IN VITRO REPRODUCTION OF *NYMPHAEA*

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INTRODUCTION

This presentation does not claim to solve the problems of *in vitro* reproduction of waterlilies (*Nymphaea* spp), but I hope that it will provide an insight into the need to develop such a technique and give some indication of progress so far. The work that I and my colleagues have undertaken only refers to the *Nymphaea* subgenus, *Chamaenymphaea*—the hardy waterlilies.

The production of aquatic plants is one of the fastest growing areas in decorative horticulture in Europe, Australasia, and North America. It is estimated that 1.5 million households in the UK have garden ponds (3) and that the UK market in waterlilies is approximately 500,000 plants each year. Home production accounts for about 50%, the remainder being imported from continental Europe and Japan Waterlilies require specialised production and are high value plants retailing between £4.25 and £60.00 each, the average selling price being about £12 00.

Crown rot disease has devastated many UK stocks in recent years. The current virulent strain of this disease is believed to have arrived on imported Japanese stock. UK growers have set up and subscribe to a Waterlily Research Fund which is supporting investigations by the Agricultural Development and Advisory Service into the isolation and control of the pathogen. It is believed that if *in vitro* propagation can be achieved, then a non-commercial benefit would be the rapid multiplication and reinstatement of wild populations in UK waterways of *Nymphaea alba*. Rivers and waterway recovery are high priorities in current environmental programmes.

RESEARCH AWARD AND MARY HELLIAR AWARD

It is against this background that in 1989 I applied to the Department of Trade and Industry for a SMART (Small Firms Merit Award for Research and Technology) Award. My project was selected and work commenced in January 1990, with the Science Department, Askham Bryan College of Agriculture and Horticulture serving as sub-contractor. Funding for the project

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could only be granted for one year as European Economic Community rules prohibit government departmental support of horticulture or ship building! With time against us, and the prospects of a rapid breakthrough fairly slim, I applied for a Mary Helliar Travel Award from the IPPS in order to visit colleagues in Czechoslovakia where, allegedly, the most advanced *in vitro* work was being undertaken. They had followed different routes and were encountering similar problems. However, their experiences precluded us from making some of the same mistakes. Their work is integrated into this presentation, although it was not as advanced as I was led to believe

THE PROJECT

The major problem with tissue culture of waterlilies was believed to be endogenous bacteria, although preliminary work by Burgess (1) was inconclusive about this. Since then little work has been done with waterlilies other than excised embryos and, until very recently, only with the closely related *Nelumbo* (2). Young plants have been produced in culture, but as the embryos are clean to start with, this does little to point the way forward. Seed-raised waterlilies are few and only the species—which have little commercial appeal—can be increased this way. The problem being addressed is with hybrid or mutant cultivars which all have endogenous bacteria present

Work was started to produce mother plants that were as clean as possible, raising them under glass in controlled conditions. The cultivar used throughout has been *Nymphaea* × *marliacea* 'Carnea'. This is well known in commerce and is an early hybrid—possibly a union between *N. alba* and *N. odorata* var. *rubra*. Given that species can be tissue-cultured from excised embryos this hybrid is also likely to respond in the same way once clean stock has been produced.

Two further areas of investigation have been followed in order to overcome contamination problems The use of sterilant, antibiotic, or antifungal treatments, alone or in combination, as well as the investigation of systems which do not require the addition of sugar in order to achieve proliferating cultures.

To date, two systems have been evaluated for growing plants as cleanly as possible. A flushing tank system with plants continually washed in a current of tap water, and a spray tank system in which plants are suspended on netting in a clear closed container and intermittently sprayed with sterilized and filtered distilled water containing soluble plant feed

Small pieces of plant tissue have been removed from each of the cleansing systems and treated with sodium hypochlorite bleach and

mercuric chloride as surface disinfectants, followed by washes in sterile distilled water. They have also been treated with each of five antibiotics and with Captan fungicide incorporated into the growth medium, and with a combination of these treatments.

The plant material used in these trials has been either strong white/green anchor roots which grow very rapidly from mother plants suspended in water or air, leaf blade and leaf stalk, and eventually bud and rhizome material. It is anticipated that this latter material will be most useful for obtaining proliferating cultures.

Preliminary investigations indicate that clean cultures are possible. Clean growing root culture and leaf sections are currently in a sugar containing growth medium. Surface contaminants can be removed easily by clean growing methods and the use of chemical sterilants. However, many cultures start clean, but progressively become contaminated by apparent seepages of micro-organisms from the interior of the tissue. It is apparent that the predominant contaminants are fungal and not bacterial as first thought. This may be the result of the cleansing conditions. A possibility being investigated is that the effect of growing stock plants in the air has selected against those microorganisms that grow best in low oxygen concentrations present at the bottom of ponds. Varying oxygen concentrations are being used to investigate this.

Since there is still evidence of internal contamination causing the death of cultures, a further series of experiments is underway to assess the effect of feeding systemically-acting fungicides to plant tissue prior to sterilization. A technique—using a root growing in air which extends into a Benlate suspension for 2 days while being warmed by the direct radiation from an infrared lamp to ensure rapid growth has, following surface sterilization, started to grow in culture. This approach seems promising and is being pursued with other plant sections and a variety of treatments.

CONCLUSIONS

With refinements it is hoped that enough material can be produced to investigate the ideal media requirements necessary to achieve proliferation. As indicated earlier, successful experiments using excised embryos of *Nelumbo* and recent work with *Nymphaea* embryos suggests that this will not be unduly difficult to achieve in a commercially exploitable way.

LITERATURE CITED

1 Burgess, G 1981 Propagation of marginal and aquatic plants *Proc. Inter. Plant Prop Soc* 31 417-422

2 Kane, M , M Jenks, and T Sheehan 1990 *In vitro* propagation studies in the Nymphaeceae American Lotus *Water Garden Jour* , 6(1), 31-33

3 *Mintel Leisure Survey* 1988 Published by Mintel Market Research Group, London