INTERACTIONS BETWEEN MICROPROPAGATION AND CONVENTIONAL PROPAGATION

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INTRODUCTION

When shoot culture *in vitro* was first recognized as a method for vegetative propagation there was a tendency to view it as a "stand alone" technique, not as one to integrate into general propagation.

There are various reasons for this. Tissue culture is a novel and highly technical process, requiring special and costly facilities more akin to a hospital than a nursery. It provided a wide range of research opportunities extending beyond plant propagation to plant improvement and was taken-up by specialist groups, often based in universities, some without contact with commercial horticulture. Initially, micropropagation was seen to have special opportunities, enabling the creation, maintenance, and exchange of healthy plant material for example.

The tendency to develop as a technology separate from the rest of propagation is only being eroded slowly. Of the 20 or so commercial tissue culture laboratories in England in 1986 concerned with vegetative propagation (as opposed to seed or other special purpose) only three were part of a commercial nursery enterprise (3).

As commercial laboratories increasingly focused on plant production they encountered problems of product acceptability. Plantlets supplied bare-root as removed from the culture flask were unfamiliar to nurserymen and many small plants died, often through over-watering. The majority of laboratories responded by establishing their micropropagules in modules or plugs, so providing liners to the nurseries and building another bridge between the two technologies.

Knowledge and technical skills in conventional cutting propagation are improving rapidly, and in many respects micropropagules behave as very small cuttings which can benefit from this progress. On the other hand, micropropagules *in vitro* comprise highly meristematic and "plastic" tissues which can be exposed to complex chemical conditions and finely controlled physical environments in a way that is not possible during auxin treatment and rooting of conventional cuttings.

To investigate and exploit the opportunities for interplay between micro- and macro-propagation will ensure faster progress in the science and practice of propagation than if the techniques are allowed to develop separately.

Nature of the opportunities. Micropropagation has broad

relevance across horticulture from the production of plants for cut flowers, herbaceous perennials, foliage plants, bulbs, ornamentals and fruit trees and shrubs, and even some vegetables.

Trees and shrubs embody most of the problems and opportunities. Many of the 8000 woody subjects grown in the UK are propagated commercially, presenting the problem of diversity. Trees exhibit strongly the problem of phase change, whereby commercial horticultural characteristics cannot be identified until the adult phase of flowering and fruiting is reached, by which time the juvenile period, with its associated ease of propagation, is passed.

Opportunities for an integrated approach include taking into culture difficult-to-propagate adult material, creating "rejuvenated" material in culture, identifying methods to culture successfully a wide range of genera, and developing less costly processes. This last objective is particularly relevant to the rooting stage where individual shoots rooted *in vitro* attract all costs, whereas these are spread over increasing numbers of shoots during the earlier multiplication stage.

Culture initiation. The importance of maximizing efficiency at the start of culture is obvious. The material may be scarce because it is of a new cultivar or healthy clone. Early losses set back production schedules seriously. For example, increasing the number of successful initial explants from 1 to 100 reduces the time required to exceed 50,000 cultures from 8 to 5 months given a 5-fold monthly multiplication rate.

Techniques that raise the rooting potential of shoots for use as conventional cuttings can assist culture initiation *in vitro*. An investigation of why an apple rootstock failed to root from cuttings, but rooted when stooled, showed that severe stockplant pruning and localized exclusion of light were important components of the stooling process (4). General dark treatments, in which stockplants are covered with ventilated tents of black polythene, raise the rooting potential in shoots of different species (Table 1).

Explants taken into culture from field stockplants grown in darkness or heavy shade survived in greater numbers than those taken from light-grown plants. The improvement was associated with a decrease in the production of oxidized phenolics. In *Quercus robur* 'Fastigiata', the frequency of detrimental phenolic oxidation was reduced from 33 to 13 per cent, and in *Garrya elliptica* 'James Roof' from 100 to 0 per cent (6).

Table 1. Effects of dark-preconditioning stockplants on subsequent rooting percentage of leafy cuttings.

	M.9 apple	*Syringa vulgaris* 'Mme. Lemoine'	*Quercus robur** 'Fastigiata'	*Tilia* sp.
Dark	90%	92%	30%	67%
Light	10	43	4	45

*1 per cent available light

'Rejuvenation' and rooting potential. It is now well-established that the rooting potential of shoots grown *in vitro* increases over a period of successive subcultures. The number of subcultures and the precise culture conditions required to obtain a high rooting potential varies with the cultivar. More than 90 per cent of the explants of the apple scion cultivar, Jonathan, rooted by the ninth subculture, while 31 subcultures were required for the cultivar, Red Delicious, to reach 79 per cent rooting (8). Deciduous azaleas show a similar but faster response (2).

The *in vitro* process can be used therefore, to achieve propagation in hitherto difficult subjects, but there may be reasons why this approach is not sensible as the sole propagation method. The cost to nurserymen of buying-in micropropagated liners may be relatively high compared to production on the nursery, and the small liners may not fit easily into production schedules.

It is of considerable importance, therefore, that plants whose rooting potential has been increased during production *in vitro* produce cuttings which retain an enhanced rooting potential in conventional cuttings taken subsequently from container-grown and field-grown stockplants. This effect occurs in genera as different as plum and rhododendron (Table 2), but the "memory" of the initial micropropagation event is not consistent. The effect on rooting potential of plum was still present in hedge-grown material derived from micropropagules nine years previously and it has lasted for at least a year in *Rhododendron* 'Hoppy', whereas it disappeared within a year or so for *Rhododendron* 'America'. Associated characteristics such as increased shoot vigour, spinyness, and slight delay in flowering support the view that 'rejuvenation' occurred.

Table 2. Rooting percentage of conventional cuttings from *in vitro*-derived stockplants compared to rooting *in vitro*, and from normal stockplants. (*In vitro*-derived and normal plum material was grown as field-hedges and *Rhododendron* material was grown in containers).

	In vitro	*In vitro*-derived	Normal
Prunus insititia 'Pixy' hardwood cuttings	100%	67%	39%
Rhododendron 'Hoppy' softwood cuttings	100	60	20

Maximizing shoot multiplication. When first taken into culture many subjects are difficult to grow, with the all-important axillary shoot production lacking. Approaches used to overcome this problem include relating *in vitro* conditions to those in which the plant does best in its horticultural environment. A low pH in the culture medium is required by *Magnolia* × *soulangiana*, the most calcifuge of that genus, and also for the acid-loving *Disanthus cer-*

cidifolius. *Magnolia* shoot production increased from 3.7 to 5.7 by reducing the medium pH from 4.5 to 3.5, and *Disanthus* increased from 2.3 to 7.2 shoots over the pH range 6.5 to 4.5, respectively.

Acid soils are often nutritionally poor, and both rhododendron (1) and *Kalmia* (5) require lower nitrogen and potassium levels than those provided in Murashige and Skoog medium. Such specific requirements explain why results may be poor when a wide range of species are processed in laboratories using relatively few standard culture media. Because economies of large-scale production are not available when specific media must be prepared for runs of a few thousand cultures, commercial micropropagation laboratories may find difficulty in producing a wide range of plants cost-effectively and may need to specialize. In laboratories attached to commercial nurseries, where overheads can be carried by the parent organization, micropropagation should be cost-effective when it is used as a special tool for a relatively few subjects.

Rooting, weaning, and establishment. As with conventional cuttings the availability of auxin is central to success in rooting micropropagules, either by increasing the auxin:cytokinin ratio *in vitro*, or applying exogenous treatments and rooting the micropropagules as mini-cuttings *ex vitro*.

After removal from closed culture vessels, micropropagules, with reduced cuticular wax on their leaves and stems, require particularly supportive environments to avoid desiccation or excessive hydration. Fog systems are particularly effective (7). In experiments at East Malling, cuttings performed better in fog produced by a centrifugal system (Agritech), then under mist, with actual leaf wetting prevented in both cases. For *Embothrium coccineum* rooting was improved from 38 to 88 per cent in fog and, although all cuttings of *Schizophragma hydrangeoides* rooted in both environments, those from the fog subsequently grew faster.

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HOW FAR DO WE GO? FUTURE DEVELOPMENTS AND OPPORTUNITIES IN MICROPROPAGATION

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INTRODUCTION

There is no doubt that amazing advances have been made since plant tissues were first cultured *in vitro* in the 1930's. Orchid propagation by both seed and meristem culture (mericloning) was an early use of these techniques. Florist crops and pot plants probably still account for the largest number of plants propagated in culture. Increasing use of micropropagation techniques is being made in hardy ornamental nursery stock and plantation crops with considerable effect being expended in investigations in micropropagation of forest species.

Currently at least 205 laboratories are in operation worldwide (3), but it is difficult to distinguish between production and research laboratories, making any realistic output estimate impossible. There are a number of units in operation or planned with a production capacity of 5 to 20 million plantlets. The theoretical capacity of a facility and what is actually produced are often widely different and the logistics of the very large units present enormous problems.

The rapid development of micropropagation and interest in its possibilities, resulted in a crisis of confidence in the 1970s. Micropropagation had begun to be perceived as a panacea for all problems but a credibility gap grew between the theory and what was actually delivered. Nurserymen became disillusioned as contracts were not always met and insufficient account was taken of limitations of the technique. These problems have been, in many cases, overcome, but there are five outstanding problems to be faced before the technique can be fully used.

The first is synchronous development *in vitro*. We need to be able to understand and control the physiology of the plant more exactly.