In summary, from my observations, a well-aerated medium is much more forgiving than a poorly aerated one. We have found that with three times more air than water in a mix, we can stick as many as 25% more cuttings per flat than with a water to air ratio of 1:1. However, the cuttings become too crowded if allowed to grow at that density. Root initials form faster and develop with more secondary rooting in a medium with greater aeration. This is possible most of the time because you can use a higher concentration of hormone with less basal burn than in a low aerated medium.

Our requirements for a propagation medium have changed over the years. the proper ratio of water to air porosity, cation exchange capacity, pH, weight, and improved quality and quantity of cuttings rooted, will usually offset the higher initial cost of a medium.

LITERATURE CITED

1. Davidson, H. and R. Mecklenburg. 1981. Nursery Management. Prentice-Hall, Englewood Cliffs, New Jersey.

Thursday Afternoon, December 12, 1985

The Thursday afternoon session convened at 1:30 p.m. with Mark Bridgen serving as moderator.

HOW DOES TISSUE CULTURE BENEFIT THE PRACTICAL PLANT PROPAGATOR?

PAUL E. READ and TERRY L. ETTINGER

Department of Horticultural Science and Landscape Architecture
University of Minnesota
St. Paul, Minnesota 55108

How does tissue culture benefit the *practical* plant propagator? A practical plant propagator might ask, is tissue culture propagation (i.e., micropropagation, or *in vitro* propagation) appropriate for my operation? If so, to what extent? How does a propagator determine whether to use tissue culture methods or not? What are the options? Which methods should be employed and on what species? These and numerous related questions need to be asked by the propagator who is considering tissue culture as a possible propagation method. In answering these questions it is important to remember how the practical plant propagator determines whether to use any practice,

material, or equipment. The answer is, as always, will it be profitable?

Propagators should not be over-awed by the science involved in tissue culture propagation. Instead, they should think of tissue culture simply as a relatively new propagation tool. Admittedly, it is a potentially powerful tool, but it still should be thought of as just another potentially useful method for asexual propagation (cloning). Cloning has traditionally been accomplished by cuttings, grafting, layering, division, apomictic seeds, and the use of special structures such as bulbs, corms, rhizomes, etc. It is now possible to add tissue culture to this list of techniques. To better put tissue culture into perspective, it may be helpful to think of the initial plant part used in tissue culture (the *explant*) as merely a tiny cutting. In fact, most tissue culture propagation can be thought of as a modification of cutting propagation. Because of this, it can be argued that many of the same principles that apply to propagation by cuttings should apply to tissue culture propagation.

WHAT ARE THE OPTIONS?

Let's first examine some of the options available to the propagator considering tissue culture, then some of the previously mentioned principles will be briefly presented. Some of the obvious possibilities are:

1) Construct a large tissue culture laboratory, hire a professional tissue culture manager and staff, and begin to propagate plants in large numbers. This is the most expensive option, at least in the short run, because of the large capital outlay required.

2) Ignore tissue culture propagation completely. This involves no initial investment, but the propagator may be passing up a potentially more efficient and profitable approach to propagating certain species. For example, micropropagation has rapidly become the propagation method of choice for *Spathiphyllum*, especially when it is to be used for landscape purposes.

3) Try an approach somewhat intermediate to options 1 and 2:

A) Test the water by trying a small-scale, low-budget approach such as that proposed to this Society several years ago by Stoltz (14).

B) Purchase your tissue culture propagules from established tissue culture producers.

i. Buy proliferating cultures, harvest the tiny shoots and root them as you would root softwood or herbaceous cuttings.

ii. Buy non-rooted microcuttings (shoots produced *in vitro*, but not yet rooted) and root them yourself.
iii. Buy rooted microcuttings. Typically these would be sold in a fashion similar to that used for bedding plants, i.e. in plugs, packs or flats.
iv. Buy liners produced by tissue culture and grow them on to a salable size.
v. Buy finished plants produced by tissue culture.

Note that buying proliferating cultures or non-rooted microcuttings would require special rooting facilities. Such facilities are more sophisticated than intermittent mist systems commonly employed for rooting cuttings. It has been shown that leaves produced in tissue culture do not have fully functional stomates and possess an anatomy that is substantially different from that of normal leaves (1,15). Therefore, conditions of high humidity (and often reduced light) are necessary to aid in the rooting of microcuttings produced by tissue culture (4,10).

It is obvious that no single option is best for all propagators. One should ask, what best meets the requirements of my particular production scheme? Can existing facilities and equipment be used or adapted to facilitate adoption of tissue culture? Ultimately, economic considerations must be the primary factor considered in making such decisions.

TISSUE CULTURE PRINCIPLES FOR THE PROPAGATOR

The principles of tissue culture methodology, hormonal manipulations, and potential applications to propagation have been well reviewed elsewhere (6,8), so the principles addressed here will concentrate on factors that a propagator typically considers when using conventional propagation methods.

1) *Check the literature.* It is always important to find out what is already known about propagating a particular plant, whether using tissue culture or conventional methods. If nothing has been published, perhaps information is available about a closely related species. Advice can also be sought from research and extension personnel working in horticulture departments at land grant universities and other research institutions. And, of course, don't forget to ask other knowledgeable propagators for their advice and unpublished experiences. Find the best information available and use it to your advantage. Check the Index of the IPPS Proceedings.

2) *Start with good stock plants.* Tissue culture requires disease-free stock plants that have received proper nutrition. Nitrogen and other nutrients provided to the stock plant have been demonstrated to influence the rooting of cuttings (5, 16). Likewise, nutrition of the plant from which the tissue culture explant is taken can have a profound effect on tissue culture success (11, 12). Furthermore, the stock plant light regime has also been shown to influence explant proliferation and subsequent rooting of microcuttings. Red light appears to increase the cytokinin levels in the tissue, which often stimulates shoot proliferation. Far-red light tends to elevate auxin levels which in turn stimulates root induction (2, 9, 12). Stock plant photoperiod and chemical treatments have also caused an increase in proliferation *in vitro* (11, 12). In addition, reduced light intensities applied to the stock plant or culture can cause higher auxin levels, thus facilitating rooting of microcuttings (2). In many cases, growth regulators such as IBA do *not* need to be applied to microcuttings, even for species where rooting stimulants would normally be required when rooting softwood cuttings (4, 9, 10).

3) *Use a good rooting medium.* Microcuttings, like other cuttings, respond favorably to a clean, well-drained medium free of pests, pathogens, and toxic substances. Microcuttings rooted in a soil-like medium usually experience less transplanting shock than those rooted *in vitro*. The pH of the medium also influences the rooting of microcuttings, especially those of species which are particularly pH-sensitive when grown in the field (3).

4) *Provide optimum environment when rooting microcuttings.* As mentioned earlier, high humidity, preferably near 100%, and light levels that are less than ambient may be desirable when rooting microcuttings. Fog systems seem to be near ideal for rooting most microcuttings. Use of a fog system or modification of an existing mist system often will enable the propagator to successfully root microcuttings. Bottom heat has also proven beneficial, while plastic tents over greenhouse benches and similar modifications may also aid the propagator in adapting existing facilities to help root cuttings produced *in vitro* (10).

WHAT NEXT?

Tissue culture is a propagation method of great potential. For some plants, it is already practical and will likely become the method of choice for many others. However, for many species, conventional methods will still be preferable to tissue

culture. For others tissue culture may be the best way to establish sufficient stock plant numbers for subsequent conventional propagation. It is likely that new chemicals and methods will be found that will enable successful tissue culture of previously difficult-to-propagate plants. For example, the use of forcing solutions similar to those used for extending cut flower longevity have been used to force softwood growth from cut woody stems. Such softwood shoots can then be used as a source of explant material for tissue culture during the winter period. The forcing solution has also proved to be a useful means of delivering growth regulating chemicals to the tissue prior to culture initiation. Forcing solutions thus may provide a simple new method of modifying tissue responses in vitro.

Somaclonal variation is another potential future application of tissue culture. It can be used as a new source of genetic variation. This phenomenon has been defined as any type of variation displayed by plants regenerated from any form of plant cell culture (7). An excellent review of this subject was published in Vol. 32 of the IPPS *Proceedings* (13). Identification of somaclonal variants in tissue cultures of horticultural crops may lead to the introduction of superior plants showing increased resistance to diseases, herbicides, drought or cold stress, or to plants possessing improved horticultural traits. These novel plant types could then conceivably be clonally propagated by conventional or tissue culture means for release to the consumer.

Practical propagators should monitor these and other developments in the world of plant tissue culture and take advantage of those that prove to be appropriate to their propagation requirements.

LITERATURE CITED

1. Brainerd, K.E. and L.H. Fuchigami. 1981. Leaf anatomy and water stress of aseptically cultured 'Pixie' plum grown under different environments. *HortScience* 16:173-175.
2. Economou, Athanasios S. and Paul E. Read. 1986. Influence of light duration and irradiance on micropropagation of a hardy deciduous azalea. *Jour. Amer. Soc. Hort. Sci.* 111:146-149.
3. Economou, Athanasios S. and Paul E. Read. 1986. Influence of pH and medium coposition on rooting of hardy deciduous azalea microcuttings. Jour. Amer. Soc. Hort. Sci. 111:181-184.
4. Garton, Stephen, Mary A. Hosier, Paul E. Read, and R.S. Farnham. 1981. In vitro propagation of Alnus glutinosa Gaertn. *HortScience* 16:758-759.
5. Kraus, E.J. and H.R. Kraybill. 1918. Vegetation and reproduction with special reference to the tomato. *Ore. Agri. Exp. Sta. Bull.* 149.

6. Krikorian, A.D. 1982. Cloning higher plants from aseptically cultured tissues and cells. *Biol. Rev.* 57:151-218.
7. Larkin, P.J. and W.R. Scowcroft. 1981. Somaclonal variation — a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet.* 60:197-214.
8. Murashige, T. 1974. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. 25:135-166.
9. Read, Paul E. and Athanasios S. Economou. 1982. Supplemental lighting in the propagation of deciduous azaleas. *Proc. Inter. Plant Prop. Soc.* 32:639-645.
10. Read, Paul E. and Cynthia D. Fellman. 1985. Accelerating acclimation of in vitro propagated woody ornamentals. *Acta Hort.* 166:15-20.
11. Read, Paul E., Stephen Garton, Carol J. Carlson, Laurel Conviser and John E. Preece. 1979. Programming stock plants for tissue culture success. *Proc. Plant Growth Regulator Working Group* 6:197-204.
12. Read, Paul E., P. Gavinlertvatana, P. Suriyajantratong, S. Garton and M.L. Brenner. 1978. Stock plants affect tissue culture success. In: K. Hughes, R. Henke, M. Constantin (Eds.), Propagation of Higher Plants Through Tissue Culture. U. of Tennessee, Knoxville, p. 249.
13. Scowcroft, W.R. and P.J. Larkin. 1982. Plant biotechnology, somaclonal variation, and varietal improvement. *Proc. Inter. Plant Prop. Soc.* 32:80-89.
14. Stoltz, L.P. 1979. Getting started in tissue culture: equipment and costs. *Proc. Inter. Plant Prop. Soc.* 29:375-381.
15. Sutter, E. and R.H. Langhans. 1979. Epicuticular wax formation on carnation plantlets regenerated through shoot tip culture. *Jour. Amer. Soc. Hort. Sci.* 104:492-496.
16. Weiser, C.J. and L.T. Blaney. 1960. The effects of boron on the rooting of English holly cuttings. *Proc. Amer. Soc. Hort. Sci.* 75:704-710.

ROLE OF CYTOKININ IN WOODY PLANT MICROPROPAGATION

JOHN W. EINSET
Arnold Arboretum
Harvard University
Cambridge, Massachusetts 02138

Abstract. Using what has now become a standard technology, woody plant micropropagation takes advantage of the effect of cytokinins in stimulating growth and causing shoot multiplication under controlled tissue culture conditions. Although the greatest impact of micropropagation involves species in Ericaceae and Rosaceae, a systematic survey of 130 species in 33 families and 16 orders indicated that the method could probably be extended to several woody taxa that are not currently being exploited (e.g. other species in order Ericales and species in families Bignoniaceae and Rubiacaea). The survey also identified taxa that are unresponsive to the cytokinins, N6-isopentenyladenine (i^6Ade), thidiazuron, and N6-benzyladenine. Apparently, woody species can be classified into three groups based on their tissue culture characteristics: 1) inherently responsive to cytokinins, 2) responsive to cytokinins after acclimation (e.g. *Magnolia* spp.), and 3)