CONCLUSIONS

Fertilized peat blocks can be used for the successful propagation of some tree and shrub species but environmental control needs to be investigated to obtain the best results.

The lack of root disturbance and damage with plants raised in fertilized peat blocks (LBC) improves establishment and early growth at potting on.

The disadvantages of the lower density of cuttings in the propagating house may be balanced by fewer potting stages and a shorter period to produce a saleable plant.

The use of fertilized peat blocks (LBC) for growing on of rooted cuttings offers better early growth and the saving of at least one pot stage, resulting in economy of compost used.

Acknowledgements. The help and interest of the following people and establishments is acknowledged Without such co-operation this paper could not have been written G W Dendy, W Crowder & Sons Ltd , G Jones Ltd , and E F G. Nurseries Ltd

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B. MACDONALD: Have you done any work with hardwood cuttings in blocks?

D. ATTENBURROW: No. Most of the cuttings used have either been soft or semi-hard. Up to now, we have had more success with the semi-hard than soft.

CURRENT ASPECTS OF COMMERCIAL MICROPROPAGATION

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The Location of Micropropagation Laboratories. Commercial micropropagation units that have arisen during the last ten years have developed either in association with or in close proximity to sites of academic research in the plant tissue culture

field. This is no accident. The successful application of new technologies requires continual access to research facilities and information exchange (Figure 1). Twyford Laboratories is an exception to this only in that it provides such facilities within the company to stimulate further developments in the micropropagation and disease indexing fields.

On a worldwide basis the principal micropropagation units are found in close proximity to Pieriks' laboratory in The Netherlands, to that of Boxus in Belgium, the late Professor Morel, Beauchesne, Tran Than Van and Nitsch in France, Zuccherelli and Rosati in Italy, and Murashige in the United States. These units have naturally concentrated on crops of local or national importance — lilies and gerberas (11) in The Netherlands, foliage ornamentals (9) and orchids (5) in the U.S.A., fruit tree rootstocks (14) and strawberries (3) in Italy and Belgium, and orchids (10), gerberas and strawberries in France.

The expertise for the micropropagation of these crops is often isolated in these areas and growers from elsewhere may be unaware of the current propagation capabilities for a particular genus.

Tropical crops grown in their own environment have received scant attention with the exception of *Elaeis* (8) and *Ananas* (13) reflecting more the lack of local tissue culture laboratories than the value of the crop. In contrast, tropical ornamentals, important in the temperate nursery industry, provide the most important genera for tissue culture laboratories in both variety and total numbers of plants.

Principal Genera Involved. Genera that have been micropropagated, sold in at least four figure quantities and judged to have been a profitable undertaking are listed in Table 1, in chronological order.

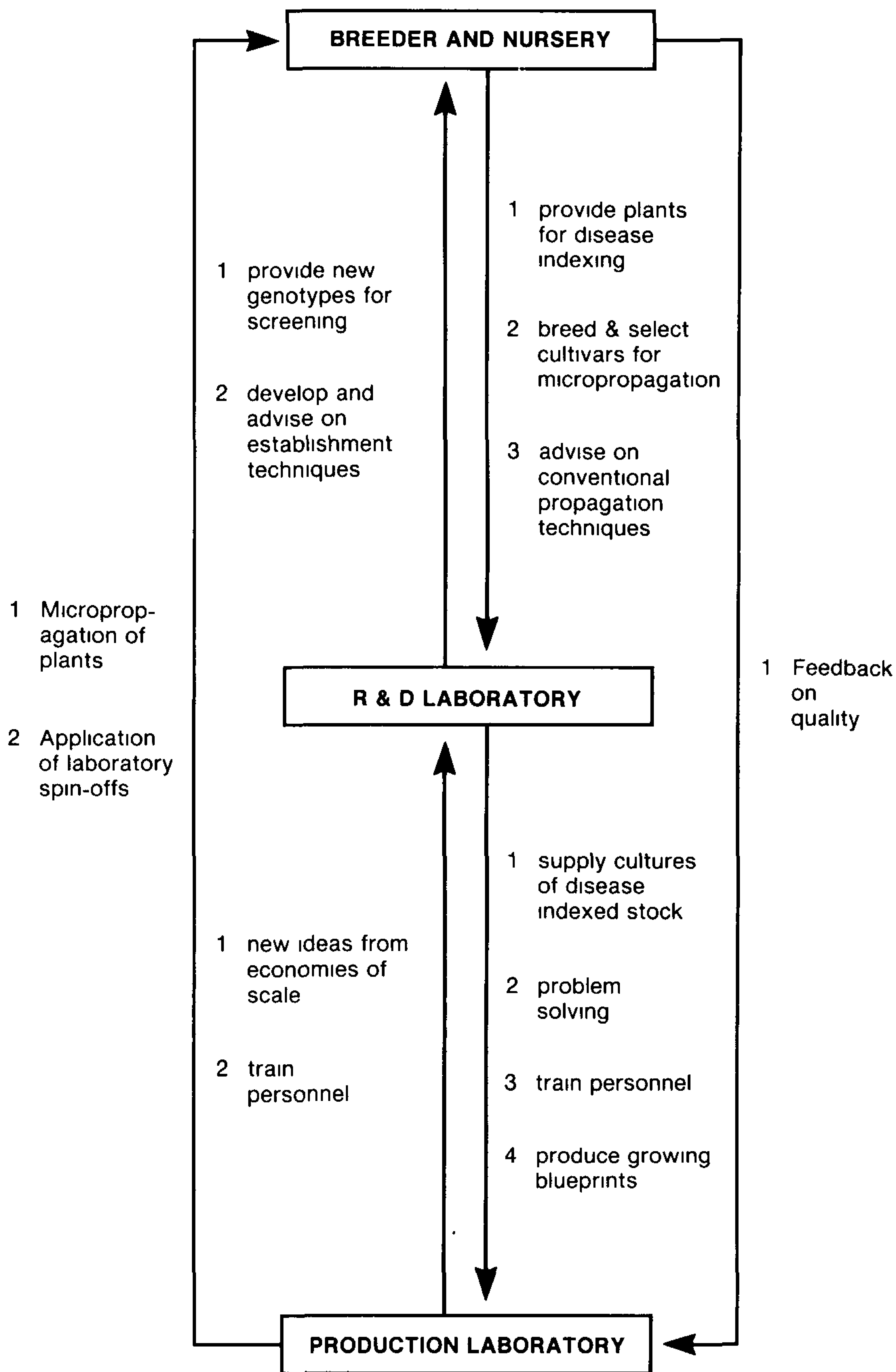

Figure 1. The interrelationships between nurseries and research and production laboratories for micropropagation

Table 1: Principal genera to which micropropagation has been successfully applied on a commercial basis, presented chronologically

FAMILY	Genera 1960	1965	1970	1975	1977	1980
ORCHIDACEAE	*Cymbidium*	*Cattleya*	*Phalaenopsis* *Dendrobium* *Vanda* *Odontoglossum*			
IRIDACEAE			*Freesia*	*Gladiolus*		
LILIACEAE			*Lilium*	*Asparagus*	*Cordyline* *Dracaena* *Allium* *Hemerocallis*	
AMARYLLIDACEAE				*Nerine*	*Alstroemeria*	*Narcissus* *Haemanthus*
ROSACEAE				*Fragaria*		*Malus* *Prunus*
FERNS				*Nephrolepis*	*Platycerium* *Davallia* *Pteris*	
COMPOSITAE				*Gerbera* *Chrysanthemum*		

FAMILY	Genera 1960	1965	1970	1975	1977	1980
ARACEAE				*Anthurium*	*Dieffenbachia* *Spathiphyllum* *Syngonium*	*Philodendron* *Alocasia*
GERANIACEAE				*Pelargonium*		
GESNERIACEAE				*Saintpaulia*		
BEGONIACEAE					*Begonia*	
MORACEAE					*Ficus*	
CRUCIFERAE					*Brassica*	
BUXACEAE						*Simmondsia*
ERICACEAE						*Rhododendron*
SOLANACEAE						*Solanum*
PALMAE						*Elaeis*
BROMELIACEAE			*Ananas*		*Cryptanthus* *Aechmea*	
ARALIACEAE					*Tupidanthus*	

Table 2. The advantages of micropropagation as applied to those genera listed in Table 1

1	Disease indexing for the production of healthy stock	*Pelargonium, Dieffenbachia, Gladiolus, Lilium, Narcissus, Fragaria, Chrysanthemum, Solanum, Cymbidium, Cattleya*
2	Rapid propagation of parent lines for F_1 hybrid seed production	*Brassica Beta*
3	Vegetative propagation of plants on a commercial scale that was hitherto impratical	*Anthurium, Cymbidium, Cattleya, Phalaenopsis, Dendrobium, Vanda, Odontoglossum, Alocasia, Elaeis, Nerine, Haemanthus*
4	Acceleration over conventional systems	*Lilium, Gerbera, Fragaria, Philodendron, Alstroemeria, Cordyline Dracaena, Malus, Prunus, Allium, Spathiphyllum, Syngonium, Saintpaulia, Nephrolepis, Hemerocallis, Asparagus, Tupidanthus, Begonia, Ficus, Simmondsia, Rhododendron*
5	Replacement of sexual propagation methods	*Ananas, Cryptanthus, Aechmea, Davallia, Platycerium, Pteris*
6	Rapid propagation of new hybrids	*Begonia, Lilium, Gladiolus, Freesia, Cymbidium, Cattleya, Phalanenopsis, Dendrobium, Vanda, Odontoglossum, Nerine, etc*

Table 3. Genera from which more than a million plants have been micropropagated for commerce

Nephrolepis	*Cymbidium*
Fragaria	*Anthurium*
Gerbera	*Philodendron*
Lilium	

Since I last talked to the Society in 1974 (12) the range of genera within these criteria has expanded rapidly with the result that tissue culture methods should now always be considered as an alternative option to conventional propagation.

Families of monocotyledons have provided the majority of genera that have been successfully propagated to date; principally the Orchidaceae, Bromeliaceae, Amaryllidaceae, Liliaceae, Araceae and Iridaceae. Genera that are easily propagated by conventional means exhibit a similar trait in tissue culture. Post graduate studies that have presented projections for the propagation of one million plus plants in a matter of months are concentrated on those families where success is more likely within the usually limited time allocated to specific projects Those genera that are more difficult to propagate often have to wait until the

pressures of commerce are applied. *Simmondsia* (1), *Phoenix* (7), *Picea* (4), *Pinus* (4), *Pseudotsuga* (2), *Eucalyptus* (6), *Elaesis* (8), and *Cocos* (7) are all genera to which these pressures are now being applied. *Elaeis, Malus* and *Prunus* are the first of many plantation crops to be produced successfully via the application of tissue culture propagation techniques. Although the benefits from these projects will not be realised for 5 to 50 years they will produce the major financial returns from micropropagation.

Development Costs. The high cost of independently initiating and developing a micropropagation system for a new cultivar or genus requires the subject to fulfill one of three criteria: —

1) high volume market demand
2) high market value of individual plants
3) co-ordination of demand and supply for a group of customers.

Commercial laboratories are unlikely to accept plants for propagation unless they are convinced both of the market and the likelihood of success.

Market demands may change, during the one to two year laboratory production stage and therefore 'a finger to the wind' is advisable.

Figure 2 illustrates the high proportion of costs attributed to labour, 75 to 85%. Growing room and material costs both vary from 5 to 15% of the total production cost. However, within all three categories of labour, growing room and materials, there is little variation in the allocation of costs for a wide range of genera. Policy on the allocation of overheads within different institutions may well affect these percentages. Mechanisation and automation of the preparation of growing containers only begins to justify investment when the production of these reaches the 5 to 10,000 per week quantity. The preparation and grading of microcuttings is a skilled task and is likely to be automated only if many millions of plants are required of one cultivar and a high wastage level is acceptable.

To reduce growing room costs, hygienic glasshouse environments will be increasingly employed to root and establish cuttings produced *in vitro.* The high cost of agar would thus be eliminated and the rooted plant would not be subjected to "transplant shock" on removal from the growing room to be potted-on in the glasshouse.

Health Status. The professional propagator should always be aware of the health status of his product. The environment provided for the growth of plant tissue cultures is frequently optimal for plant pathogens and saphrophytes. Unless specific precautions are introduced into the culture system the micropropagated plants may emerge with a microflora more damaging than when they entered.

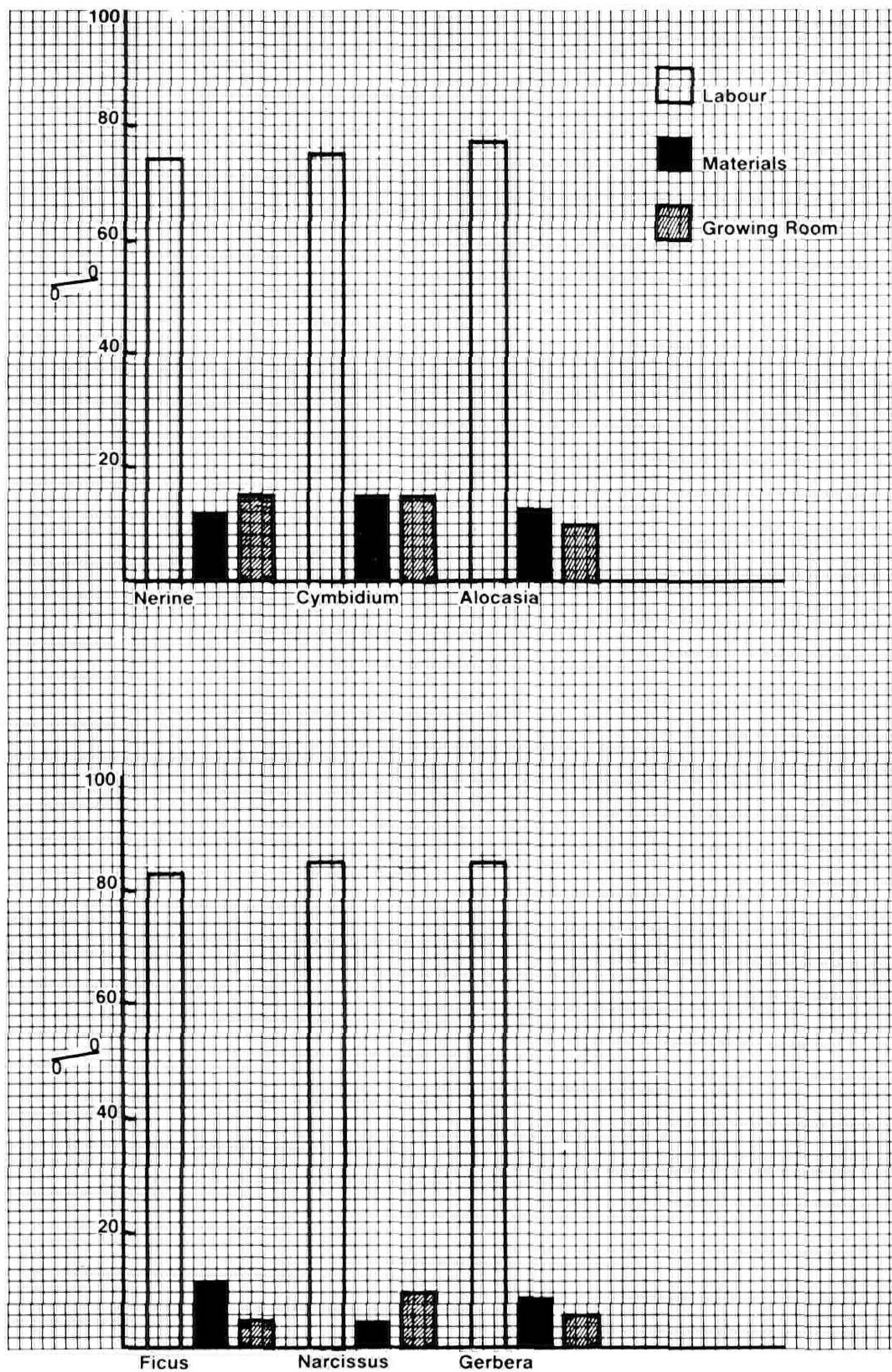

Figure 2. Production costs in labour, materials, and facilities for micropropagation of several genera. Percentage of total costs.

Disinfection and disease indexing. The implementation of disinfecting and indexing procedures and monitoring of the health status of tissue cultures may be categorised thus:

1) Disinfection procedures
2) Disease indexing
3) Maintenance of the indexed and aseptic state.

Disinfection procedures commence with an incubation period under low relative humidity but otherwise optimal growth conditions to reduce the surface microflora. This should be followed by a combination of chemical, vacuum or agitation treatments of the explant, be it bud, leaf, stem or root, to complete stage one. The disinfection should be checked by culturing tissue from each explant on media that will support the growth of a comprehensive range of bacteria and fungi.

Specific pathogens may be detected by culturing tissue on defined nutrient media. To eliminate these and produce disease-indexed tissue skilled culture work is necessary, followed by regular monitoring of the status of the tissue.

Virus indexing may involve any or several of the techniques of chemo- or thermo-therapy, and testing by use of electron microscopy, serology or indicator plant tests.

To maintain the "indexed" state of the culture re-infection from other plant material via insufficiently sterilised instruments must be prevented. Dipping the handling instruments in alcohol and igniting the solvent provides insufficient heat to sterilise. A longer exposure to a hot flame is required or immersion in a chemical sterilant.

The ubiquitous *Bacillus subtilis* is a frequent contaminant of tissue cultures. Growth of the bacillus appears to be inhibited by cytokinin compounds and the extent of contamination may only be revealed when these compounds are omitted from the medium at the root induction stage. Such high levels of contamination as may then appear often inhibit root growth and reduce establishment success, particularly when plants may be in transit for several days. Considerable attention to sterilisation techniques and working procedures of operators is required to maintain control of this problem. Training in the detection of decreased vigour, stunted root growth or the characteristic appearance of the bacillus colony as it penetrates the nutrient medium from the plant, is vital in order that these cultures are destroyed to prevent the entire stock from becoming contaminated.

Certification schemes and international exchange. The U.K. Ministry of Agriculture is currently introducing a scheme for the inspection and certification of disease indexed stock of tissue culture origin. This will enable its integration into the certifica-

tion schemes for "S.S." material that already exists. Organisations wishing to enter the scheme will require written prior approval from the Ministry of Agriculture of the culture method used, including specification of the number of subcultures to which the tissue has been subjected, and the hygiene in the laboratories and glasshouses.

Disease-indexed tissue cultures provide ideal plant material for international exchange. Evidence of constant monitoring of the health status of cultures combined with an effective phytosanitary inspection service should enable many of the entry and quarantine restrictions imposed between states to be overcome.

As there is now an international source list of virus-tested fruit tree material produced by Long Ashton Research Station, Bristol, England; so we look forward to similar lists of hardy nursery stock and perhaps ornamentals in the future. Exchange of tissue cultured material could occur independently of season and a tissue bank, maintained under the correct conditions, would require little attention and provide effective isolation from disease.

In terms of clonal uniformity, quality of product, and health status, those concerned with micropropagation should always aim to produce plants of the highest quality.

Problems Encountered by the Micropropagation Laboratory.

Juvenility. Successful initiation of *in vitro* cultures is inversely proportional to the age and state of lignification of the tissue and can also be influenced by season. Juvenile tissue should always be sought for propagation Vernalization may be necessary to break dormancy. Disinfected material of herbaceous origin may be more readily induced, with the application of cytokinins, to form juvenile tissue than that of woody plant material.

Cultures from woody plants are most successfully initiated during the early stages of the active growth period. At other seasons phytotron facilities may be required to induce growth. Juvenility in coniferous species has been induced by grafting shoots from mature trees on to seedlings of the same species Tissue is later selected for culture once the graft has united and the scion exhibits more juvenile characters. The parameters by which forest and plantation trees are selected are only exhibited in the mature plant from which tissue cultures are often difficult to initiate. A repeatable method of inducing juvenility is a necessary precursor of the micropropagation of many tree crops.

Seasonal variation. Most horticultural crops in temperate areas are grown under seasonal systems with which the tissue culture propagator is required to co-ordinate. This results in a

seasonal imbalance of work in the laboratory and consequently loss of efficiency and increasing costs.

Development from culture initiation to delivery to customer may take one to two years and skilled forward balancing of orders is essential.

In vitro environment. Plant tissue grown *in vitro* is highly susceptible to variations in environmental conditions. Light, in terms of intensity, wave-length and photoperiod ranges is less critical than temperature. Temperatures, even within the limited range of 21° to 27°C, can limit growth. *Alocasia* and *Anthurium* exhibit reduced growth rates at temperatures above 25°C whilst optimum rates are obtained from *Lilium* at 21°C, and rooting of *Malus* and *Prunus* rootstocks at 27°C.

The hormonal constituents of the medium may be varied to control the morphology of the tissue at each stage in its production and it is these, frequently applied in levels of <1 ppm, that require the closest monitoring of all the medium constituents. Charcoal, sugar source, and total nutrient levels are other significant variables.

Some genera exhibit reduced growth rates when grown in certain plastic containers, compared with glass. Composition of the growing container is a factor to be considered in its selection along with shape for efficient use of growing room space, recycling capability, and ease of handling.

Production blueprints may be written for individual genera or clones. Considerable flexibility should be allowed for new clones. Delays in subculturing tissue of familiar clones may initiate unforeseen changes in morphology which could jeopardise production schedules.

Packing and transport. The plant produced from the *in vitro* environment is a challenging subject for those responsible for its packing and transport. In-transit handling methods may damage plants shipped in agar. Quality can be improved and freight costs reduced by despatching plants bare-root, packed in enclosed containers to prevent wilting. Removal from the growing container, washing, grading and packing should be carried out swiftly. Accurate labelling, particularly of disease-indexed stock, is necessary at this stage and should be carried through to the nursery. Transport to the growing area must be as swift as possible — within 3 days. To reduce temperature variance to a minimum, insulated packaging is essential with a specified temperature range, whether high or low, printed on the outside of the carton.

Plant establishment. The field or glasshouse establishment of plants produced from tissue cultures is a skill of which there are currently insufficient practitioners. To improve understanding of

this skill consideration should be given to including training in the handling and establishment of micropropagated plants in the syllabus of plant propagation courses.

The plants arrive at the potting bench from an environment which provided a 100% relative humidity and "balanced" temperature, light, and nutrient supplies. The emphasis should be on minimising checks to growth that may, in genera such as *Malus* and *Prunus*, lead to the onset of dormancy. Leaf turgidity must be maintained during the potting process. This "soft" plant, which will frequently have a residue of agar medium amongst the roots, and that may contain sugars, provides an ideal subject for attack by pathogens and a programme of preventative pesticide treatment must be implemented immediately the plants are potted. Micropropagated plants will only perform well if the highest standards of hygiene, including the sterilisation of compost, pots and benchwork, and cultural practice are observed.

The Future. The market value of the young plant dictates the feasibility of micropropagation. The technique is often in direct competition with conventional methods as in *Lilium*, *Ficus*, *Gerbera*, and *Prunus* rootstocks. However, the independence of seasonal supply, the propagation of plants for which there was no previous practical method, disease indexing, and clonal uniformity all combine to increase the value of this technique beyond its conventional competitor.

The barrier that propagation once presented to the introduction of commercial cultivation of many cultivars is gradually lifting as micropropagation is more widely applied Indeed ease of propagation by tissue culture may become a selection parameter. Whereas the introduction of plant breeding rights has provided greater security for the breeder, the advent of micropropagation and the increasing appetite of the consumer for different products demands a more rapid flow of new cultivars of ornamental plants.

The functioning of the larger micropropagation laboratory is in many ways more closely related to a small factory than a conventional horticultural unit except for one very important difference. The product is a living one, continually changing in its requirements and responding to its environment. Monitoring and amending schedules and blueprints to guide a clone of plants through the research, development and production phases of the micropropagation laboratory and into the nursery forms a new and challenging area of horticulture.

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P. GAUT: Are you involved in the anther culture method?

M. STOKES: No.

B. RIGBY: Do you feel there are applications for haploid culture?

M. STOKES: There are applications on the breeding side. The plant breeder is interested in acquiring haploid plants for given purposes, hence the interest in anther culture.

A. CARTER: To give some idea on rhododendrons, how many would need to be ordered and roughly what price per unit would they be?

M. STOKES: Depending on the cultivar, the cost of the small size as displayed on the table would be probably 50 to 60p; for the plant direct from tissue culture, 20 to 30p.

D. CLARKE: How do you see this propagation method in hardy nursery stock during the next 5 years?

M. STOKES: I think it will depend on co-operation among growers and research stations in establishing which cultivars could be employed economically.

VOICE: What is the time limit between the growing *in vitro* and purchasing smaller rhododendrons?

M. STOKES: Between 1 year and 18 months.

D. CLARKE: Does that include the development time as well? Would that time be from when you have the first plant from the nursery to the point when you receive 10,000 back?

M. STOKES. Providing there were no hiccups and the cultivar responded well — yes, but if there were any hiccups it could be longer.

TEACHING MICROPROPAGATION

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Teaching micropropagation can come into various categories. It is listed in the syllabus for Higher Grade Horticultural Science in the Scottish Certificate of Education for Schools.

At the West of Scotland College it is taught to all Ordinary National Diploma students in their first year in a laboratory class and, in the third year, students from time to time have chosen some aspect of micropropagation for their third year individual projects. It also comes into the crop option of the M.I. Biology course, the B.Sc. students have a laboratory class to introduce the subject to them and as a group may tackle a micropropagation problem. When it comes to the Honours year thesis, two students have chosen some aspect of micropropagation as their remit.

At universities and polytechnics, where post-graduate courses are available, micropropagation can be part, if not all, of the investigations carried out. Under these circumstances more time is available to devote to the culture and the problems which can arise

At the West College we are fortunate in having in the Biology building, equipment available to carry out micropropagation work, i.e *laminar flow benches* (bench with sterile air coming from the back and flowing over the work area), *autoclaves* (pressure cookers to kill bacteria, etc.), *incubators* (heated cabinets