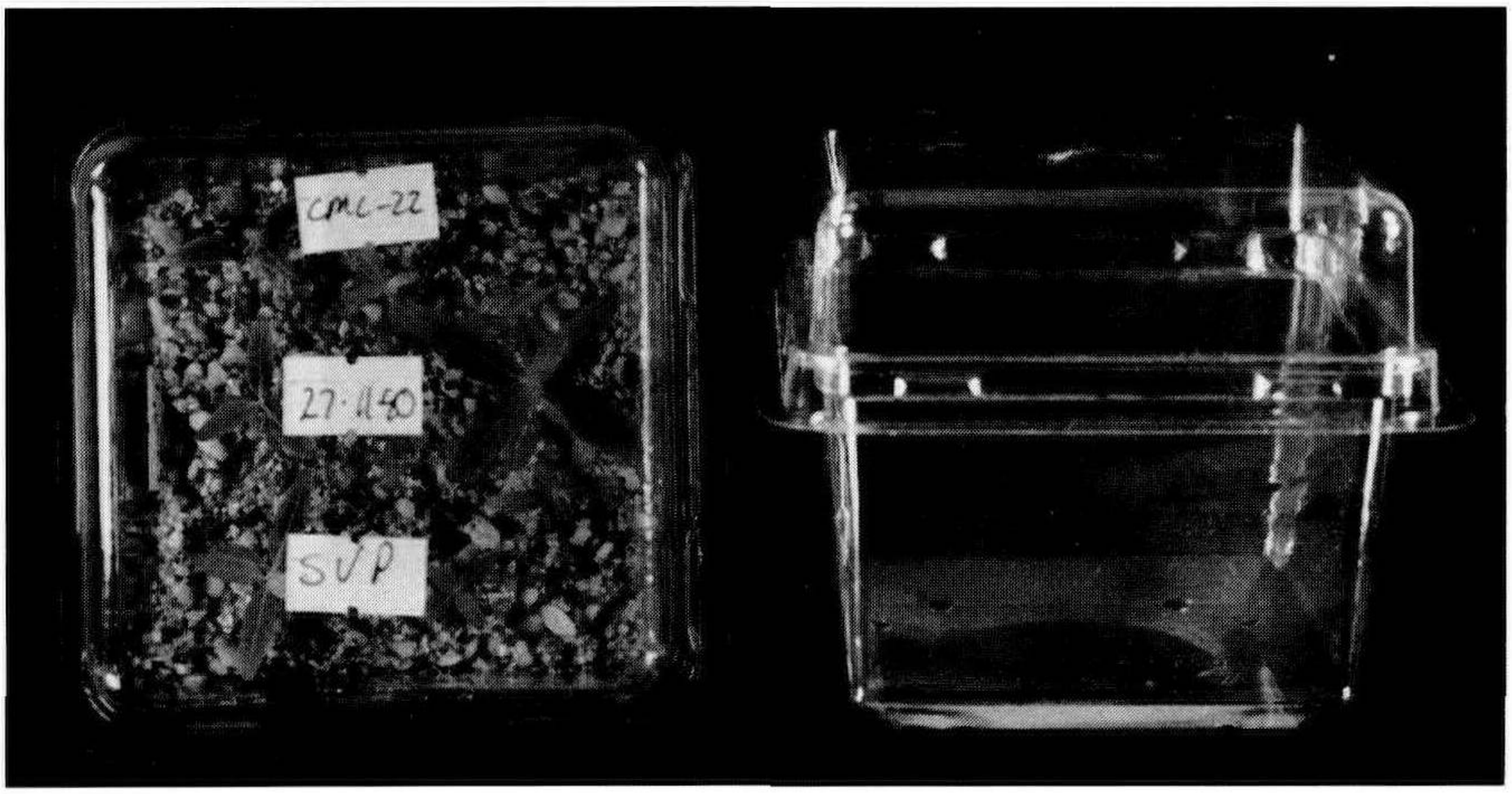

Figure 3. Rooted plants growing in 'miniature greenhouses' made from plastic food containers.

7) **Techniques for cutting shoots.** Sterile transfer and handling techniques are the most important tasks to master with microrpropagation. Test your handling procedures, instrument sterility and possible sources of contamination of cultures by exposing containers of microbial medium inside the transfer chamber.

It is amazing, when learning sterile transfer techniques, how many times you touch sterile surfaces and plants, and transfer contaminants to the cultures. With practice, careful examination of procedures, and with help from a competent operator, the skills of sterile transfer can be quickly learned.

Transfer of shoots to shoot multiplication medium is much faster and results in higher multiplication rates if clumps of shoots, rather than single shoots, are transferred to fresh medium (Figure 4). Single shoots at least 15 mm long are best for placing onto a rooting medium or setting as micro-cuttings. These single shoots can be selected from clumps of shoots or they can be encourabed by growing under low intensity light, or by adding gibberellins to encourage etiolation (George and Sherrington, 1984).

Rates of transfer using the above techniques should be equivalent to the rate of setting cuttings.

LONG-TERM STORAGE OF PLANTS

Shoot cultures growing rapidly require regular subsulturing at about monthly intervals. This is desirable if you want large numbers of shoots but it can represent a high labour cost if you are subculturing shoots only to maintain the line.

We have been able to store a large number of the species listed in Table 1 in a seed germination cabinet at 10° C, or as rooted plants *in vitro* at about 25° C for months or years (Hartney and Svensson, 1990). Under these conditions growth diminishes to a low level. Storage of cultures in such simple facilities may be easier than maintaining disease-free, clonal mother plants for cuttings propagation.

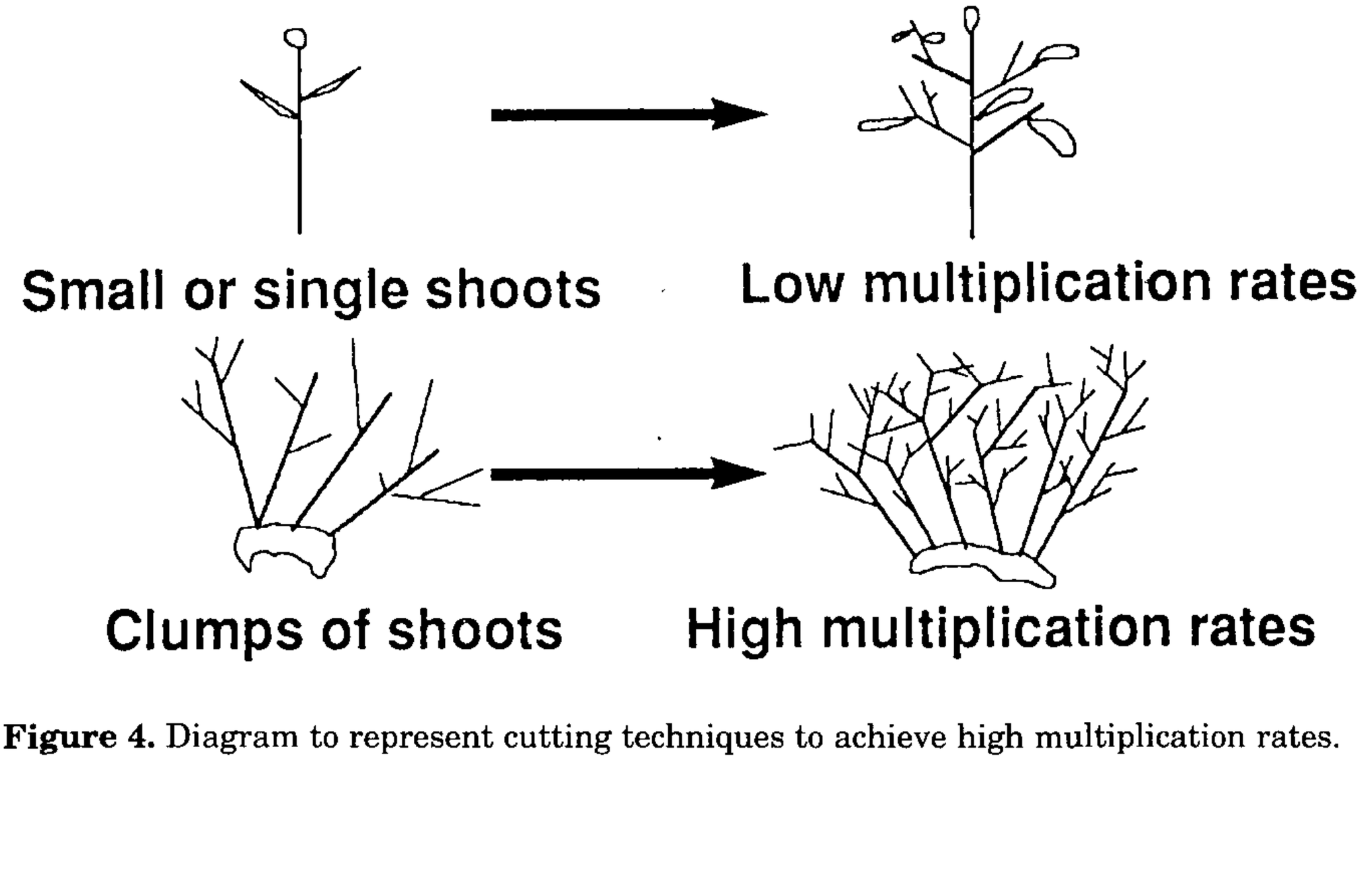

Figure 4. Diagram to represent cutting techniques to achieve high multiplication rates.

Long-term storage of cultures enables one to stock-pile plants to meet high seasonal demands such as for a restricted planting season or seasonal markets.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Tim Vercoe, Mr. David Spencer, Mr. Alan Brown and Mr. Robin Cromer for reviewing the manuscript; Mrs. Karin Munro and Mrs. Eva Morrow for word-processing services, and Mr. Joe Oros for photography.

LITERATURE CITED

Australian Science Technology Newsletter. 1990. Super trees rehabilitate salt-damaged land and yield crop. Aust. Sci. Technol. Newsl. 2:1.

de Fossard, R.A. and **R.A. Bourne.** 1977. Reducing tissue culture costs for commercial propagation. Acta Hort. 78:37-44.

Drew, R.A. 1991. Optimizing root initiation by controlling exposure to auxin. Comb. Proc. Intl. Plant Prop. Soc. 41: 68-73.

George, E.F., D.J.M. Puttock and **H.J. George.** 1987. Plant Culture Media. Exegetics Ltd, England.

George, E.F. and **P.D. Sherrington.** 1984. Plant Propagation by Tissue Culture. Exegetics Ltd, England.

Hartney, V.J. and **J. Svensson.** 1990. The role of micropropagation for Australian tree species. (In press, Commonwealth Agricultural Bureaux).

Hartmann, H.T. and **D.E. Kester.** 1983. Plant Propagation - Principles and Practices. Prentice-Hall, Inc; USA.