fertiliser program we have achieved a reliable method of controlling growth. We have developed this system over a period of 5 years.

With a spacing of 800 macro-pots per acre one can imagine how little area is required to provide a substantial, if not total, supply of all the propagation material required. The material is on site and ready when you need it.

FROM TISSUE CULTURE TO FOREST TREES

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Abstract. Micropropagation of eucalypts and many other kinds of forest trees is now technically possible. There are several methods available to reduce the cost of producing plants by micropropagation and there is potential for integrating tissue culture techniques into nursery systems developed for seedlings. However, the largest cost in micropropagation is the labour and time entailed with manual subculturing techniques. Automatic, intelligent machine systems could overcome this restriction and revolutionize clonal forestry.

INTRODUCTION

Clonal plantations of forest trees are now a reality in many parts of the world. It is the main method of establishing plantations for species that can be easily propagated vegetatively, like poplars. Even for species that are more difficult to propagate, such as the eucalypts, clonal plantations are being established on a large scale: Brazil plants more than 10,000 ha per annum (3), the Congo over 6,000 ha (6) and France plans 2,000 ha (14). Most of these plantations are established from rooted cuttings of hybrid eucalypts. This development in forestry is not surprising since it enables clones to be planted which are adapted to specific sites or management objectives. Such clones might have been selected for maximum growth or adaptation to harsh environmental conditions such as mining dumps or saline soils. Clonal forestry also allows hybrid vigour to be exploited.

When we examine the methods of propagation of commercial plant species we find that vegetative propagation is the preferred method for most of the high-valued horticultural plants, e.g., perennial fruit crops and ornamentals. However, broad-acre crops, such as annual cereals, forage crops, and vegetables, are usually propagated by seed because each plant

has a low value and the present techniques of vegetative propagation are too labour intensive and expensive to produce clones in large numbers.

Forest trees fall in between the horticultural crops and the broad-acre crops. On the one hand, forest trees are similar to horticultural fruit trees, being perennial; seed improvement programs are both difficult and slow. For example, each cycle of selection in *Pinus radiata* takes about 15 years. On the other hand, forest trees are similar to broad-acre crops as each tree has a low value at the time of planting (approximately $0.05 for bare-rooted *Pinus radiata* and up to $0.90 for large container-grown eucalypts). Forest trees are also required in large numbers (millions per annum) over a short planting season and many are difficult to propagate vegetatively.

COMMERCIAL MICROPROPAGATION OF FOREST TREES

A joint project between CSIRO's Division of Forest Research and Alcoa of Australia is examining whether micropropagation could be used as a method of producing clones for forestry plantations. If this is to become a reality it will be necessary to overcome a number of problems which are commonly found in commercial micropropagation laboratories and to reduce the costs of plants produced by micropropagation.

Micropropagation has the advantages over cuttings of a much higher multiplication rate, a greater degree of control and small space requirement. Media have been developed for the propagation of many forest species and small-scale commercial operations are being undertaken with eucalypts in our laboratory (10) and for *Pinus radiata* (1) and poplars (4) in New Zealand.

Salinity is an enormous problem world-wide (15) and a large and increasing problem in Australia where about 43 million ha are affected (5). During the CSIRO/Alcoa project emphasis is being placed on the micropropagation of salt-tolerant eucalypts. These clones (Table 1) were selected at the University of Melbourne (17), the Forests Commission of Victoria (16), and the Forests Department of Western Australia (2) by subjecting seedlings to an increasing salt stress in hydroponic growing systems.

Field trials are being established in Australia and overseas to determine which clones are most suitable for particular locations and to determine the role of such trees in reclaiming salt-affected areas.

Table 1. Salt-tolerant clones of *Eucalyptus*. All clones tolerated a NaCl concentration above 640 mmol l^{-1} under laboratory conditions.

Species and Clone No.**	Locality name+	Location of original seed source* Lat. (°S′)	Long. (°E′)	Alt. (m)
E. camaldulensis				
41	Umberumberka Creek, NSW	31°55′	141°14′	230
42	Wiluna, WA	26°34′	120°03′	490
43	Victory Creek, WA	28°31′	120°59′	400
44	Wooramel River, WA	25°45′	114°16′	11
45	Gum Creek, WA	26°31′	120°02′	490
46	Wooramel River, WA	25°45′	114°16′	11
47	Irwin River, WA	29°16′	115°00′	20
48	Wooramel River, WA	25°45′	114°16′	11
49	De Grey River, WA	20°11′	119°11′	46
52	Umberumberka Creek, NSW	31°55′	141°14′	230
73	Finke River, NT	24°30′	133°15′	550
74	No location given	—	—	—
77	Minilya River, WA	23°49′	114°01′	10
78	Pentecost River, WA	15°48′	127°53′	30
79	Swanport Bridge, SA	35°07′	139°17′	8
82	Umberumberka Creek, NSW	31°55′	141°14′	230
83	Victory Creek, WA	28°31′	120°59′	400
84	Victory Creek, WA	28°31′	120°59′	400
85	Wiluna, WA	26°34′	120°03′	490
87	Wilpena Creek, SA	31°29′	139°21′	95
88	Wooramel River, WA	25°45′	114°16′	11
89	Wooramel River, WA	25°45′	114°16′	11
93	De Grey River, WA	20°11′	119°11′	46
94	De Grey River, WA	20°11′	119°11′	46
95	De Grey River, WA	20°11′	119°11′	46
96	Gum Creek, WA	26°31′	120°02′	490
97	Gum Creek, WA	26°31′	120°02′	490
125	Swanport Bridge, SA	35°07′	139°17′	8
126	Lake Hindmarsh	36°03′	141°53′	72
127	Wilpena Creek, SA	31°29′	139°21′	95
128	Lake Agnes, VIC	35°26′	141°56′	55
129	Swanport Bridge, SA	35°07′	139°17′	550
130	Finke River, NT	24°30′	133°15′	550
131	Hamilton, Vic.	37°24′	142°02′	250
E. macarandra				
61	No location given	—	—	—
E. spathulata				
72	No location given	—	—	—
E. wandoo				
123	No location given	—	—	—
124	No location given	—	—	—
250	No location given	—	—	—
251	No location given	—	—	—
252	No location given	—	—	—
253	No location given	—	—	—
254	No location given	—	—	—
255	No location given	—	—	—
256	No location given	—	—	—
257	No location given	—	—	—

* Clones were derived from individual seedlings of a particular seed source.
** CSIRO Division of Forest Research
\+ NSW (New South Wales); NT (Northern Territory); QLD (Queensland); SA (South Australia); WA (Western Australia).

REDUCING THE COSTS OF COMMERCIAL MICROPROPAGATION

1) The largest cost of producing plants by micropropagation is the labour involved in transferring shoots from one medium to another. Labour costs are about three times the cost of all other parts of the micropropagation procedure. Methods of reducing labour costs are:

a. Reduce the amount of time spent on each container. For many species it is possible to cut the shoots randomly into small clumps rather than dissecting out small individual shoots.

b. Reduce the number of steps for propagation. Shoots of some clones can be rooted directly as miniature cuttings thus avoiding the transfer step to a sterile rooting medium. However, this may increase the risk of plant loss, a factor which has to be carefully considered when a large number of plants are involved. Rooted plants can also be hardened in the same containers used for rooting. This may enable a higher success rate on transferring plants to a potting mixture and ultimately to the field.

c. Micropropagation could be integrated into an automated nursery system. We are at present evaluating the use of a seed-chain for root formation, hardening, and automatic transfer to larger pots (18). This system has been developed for automatic seeding and sorting of germinated seedlings at very high rates (thousands per hour). There is no inherent reason that it could not be adapted to micropropagation.

   Alcoa has developed a vacuum-operated device to enable rapid transfer of the rooted plants from agar-solidified medium to a seedling tray.

d. Sets of instruments can be pre-sterilized and so reduce the considerable time involved in flaming instruments in the sterile transfer chamber.

2) The time taken in media preparation and the cost of media can be reduced if the plants can be grown in liquid rather than an agar-based medium. Most of the eucalypts we have in culture grow better on a liquid rather than an agar-solidified medium.

3) Space can be saved in the growing room by culturing in stacks of containers. The stacks can also serve as part of a batch processing system where the stack of containers is handled as a unit for media preparation, for sterilization, in the growing room, and for despatch.

4) The cost of cleaning containers and removing labels can

be eliminated by culturing in cheap disposable containers such as polypropylene (autoclavable) take-away food containers.

5) Slow-growing, systemic contaminants are a serious problem and their elimination and detection may be a large cost in commercial micropropagation (12,13). Most of the rapidly-growing microbial contaminants are readily detected in tissue culture and such cultures are discarded. However, we have found that some of our cultures, and cultures from other laboratories, have deteriorated for no apparent reason. Some of these cultures contained a slow-growing bacterium which was not apparent on the plant medium for several months. It could not always be isolated on a number of microbial media, suggesting that it may be systemic or not uniformly distributed throughout the plant. The significance of such organisms as plant pathogens has not been determined but in some cases they may be very important (7).

The usual solution to the problem of systemic pathogens is to devise detection procedures, especially for the initial explants, and then to discard all contaminated cultures. This method is reliable but it may represent a large cost to a commercial laboratory when it finds (say through improved detection procedures) that hundreds of containers may be contaminated.

Antibiotics have not been generally successful as they often only retard the growth of the micro-organisms or severely inhibit plant growth. Further studies are required using systemic fungicides (9) and other anti-microbial agents in tissue culture.

6) Record-keeping and checking for errors is a major task if a laboratory is handling many clones; the maintenance of accurate records for the identity of clones (such as certified cultivars) is vital. In our laboratory we are handling about 200 clones, a minimum of 20 containers per clone on a 3-weekly subculturing interval. This represents over 130,000 records per year.

To reduce the labour and errors with record keeping we have developed an interactive data base system which gives details of each clone (such as its original source, salt tolerance, etc.) and maintains a complete history of all subculturing operations for each clone (19). The basis of the system is to keep track of sets of numbered containers, what medium is in the container, which clone, when it is due for subculturing, where it is located in the culture room, etc. Comprehensive error-checking is also performed and the information can be interrogated in many ways.

However, even when all of the above problems have been solved we still have a major hurdle to overcome if micropropagation is to play a role in clonal forestry. This problem is the large number of plants required over a restricted planting season. Each operator in the transfer chamber can place about 1,000 shoots per day onto an agar based rooting medium, as well as maintain the clone on shoot-multiplication medium. Compare this rate of production to the annual planting rate of forest trees in Australia; about 40 million trees (8) (assuming a planting density of 1,000 trees per ha). Even if only 10% of this area was planted to clones it represents 4 million plants which is equivalent to 4,000 days of subculturing (or 15 operator-years of subculturing, assuming 1,000 plants per day). Clearly, this is a large task if the clones have to be produced over a restricted planting season.

Apart from having a large number of operators working in sterile transfer chambers, one solution to this problem is to store either rooted plants or shoots as cultures in cool storage. This enables the work-load of producing plants to be spread over a longer time period. A more satisfactory solution to this problem is to develop machines that can duplicate the repetitive operations of the human hand in dissecting shoots and transferring these to a sterile medium. The Centre for Research on Intelligent Systems at Deakin University, Victoria, is developing a microprocessor-controlled machine to do such a task. A prototype is being developed which will have a production rate of 1 plant per second (85,000 plants per day or 1 million in 12 days). Such developments are essential if micropropagation is to play a significant role in producing clones for forestry plantations, or for broad-acre crops.

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OUTDOOR PROPAGATION ON HEATED BEDS

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Canning Plant Farm has about 6.5 hectares of container-grown nursery stock in Perth. Like most nurseries we used to do the cutting propagation in glasshouses and polyhouses.

Whilst in America we noticed that at some of the large nurseries a great amount of cutting propagation was being carried out in the open under mist. The material appeared to be in good condition with no sign of disease. Upon arriving home we decided to try this method.

The selection of the site was most important as we soon found out, as under our conditions the winds were quite severe. The hot Western Australia summer also caused some problems.

It was necessary to choose a site which was well protected