UPDATE ON TISSUE CULTURE OF WOODY PLANTS

BRUCE BRIGGS AND STEVE McCULLOCH

Briggs Nursery, Inc.
4407 Henderson Blvd.
Olympia, Washington 98501, U.S.A.

Our first experience with tissue culture was in the late 1960's with Dr. Wilbur Anderson of the Western Washington Research Station in Mt. Vernon. Dr. Anderson (2,3), an early student of Dr. Murashige (23) of the University of California at Riverside, was anxious to do tissue culture research with woody plants. We were very excited to have him in our area and spent many hours moonlighting with him and two other nurserymen to get a breakthrough on growing woody plants using tissue culture.

My eldest son, who at that time was in junior high school, also joined us as he was very interested in research, and we put him to work making media. Along with fellow nurserymen, Les Clay and Bob Hart, we worked first on trying to get plants established in aseptic culture.

Among the problems in those early days was a lack of materials, such as the cytokinin, 2iP. Actually, it is amazing how little we were off compared to research that was being done with herbaceous plants. It was really a matter of adjusting techniques and refining the medium (24).

Later Dr. Anderson received research grants, and through many tests was able to refine the medium to determine the level of nutrients in which rhododendrons grew best.

In those days it was certainly an aid to everyone to understand how the plant that one is working with grows outside the laboratory, and what its nutrient requirements are. Even with this knowledge, we were frustrated in not being able to produce rhododendrons from meristems. They turned brown almost immediately. Looking back, it was fortunate that our first cultured rhododendron was a dwarf hybrid, 'Rose Elf'. With this plant, we could be way off on the medium and the plant would still grow. After one gets the plant started it is possible to refine the medium so that the plant grows much better (21). This is true of many plants that are easily grown in tissue culture.

In the early days when Lydiane Kyte (16) was with us, our work involved developing the correct medium on which to grow rhododendrons. At that time, we found ourselves varying our growth regulators trying to get the maximum number of shoots. At times the plants looked almost like moss on the medium. From this experience and others, we learned to always use the lowest cytokinin concentration that will give the shoot production you wish. What we are really concerned about is not how many shoots we get, or

how many micro cuttings we can get from a jar, but how many plants from a jar that grow into strong healthy plants outside it.

As we progressed, rhododendrons did come out of the lab successfully. In addition to ourselves, three other nurserymen worked on how to make them grow outside the laboratory. It was interesting because we all took a different approach and each of us succeeded in making our system work.

Our establishment system involved sticking micro cuttings into a 4 in. pot using a well-drained soil. Almost all our plants are stuck, and then these are graded when they are transplanted. Grading should be done, since plants of one cultivar coming out of the lab do not all grow at the same speed, nor are they all the same initial size. We felt the pot size and drainage was important because we had two things to accomplish. First we had to root the cutting, and then we also had to continue growing that cutting, after it was rooted, within the same medium. We prefer to root everything, if possible, outside the lab. In most cases, it's simply cheaper.

Within this area we would like to become more mechanized. Although we feel we now have a very good system, perhaps a plug system, more commonly used in the bedding plants industry, might be more space and labor efficient. The problem that we have had with plugs is that they have a very small volume of soil and, if you do not follow good cultural practices, including growing on a capillary mat or sand bed, you end up with them drying out. We are still trying to devise methods of using a plug system. Whether it is in the lab or outside, we want to prevent stress in order to have the best production from tissue culture. The key to producing a good final product is to start with high quality, and keep the plant continuously growing.

Many things have changed with time (10,18). In our lab we have more and better chemicals, we have learned what types of growing media to use (27,28), and we have learned how to refine our media. For example, rhododendrons or other woody plants coming out of the lab sometimes look vitreous or waterlogged, or even variable in shoot quality and numbers. When these conditions exist, we do not have the type of plant we really want. One needs a plant culture that has reached the stage that produces quality shoots of uniform growth, so that when the plant comes outside it will continue to grow in a stabilized manner.

Shoot tip propagation from tissue culture is just another propagation method, and in areas where it works it certainly has its place. I feel many times the system is excellent, but problems may occur caused by the people using the system.

When I compare the plants that we produced in the early 1970's with what we are now producing, I am amazed at how we have improved the uniformity and growth of our cuttings. We try to keep contamination at the lowest possible level but we have not found a

way to be absolutely clean. Many times the bacteria that may be in the tube is not that harmful, except that it becomes very evident when you put it in cold storage, or when you fail to subculture often enough. Perhaps in the future there will be an antibiotic (20) that will control these things within the jar.

One of the greatest tools we have in our laboratory is our cool room. We maintain a cold storage temperature of 5°C and 60 percent relative humidity in a room some 10 by 8 by 24 ft. Our cool room acts as a stock block, in that when we produce enough plants for the year they are held until we need more. Or if our production gets ahead, we can store plants for a few weeks. It is true that certain plants will not store very well. We are learning more all the time about how to maintain plants through cold storage. For example, a few years ago when I was in Belgium, Dr. Boxus (5) had strawberries which seemed to be very easy to store, but I saw other plants that he had stored four or five years which, although they survived, did not store as well.

We have used many types of growing containers and we still use some test tubes when studying new plants. Our main growing containers are still baby food jars. We try to streamline all our production so that we can save man-hours in handling our product and this area is a good example. We fit the jars into a basket, autoclave them, place them on carts to cool, transfer plants into them, and finally place them on lighted shelves in the growing room. They come out of this room in the same baskets, which saves a lot of moving of jars. As another example, we sterilize disposable paper towels in a towel holder in the autoclave. We then use the towel as a sterile cutting surface in the laminar hood.

Over the years we have found certain things that can help the process of rooting tissue-cultured plants, (6) but this still remains an art as well as a science. You have to be able to look at a plant and determine where you are in the range of media, you have to make adjustments and continue to make them because a plant may change with time on the same medium. You will find with some plants that a small adjustment of the growing medium, perhaps lowering the nutrient concentration, or eliminating some other element, or maybe just lowering the amount of light, seems to help the initiation of roots.

Researchers in Europe, particularly France and Belgium, have done a lot of work with varying daylength to initiate roots. This has helped rooting, especially with apples (14) and some kinds of trees. We have not seen this positive response to light. I am not sure that what we are doing is so different, but we don't seem to get the same response. Recently, Dr. Anderson at Mt. Vernon has published a very interesting paper on his work with vegetable crops that refused to root. By reducing the amount of light to less than 12 hrs they responded very well to rooting in the greenhouse.

We root and establish plants outside the lab using three different systems. We may go out to a plastic-tented area that is completely enclosed, especially in the winter. In the spring we may use a mist system on an open bench. We find ourselves in the summer using a fog system. Most of the time we like to use a mist system, in conjunction with either fog or a closed tent, to make sure that the small cutting coming out is not put under stress from lack of water. Whatever system you choose, the important thing in rooting tissue-cultured plants is to always keep the plant growing. Do not let it go into a rest period because it may be difficult to get it back into active growth.

The main thing we have observed over the last 15 years has been something we learned very early: do not stress a small plant from tissue culture by planting it in an open field where it may be hot or dry. The plant may not die, but it will not grow fast. Several years ago we planted very small tissue-cultured rhododendrons in the open field. They looked fine, they lived and eventually did well, but they were very slow to grow. They were also slow in changing from the juvenile to the adult stage, in which the plant produces large mature leaves and achieves a normal flushing growth pattern. Some growers in the Portland, Oregon, area working with tissue-cultured trees have found it helpful to grow the plants with drip irrigation. The drip tube is placed beneath the trees when they are planted in the field. It provides adequate water to the plant at all times and allows the grower to control what they are doing. Here the results were similar to those of a small bedding plant sitting on a capillary mat in a greenhouse.

This is the area in which nurserymen probably have the most trouble—the transition from controlled conditions within a greenhouse to the open field—specifically, when the plant is too small and not ready to go there.

Another important point is to grade all plants for uniformity, because growing conditions and water requirements will then be the same for all plants in a row. We encourage the bedding of plants at a small stage, or growing them in a greenhouse to develop a large enough rootball so the plant can better sustain itself. As a result, we now do not see a lack of growth or uniformity.

Many plants from tissue culture will grow faster than from a cutting, but like all things there are exceptions. Some of the outstanding plants that seem to respond for us from tissue culture are Exbury and other deciduous azaleas. They can be produced the year around, you do not have the loss you may experience when growing them from conventional cuttings, they branch better, and they grow faster. It does change your production schedule because you then have to do more shearing. We shear many young plants—including rhododendron, azaleas, lilacs and several others—very low to make them compact. This is one of the major things that growers that are

accustomed to producing cutting-grown plants need to learn. You do need to learn to handle a plant in accordance with its pattern of growth.

It is interesting why plants like lilacs from tissue culture seem to grow much faster and branch more than cutting-grown plants. Young's weeping birch seems to grow tremendously out of the lab. However some trees, like *Styrax japonicus*, have been very hard for us to get the uniformity and habit of growth that we can get from others. We need to realize that not all things coming from tissue culture respond the same. A tissue culture plant may grow faster or slower as a juvenile plant than a normal cutting-grown plant. We have to focus on those plants that respond well to tissue culture and work on what is wrong with those that do not do as well. A good example is *Kalmia*, which can be very difficult to grow. After years of growing this plant, we found that it needs to be pruned very heavily when it is small, fertilized often but not heavily, and that it grows best in a well-drained soil.

We have advanced in the field of conifer tissue culture, thanks to many people within the industry and universities. These researchers are trying to answer questions as to why most conifers with episodic growth are more difficult to culture. Some of the easiest conifers to grow *in vitro*, like *Thuja*, loblolly pine, and *Sequoia*, respond very well, and production seems to be progressing on these plants. Conifer tissue culture research worldwide includes Weyerhaeuser in Washington (7), Dr. Boulay (4) in France, Les Clay (15), Dr. Thorpe in Canada, and Dr. Aitken-Christie (1) in New Zealand.

Dr. Don Durzan from University of California at Davis (11), with fellow researcher Dr. Gupta (12), has and is now continuing research on embryogenesis of conifers (13). In the future this may prove to be an economical way to propagate conifers. At present, research continues in developing techniques to tissue culture these hard-to-grow conifers, but I am sure we will advance in this area as we did with non-conifer plants.

Tissue culture is an avenue to improve a plant, or possibly stabilize it, as Dr. Mapes (22) did with plants in the pineapple or Bromeliad family. She was able to improve stability using tissue-cultured plants compared with plants from divisions of pineapple growing in the field.

In the 1950's, Dr. Mapes studied under Dr. Steward (26), one of the pioneers in single cell research on carrots. At a meeting, I remember expressing my concern to him about how they would be able to take a single cell and stabilize it to the point where one would have good uniformity. He told me that genetic uniformity can be greatly controlled by the medium and the chemicals that the plant is grown on.

Remember: grow the best plants that you can and refine the

growth medium enough to achieve quality. Always be aware of the plants in culture and restart them if problems appear (19). Many times quality lines can be improved by adjusting culture practices in the lab, such as light, heat, media, humidity, contamination, and the upgrading of shoot tips to maintain the very best standards for growing outside.

Quality begins in the lab and ends with the grower of the product.

LITERATURE CITED

1. Aitken-Christie, J. and A. Coker. 1986. Surfactants aid uptake of cytokinin and urea into juvenile *Pinus radiata* plantlets. *Proc. Inter. Plant Prop. Soc.* 36:499–504.
2. Anderson, W. C. 1975. Propagation of rhododendrons by tissue culture: Part 1. Development of a culture medium for multiplication of shoots. *Proc. Inter. Plant Prop. Soc.* 25:129–134.
3. Anderson, W. C. 1980. Mass propagation by tissue culture: principles and techniques, pp. 1–10. Proc. of the Conference on Nursery Production of Fruit Plants through tissue culture—applications and feasibility, USDA, Beltsville, MD.
4. Boulay, M. 1976. Recherches sur la propagation du Douglas par culture *in vitro*. *Afocel*, 83–145.
5. Boxus, P. 1974. The production of strawberry plants by *in vitro* micropropagation. *Jour. Hort. Sci.* 49:209–210.
6. Briggs, B. and S. M. McCulloch. 1983. Progress of micropropagation of woody plants in the United States and Western Canada. *Proc. Inter. Plant Prop. Soc.* 33:239–248.
7. Cheng, T. 1978. Clonal propagation of woody plant species through tissue culture techniques. *Proc. Inter. Plant Prop. Soc.* 28:139–155.
8. Cohen, Daniel. 1981. Micropropagation of *Zantedeschia* hybrids. *Proc. Inter. Plant Prop.* 31:312–316.
9. Debergh, P. C. 1987. Micropropagation of woody species—state of the art on *in vitro* aspects. *Inter. Soc. Hort. Sci.* Symposium: On Vegetative Propagation of Woody Species, Universitá di Pisa, Italy, p. 31.
10. de Fossard, R. A. 1976. Tissue Culture for Plant Propagators. University of New England, Australia.
11. Durzan, D. 1984. Tissue culture for forest improvement. Symposium: Propagation of Higher Plants Through Tissue Culture: III Development and Variation, Univ. of Tennessee.
12. Durzan, D. and P. K. Gupta. 1987. Encapsulation and storage of somatic embryos of woody perennials. *Inter. Soc. Hort. Sci.* Symposium: On Vegetative Propagation of Woody Species, Universitá di Pisa, Italy, p. 82.
13. Gupta, P. K. and D. J. Durzan. 1987. *In vitro* establishment and multiplication of juvenile and mature Douglas-fir and sugar pine. *Inter. Soc. Hort. Sci.* 212:II:483–487.
14. Jones, O. P. and S. G. S. Hatfield. 1976. Root initiation in apple shoots cultured *in vitro* with auxin and phenolic compounds. *Jour. Hort. Sci.* 51:495–499.
15. Kurz, M. L. 1985. Tissue culture of conifers. *Proc. Inter. Plant Prop. Soc.* 35:206–216.
16. Kyte, L. and B. Briggs. 1979. A simplified entry into tissue culture production of rhododendrons. *Proc. Inter. Plant Prop. Soc.* 29:90–95.

17. Loreti, F., S. Morini, and G. Carrozza. 1981. Observations on micropropagation of M27 apple rootstock. *The Plant Propagator*, 27:2.

18. McCown, B. H. and G. B. Lloyd. 1983. A survey of the response of rhododendron to *in vitro* culture. *Plant Cell Tissue Organ Culture* 2:75–85.

19. McCown, D. D. 1987. Causes of non-uniformity of micropropagated stock. *Proc. Inter. Plant Prop. Soc.* Vol. 37.

20. McCulloch, S. M. and B. Briggs. 1982. Preparation of plants for micropropagation. *Proc. Inter. Plant Prop. Soc.* 32:297–304.

21. Maene, L. J. and P. C. Debergh. 1987. Optimalisation of the transfer of tissue cultured shoots to *in vivo* conditions. *Proc. Inter. Soc. Hort. Sci.* 212:I:335–342.

22. Mapes, M. O. 1973. Tissue culture of bromeliads. *Proc. Inter. Plant Prop. Soc.* 23:47–55.

23. Murashige, T. 1974. Plant propagation through tissue cultures. *Ann. Rev. Plant Physiol.* 25:135–66.

24. Nitsch, J. P. 1969. Plant propagation at a cellular level: a basis for future development. *Proc. Inter. Plant Prop. Soc.* 19:123–132.

25. Read, P. E. and Y. Qiguang. 1985. Novel plant growth regulator delivery systems for in vitro culture of horticultural plants. *Acta Hort.* 212:I:55–58.

26. Steward, F. C., A. E. Kent, and M. O. Mapes. 1967. Growth and organization in cultured cells: sequential and synergistic effects of growth regulating substances. *Ann. N.Y. Acad. Sci.* 144:326–334.

27. Zimmerman, R. H. 1978. Tissue culture of fruit trees and other fruit plants. *Proc. Inter. Plant Prop. Soc.* 28:539–545.

28. Zimmerman, R. H. 1985. Comparison of rooting methods for apple cultivars *in vitro*. *Acta Hort.* 212:I:303–314.

NOTCUTTS' EXPERIENCES WITH MICROPROPAGATION

DAVID C. HANNABY

Notcutts Nurseries Limited
Woodbridge, Suffolk

INTRODUCTION

Notcutts' interest in micropropagation began in the late 1970's when the company realised the potential for the technique on a modern nursery. What was less clear was what specific role would develop for micropropagation in the nursery stock industry and what production levels for the technique would be appropriate.

Initially micropropagated plants were brought in from commercial laboratories and closer links were formed with one of the UK laboratories. However, it soon became apparent that an on-site laboratory was necessary and in 1980 a laboratory was constructed within the propagation unit at Woodbridge.

The laboratory now produces approximately 120 subjects and represents perhaps 10 to 15 percent of Notcutts' production.