JIM WILL: We are now producing about 7½% of our roses by micropropagation. The cost of micropropagating roses is about twice that of field production. But we have many cultivars that cannot be produced by field-budding techniques. In the future these will be produced by micropropagation. Those, such as 'Mr. Lincoln' and 'Queen Elizabeth', which are easily field-produced, will not be micropropagated.

PHIL BARKER: In propagating different cultivars by micropropagation do you find distinct differences in the root system of these cultivars?

JIM WILL: We have more significant differences in shoot development in cultivar variation. With roses there is no particular difference in root development.

PHIL BAKER: Are the root systems in micropropagation different that what they are in conventional propagation — using the same cultivar?

JIM WILL: There is very little difference in the root system between a micropropagated and a seedling-grown rose.

BRUCE BRIGGS: In rhododendrons, the root system of a tissue-culture propagated plants is usually much heavier than a cutting-propagated plant, because of the way the tissue-culture plant develops — somewhat similar to a seedling grown plant, more of a mass of roots.

CHARLES TUBESING: A question for Jim Will. Do you encounter any viruses in your rose stocks?

JIM WILL: We use only stocks that have been heat-treated and viruses-indexed at the University of California, Davis, for propagation in tissue culture. Viruses have been one of the significant problems in greenhouse roses throughout the industry. Virus expression will appear in tissue-cultured material if clean stock is not used.

OPTIMIZATION OF TISSUE CULTURE MEDIA

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When Murashige and Skoog developed their medium for plant tissue culture over 20 years ago, it was seen to be a great breakthrough in *in vitro* plant propagation. By varying the concentrations of various plant growth regulators, researchers found they could grow a wide range of herbaceous plant species, plus some woody species.

A breakthrough for those interested in the tissue culture of ericaceous plants arrived with Anderson's medium for rhododendron. Further improvements in the tissue culture of woody plants came with McCown and Lloyd's Woody Plant Medium.

The traditional approach to starting a new plant species or cultivar into tissue culture is to try one of the three media mentioned above with various levels of cytokinin and auxin. Some cultivars, especially of woody species, refuse to respond to this method, or even to other media, such as Boxus's or de Fossard's. Other cultivars, although they can be grown in tissue culture, give less than satisfactory results economically, or yield poor quality plants.

We at Microplant Nurseries have faced this problem time and again, in that we mainly grow clones of woody species which have not previously been cultured *in vitro*. In response to this challenge, we have followed the lead of John Driver in developing a systematic approach to optimizing plant tissue culture media.

To begin this process for a particular plant, we search the literature for any reports of tissue culture of this or a related plant. This may only give us a starting point, as successful culture in research does not always translate into success at the commercial level. If no published report is available, or if the results of such reports are unsatisfactory, we will start the plant on three or four different media, such as Murashige-Skoog (MS), Woody Plant Medium (WPM), or Boxus (LB). Another approach, as with a new apple clone, may be to try three or four different media previously developed for different apple clones. After two or three cycles on these media (about two to three months), we visually evaluate the plant's growth on each medium and pick the best one. This may be all that is necessary to achieve eventual production of the clone.

If there are still problems with the plant, we will try to improve, or customize, the medium. We start the customizing process by dividing the chemical components of the medium into their inorganic groups, namely the nitrate group, the sulfate group, the phosphate group, iron EDTA, and calcium chloride, or the halide group. Each group will then be scanned separately. Scanning a chemical or chemical group is done by placing shoot tips on a series of media containing three to five different levels of the particular chemical or chemical group. We use 6 to 12 replicates per treatment, depending on how much material is available. Each week, the heights of the shoot tips are measured and the number of leaves are counted. The shoot tips are transferred every week to fresh medium of the same composition. At the end of three weeks, the results

are analyzed. The optimum level is determined by the amount of growth. At this stage we may also compare different formulations, such as MS nitrates compared to WPM nitrates.

Determining the optimal level of each group may sufficiently define the medium for commercial production. If there are still problems that prevent production, or if plant quality is poor, further refinements will be tried. This is done by dividing each inorganic group into its constituent parts, such as ammonium nitrate and potassium or calcium nitrate for the nitrate group, and scanning each one separately. This does greatly increase the length of time necessary to optimize the medium, but for some plants the extra steps are worth the effort.

In order to determine the optimum value for a given medium component, it is necessary to plot a curve based on the growth data and pick the high point on the curve. There are computer programs available which will do this. We use one obtained from John Driver that computes a polynomial regression analysis. At times, no bell-shaped curve results from the data. This is due either to being in the wrong concentration range or to an interaction between chemicals in the medium. Either the experiment is repeated, with a higher or lower range of concentrations, or else we move on to the next component and repeat the experiment later.

This system is useful for determining the optimum level of other ingredients of the medium, such as vitamins, sucrose, or growth regulators. Using this method of optimizing media has led us to some interesting observations on the differences between cultivars of the same species. For example, one crabapple cultivar we grow requires sorbitol for growth and will not grow on a sucrose-based medium. Another crabapple cultivar will not grow at all on sorbitol, and its growth suffers if any sucrose is replaced by sorbitol. These reactions are straightforward and the effects can be observed visually. Yet a third crabapple benefits from the presence of both sucrose and sorbitol, an effect not readily observed without careful measurement.

With modifications, this system can also be used to optimize rooting media. We are currently trying to optimize rooting media for one apple clone which grows well in multiplication and also roots well, but the tips turn brown and stop growing in the rooting medium. In another case, we are trying this method in an attempt to increase the rooting percentage of a maple clone.

Optimizing the medium may sound slow and painstaking, but the results have been worth the effort. By using this

system we have achieved improved rooting percentages and/or improved plant quality in apples, maples, blueberries, and other plants. Although optimizing plant media is not the answer to all our problems, it is a very important tool for us at Microplant Nurseries.

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LONG-TERM STORAGE TECHNIQUES FOR IN VITRO PLANT GERMPLASM[1]

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National Clonal Germplasm Repository
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The United States Department of Agriculture has established a National system of Plant Germplasm Repositories whose goals are to collect, maintain, evaluate, and distribute plant material of economically important crops. These crops are stored as seeds or as living plants. The Corvallis Repository is a clonal repository responsible for the maintenance of pears, filberts, mint, hops, and all the small fruit crops. Plant

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[1] Contribution of the Oregon Agricultural Experiment Station in cooperation with the U.S. Department of Agriculture, Agricultural Research Service. Technical paper No. 7762 of the former.

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