UPDATE ON TISSUE CULTURE OF WOODY PLANTS

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We are pleased to relay some knowledge and experience gained from some 20 years of tissue culture work at Briggs Nursery. Our first experience with tissue culture was in the late 1960s with Dr. Wilbur Anderson of the Western Washington Research Station in Mt. Vernon. Dr. Anderson, an early student of Dr. Murashige of the University of California, Riverside, was very anxious to do tissue culture research with woody plants. He was hired to do research on field cole crops like cabbage and broccoli. We spent many hours with him and two other nurserymen to get a breakthrough on growing woody plants using tissue culture. My oldest son, who at that time was in junior high school, was very interested in research so we put him to work making tissue culture media. Along with fellow nurserymen, Les Clay and Bob Hart, we worked first on trying to get plants established in 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 with woody plants compared to research that was being done with herbaceous plants. It was really a matter of adjusting techniques and refining the medium. Later Dr. Anderson received research grants, and through many tests he was able to refine the medium to determine the level of nutrients in which rhododendrons grew best.

It was certainly an aid to everyone to understand how the plant 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 the medium could be way off and the plant would still grow. After it is started it is possible to refine the medium so that the plant grows much better.

In the early days when Lydiane Kyte was with us, our work involved developing the correct medium on which to grow rhododendrons. We varied our growth regulators trying to get the maximum number of shoots. At times the plants looked almost like moss on the medium. We learned always to use the lowest cytokinin concentration that gives the shoot production wanted. This produces a plant superior to those produced on a higher cytokinin medium. What we are really concerned about is not how many

shoots or how many microcuttings we get from a jar, but how many plants from that jar grow into strong, healthy plants

As we progressed, rhododendrons did come out of the lab successfully. In addition to ourselves, three other nurserymen worked on growing them 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 microcuttings into a 4 in. pot having a well-drained soil. All our plants are graded when transplanted. Grading should be done, since plants coming out of the lab in one cultivar do not all grow at the same speed, nor are they 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, then we also had to continue growing that cutting in the same medium after it was rooted. Even though I know people report on rooting plants inside the lab, we prefer to root everything, if possible, outside the lab. In most cases, it's simply cheaper to root plants directly in the greenhouse.

We would like to make this process more mechanized. Although we feel we have a good system, using plugs might be more space- and labor-efficient. Plugs 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, they dry out rapidly. We are still trying to devise methods of using a plug system. Whether it is in the lab or outside, one must prevent stress to have the best production. The key to producing a good final product is to start with quality and keep the plant continuously growing.

Many things have changed with time. We have more and better chemicals, and we have learned how to refine our media. We must have a culture that produces quality shoots of uniform growth so that the plant will continue to grow in a stabilized manner outside of the laboratory.

Shoot-tip propagation from tissue culture is just another propagation method and, in situations where it works, it certainly has its place. However, if it doesn't improve a situation, it may be wise to stay with conventional propagation methods because they also have a place.

I am amazed at how we have improved the uniformity and growth of our cuttings since the 1970s. However, we have not found a way to be absolutely clean. Many times the bacteria in the tube are not that harmful, but become very evident when put in cold storage or not subcultured often enough. We have tried many antibiotics, but at the present time we do not have any that effectively control bacteria in our shoot cultures.

One of the greatest tools we have in our laboratory is our cool room. We maintain a 10-x 8-x 24-foot room at a temperature of 5 °C and 60% relative humidity. The cool room acts as a stock block; 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. Certain ones will not store very well, but we are learning more all the time about how to maintain plants through cold storage.

We have used many types of growing containers and still use some test tubes when studying new plants. Our main growing containers are baby food jars. We try to streamline all our production so that we can save man-hours in handling our product. 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, but this still remains an art as well as a science. One has to learn and acquire the ability to look at a plant and decide how to make adjustments and continue to make them. One must sense in some cases that just lowering the amount of light will help the initiation of roots. People in Europe vary day length to improve root initiation. This has helped rooting, especially with apples and some trees. We have not seen this positive response to light. However, Dr. Anderson has found that reducing the amount of light to less than 12 hours daily improved rooting of certain vegetable species.

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. In the summer we use a fog system. Most of the time we like to use mist in conjunction with either fog or a closed tent, to make sure that the cutting is not put under stress due to lack of water. The important thing in rooting tissue-cultured plants is to 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 we learned very early: do not stress a small tissue-cultured plant by putting it in a hot, dry, open field. 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 and were slow in changing from the juvenile to the adult stage, in which the plant produces large mature leaves and

has a normal flushing growth pattern. Some growers in the Portland, Oregon, area have found it helpful to grow tissue-cultured trees with drip irrigation. The drip tube is placed beneath the trees when they are planted in the field. The transition from controlled conditions within a greenhouse to the open field is the most troublesome stage in tissue-culture production.

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

Many plants from tissue culture will grow faster than from a cutting, but there are exceptions. Some of the outstanding plants from tissue culture are Exbury and other deciduous azaleas. They can be produced the year around, have higher survival rates, branch better, and grow faster. It does change the production schedule because you must do more shearing We shear many young plants; including rhododendron, azaleas, lilacs, and several others very low to make them compact.

Tissue-cultured lilacs 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 other trees. We need to realize that not all plants coming from tissue culture respond the same. We have to focus on those plants that respond well to tissue culture and work on what is wrong with those that do not. *Kalmia* can be very difficult to grow. After years of growing this plant, we found that it needs to be pruned very heavily when it's small, fertilized often but not heavily, and grown 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. *Thuja, Pinus taeda* (loblolly pine), and *Sequoia* respond very well, and production seems to be progressing on these plants.

Tissue culture can be a way to improve or possibly stabilize a plant as Dr. Mapes did with plants in the pineapple or bromeliad family. In the 1950s Dr. Mapes studied under Dr. Steward, one of the pioneers in single-cell research on carrots. I expressed my concern to him about stabilizing a single cell enough to 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. To me this was quite an insight. Many times a minor change can become major in the way it affects the quality and production

of a plant in the lab. I am sure many of those working on tissue culture can give examples from their own labs where this observation of Steward's is true.

In summary, we have come a long way. Many people have been involved, many ideas have been exchanged. The motto of this group, "To seek and to share", certainly has helped many of us. We should remember that the system is working well. 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. Many times quality lines can be improved by adjusting culture practices in the lab involving light, heat, media, humidity, contamination, and upgrading shoot tips. Remember, quality begins in the lab and ends with the grower of the product.