

general left me with a great respect for the Englishman's regard for the finished product. The propagation of a species seems to be less of a challenge to him than its culture through to maturity. Due to the destructive ice ages, England has very few indigenous plant species. Hence the Englishman is an avid plant collector and he derives a great deal of satisfaction in establishing introduced species in new environments.

Marketing. Aalsmeer Co-operative flower and plant market is a revelation in marketing technique. The system not only assures quality production from growers, for unsold plants are destroyed, but also circumvents the middleman commission agent. All plants are purchased by merchants on the day of sale. Plants are sold under auction on a diminishing value system which precludes buyers from "fence sitting". The mechanization and efficiency of the market is such that the Co-operative is financed by 4-1/2% of gross turn-over. Most Australian agents charge 10 to 15% for the service of selling, without accepting any risk.

As a group we are indebted to our tour organizers headed by Mary and Ed Bunker, Thomas Chang who was our host in France and, particularly, Ann and Richard Martyr of Pershore College, England, who spared no effort to make our tour the success it was. We gained greatly in knowledge and even more importantly in friendship.

A WORLD TOUR OF COMMERCIAL NURSERIES USING TISSUE CULTURE PROPAGATION

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Tissue Culture Propagation Overseas is Expensive. One over-riding impression that I gained from visiting nurseries using tissue culture techniques was the large financial investments made in them and the apparent lack of thought and planning relative to cost-cutting methods. Investments of \$50,000 were common and several nurseries had invested in excess of \$100,000. The nurseries I had visited were, *ipso facto*, still in business and according to them business was not only good, it was very good. The majority of these nurseries tissue culture propagated for themselves, i.e. plants from culture were potted up in their nursery section and sold along with plants propagated by other means. One nursery that I visited propagated all of its plants by tissue culture; most nurseries propagated part only of their plants by tissue culture.

The two most expensive parts of tissue culture propagation facilities were the inoculating and incubating areas. Whereas some had designed, or bought, relatively cheap laminar flow transfer chambers many had several expensive laminar flow chambers. I remain sceptical about the need for these chambers and believe that with good aseptic techniques and simple equipment, like glass tunnels, there should be only a low level of contamination due to microbes in the air. But there is no doubt that these laminar flow chambers are very efficient and allow risks which could not otherwise be taken; in addition, I was told that for work in humid sub-tropical regions, such as Florida, they are a necessity.

Personnel Employed in the Inoculating Area. I inquired at various nurseries about first the type of person employed to do the routine inoculation for multiplication and, second, the rate of transplanting that was achieved. It was fairly general for nurseries to train people themselves to do this work but the type of person being sought ranged from those who would do precisely what they had been told to do without any deviations, to one nursery that employed university women graduates only. The first type of personnel averaged less than two explants per minute (or about 600 to 800 explants in a working day) whereas the latter, employing women graduates, averaged seven explants per minute but kept them at the inoculating job for four hours only per day, the remainder of day being spent on other tissue culture tasks. The graduates employed were not necessarily science graduates, in fact I gained the impression that they were mainly arts graduates, and the reason for their dramatic increase in rate of inoculation lay in qualities associated with "critical" thinking. The job is essentially a "mindless" one: with a laminar flow cabinet, the contents of one flask are emptied into a sterile Petri dish, where they are divided into pieces and each piece is then either placed into an individual tube or flask with fresh medium or several to many pieces are placed in large flasks. It is quite easy to train a person to do this and after a short while such a person can do this all day without any great strain on the mind. In the case of graduates, this type of job soon gets very boring and, in my view, leads to one of two results: the graduate either leaves the job, or finds some way to make it more interesting, e.g. becomes more involved in finding quicker ways of doing it, learns more about each species, keeps an eye open for abnormalities. Even so, this work for graduates for eight hours a day, five or six days a week, would eventually become boring to most, but, to me, the single nursery that used graduates had reached a very satisfactory multiplication rate by at least in part realizing that an active critical mind was inval-

able and by realizing that a four-hour period of inoculation alternated with a four-hour period of some other activity was a necessity for such graduates.

The Incubation Area. The majority of nurseries visited incubated their cultures indoors in rooms illuminated entirely by artificial light. Energy costs for illumination, heating and cooling, are high, and likely to get higher, and the cost of construction of "shelves" and light banks is also high. Many nurseries used round-bottomed tubes and overhead lights which in turn necessitated sloped tubes and supports for the tubes. Large Erlenmeyer flasks (500 ml) were used by orchid and other tissueculturalists, and others used Mason jars and placed these on their sides thus obviating the need for supports and maximizing the amount of light reaching the cultures from overhead lights. One nursery used cheap pie dishes made of aluminum foil and fitted these with (clear) polystyrene lids; another used polystyrene sandwich boxes. One nursery did all of their incubation in Mason jars in a greenhouse, and claimed that although their multiplication rate was slower, their energy costs were much lower and that their cultured plants suffered fewer losses on transplanting to soil.

Preparation Area. I found less thought put into the preparation area than elsewhere in the tissue culture operation. Quite frequently very expensive autoclaves had been bought, but media was still being dispensed by hand or by funnels. Where relatively large volumes of medium per flask were used, e.g. 100-150 ml medium per 500 ml flask, the quickest way of dispensing that I saw was by means of a soupladle. Disappointingly, I saw no signs of a move towards semi-automotive methods of vessel-washing, vessel-drying, vessel filling with medium, lid-fitting and autoclaving.

Costing of Tissue Culture Propagation. Details on cost-analysis were as difficult to obtain from tissueculturalists as they are from nurserymen in general. One orchid tissueculturalist said that his laboratory regularly inoculated 84 flasks per day with 50 plants per flask and, after a 7 month incubation period, sold each plant for US \$1.50 (final prices varied depending on quantity and species). This laboratory employed three inoculators (who were also shareholders in his company) and there were two or three other people (including himself) helping in other tissue culture activities. Thus each day, US \$6,300 ($84 \times 50 \times \1.50) of business was created.

I inquired at several places of the proportion of their time spent on research to improve, for example, the multiplication rate for a species. Mostly, this proportion was very little, or nil, reliance being absolutely on research done under contract or available in scientific publications. One nursery was offering a

service in research and development but with a Catch-22: They charged US \$35 per hour during the research and development stage and at the end of this stage negotiated a multiplication contract from 18 to 25 cents per plant — but the investor was not told anything about the results of the research, which were the secret of the nursery concerned.

The derivation of a cost for a tissue cultured plant depends on a number of factors, which include the degree of difficulty in achieving its culture, its rate of multiplication, and the normal selling price of the species. One view is that the sale price of a tissue cultured plant should be in excess of that of a plant (of the same cultivar) propagated by other methods. This view is based, I think, on the frequent multi-crowned nature of a tissue cultured transplant which results in the formation of a bushy habit which “fills” the pot better than other propagated material. But another reason for this view is based on the potential of this technique to produce better plants because of its freedom from microbes and viruses — this presupposes that tissue cultured plants have this freedom, and this could not be assumed to hold for the majority of plants presently being propagated by tissue culture.

Conclusions. The value of this study tour lay in the detailed discussions held with many people with experience in commercial tissue culture operations. Ideas for cost-cutting possibilities had been developed prior to this tour (2), and many of these became points for discussion during the tour. More and more, I see the need for semi-automation in the preparative stages, and the need to use solar energy for incubation. Procedures, including constitution of the culture medium used for a particular species, might have to be modified when the decision is made to use greenhouse rather than indoor, controlled environment facilities.

Many of the discussions led to increased emphasis being placed on certain areas of research (1). These areas include: (1) embryogenesis and morphogenesis, (2) stability of genotypes, (3) organ culture, (4) juvenility, (5) constituents of culture medium, (6) incubation conditions, (7) genotype responses, (8) nature of the explant, (9) hardening-off, (10) “finger-printing” of clones, (11) germ plasm preservation, (12) mutation breeding with adventitious buds. These are not listed in order of priority. Increased knowledge in all of the above areas of research is likely to bring us closer to the day when we can tissue culture propagate all species.

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LITERATURE CITED

1. de Fossard, R.A. 1977. Tissue culture in horticulture — a perspective. *Acta Horticulturae*. 78:455-459.
2. de Fossard, R.A. and R.A. Bourne. 1977. Reducing tissue culture costs for commercial propagation. *Acta Horticulturae*. 78:37-44.

PROGRESS TOWARD CLONAL PROPAGATION OF EUCALYPTUS SPECIES BY TISSUE CULTURE TECHNIQUES

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Abstract. Large numbers of clonal trees of eucalyptus species have been obtained by culturing nodes of seedling or coppice material. Adult nodes of two species have successfully produced multiple buds. Shoot systems have been established from such buds and research is being directed towards the establishment of healthy plants by inducing these shoots to form roots.

Culture media are discussed and the composition of the most successful media for the production of multiple buds and for rooting are given.

Problems concerning microbial contamination of field collected material are discussed and methods for reducing consequent losses are suggested.

A routine for the establishment of test tube plants in soil is described.

REVIEW OF LITERATURE

A number of attempts have been made to propagate *Eucalyptus* species by tissue culture techniques (4,7,8,9,10). Successful regeneration from lignotuber material has been reported for *E. citriodora* (1) and from seedling hypocotyl callus for *E. alba* (11). Two approaches have been used to propagate from nodal cultures; one is to produce multiple buds and shoots in aseptically cultures and then to induce these shoots to form roots (8,9,10). The other approach is the direct induction of roots and shoots on an initial nodal explant (2,3,7,10). Seedling nodes of *E. ficifolia* have been induced to form multiple buds and subsequently roots (8,9,10). Adult nodes of *E. ficifolia* and *E. polybractea* and seedling nodes of *E. regnans* have produced multiple buds (10). Suitable concentrations of chemical constituents of multiple bud media have been established by the broad spectrum approach (5,6). *E. grandis* plants have been successfully established from cultured nodes of seedlings, coppice and young trees (2,3,7,10). Microbial contamination has been a serious problem with field-collected material (8,9,10).