A SMALL SCALE TISSUE CULTURE LABORATORY

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I started as a backyard nurseryman some 40 years ago. When I raised a few plants I found out that I had to buy a license to sell them, so on March 19, 1948, I got my first license. That was 33 years ago. When I outgrew the backyard I bought a small ranch, on the outskirts of Snohomish, Washington. De Wald Nursery sits on 7½ acres along the East bank of the Pilchuck river.

My latest project has been starting a small scale tissue culture laboratory for rhododendrons. Tissue culture is a very complex and special way of propagation but at my age and with no more scientific knowledge than I have I can make it work. There is a vast future in tissue culture for many of you younger and more qualified people.

If you have any success at all with tissue culture it will not let you stay small. It multiplies and grows. To start with I took an old refrigerator, then installed heat cables on a thermostat, Gro-Lux lights on a timer, and a fan to circulate the air and keep it cool. In the refrigerator I built 5 shelves. Each shelf held one rack and each rack held 77 tissue culture tubes at the proper angle. This was a total of 385 tissue culture tubes.

One of the first and most important things to remember about any tissue culture set-up is to keep it clean of all contamination at all times. When I started my tissue culture of rhododendrons, I did not do it alone. I worked with Dr. Wilbur Anderson, from Mt. Vernon, Washington, who perfected the formula for starting, multiplication, and rooting rhododendron explants. I went to his laboratory where he furnished me with the filled and sterilized test tubes. I would bring my rhododendron cuttings with me then we sterilized and trimmed them for the test tubes. Transferring the cuttings to the test tubes was done by using a laminar flow hood. I would then place these tubes in my make-shift lab (the old refrigerator) for growing. I also attended a short course on tissue culture propagation of rhododendrons, sponsored by Dr. Anderson and Randy Burr at Skagit Valley College, Mount Vernon, Washington.

In November or December, 1979, I outgrew the old refrigerator and built a room 10′ wide and 28′ long upstairs in my storage shed. This room contained my kitchen, transfer hood, and 2 racks, 4′ by 8′ by 64″ high, with 4 shelves. There are two

Gro-Lux lights to each shelf. Each rack has the capacity for about 4,000 tubes. The racks are mounted on wheels so they could be moved for cleaning and easy access to the shelves.

Now the media are prepared, the tubes filled and sterilized here. Also the old tubes are opened and the plantlets transferred to clean tubes under a hood that I built for this process.

One year later this room became too small. I had one rack completely full and the second well on its way. Again, it was time to enlarge so I extended the room to 40 feet. This room will now hold 5 racks. Each rack will hold 4,000 tubes, so this will give me about 20,000 tubes total. This should give me all the pleasure and trouble that I will be able to stand. So then I had to build another room for the kitchen and transfer room.

At this stage in Dr. Anderson's course, I became an outlaw with the scientific way. I skipped Dr. Anderson's third stage, which is the rooting stage. I did this to save time and space. I took the cuttings directly from the multiplication medium in the test tube and placed them in the same medium that I use to root rhododendrons in the conventional way. This medium contains 1 part peat moss and 2 parts perlite. It was placed in plastic cocktail glasses to within one inch of the top and covered with a plastic petri dish lid. Before placing the tiny cuttings in the medium they were dipped in Hormodin #3, the same as I do with conventional cuttings. Each glass holds 20 cuttings. These glasses are then placed in a 12″ by 24″ flat filled with the same medium. Each flat holds 18 glasses. The flats are then placed on electric heat cables set at 65° to 70° F. The results that I have had this way have been so good, I see no reason to change my procedures at this time.

The small cuttings, ⅜″ to ¾″ high, have a good root system in about 6 weeks. These tiny plants are then placed in the greenhouse in beds with a fine bark mixture. The mixture contains in 4 cubic feet: one 5-gallon bucket of alder sawdust; and 1 scoop shovel of used starting mix (1 part peat moss + 2 parts perlite). The balance of the 4 cubic feet is fine bark. To this I added dolomite lime, sulphur, and trace elements. This mixture is then placed in a bed on the ground in the greenhouse. The bed is 42″ wide and 100′ long and has electric heating cables under it. The fine bark mixture is then placed 4 to 5 inches deep in the bed. This is the same method that I use in rooting cuttings the conventional way. From here I add more fertilizer and watch for all the problems that occur in raising any plant.

At the present time I have roughly 12,000 rooted rhododendron cuttings started in tissue culture. They range from ½″

to ¾" tall just rooted plants to those 10" to 12" tall. The latter plants are 'Jean Marie de Montague'. They were rooted cuttings a little over a year ago and it will take 2 more years to complete the cycle. At this stage the plants are developing nicely.

It is hard to give a talk when most of your thoughts are still unanswered questions, such as: time to take the cuttings, how long to sterilize, hardness of the wood, and all the other things that a grower must know even for conventional propagation. There are so few of us into tissue culture yet that is a real problem to get such information and material. I can't even get some rhododendron cultivars to start in the tubes. So there is much for me to learn. One of the biggest problems is to learn the different chemicals for the different rhododendron cultivars and how to change the chemicals for the starting and for the multiplication media for each individual plant. I have used the starting medium at ½ strength, regular strength, and double strength.

There is no one that goes any place alone in this world. You have to listen to those who know, and watch what they are doing. Also I think much will be learned by our mistakes and by keeping in close contact with each other to exchange information. Then by applying what looks reasonable to you in your way of accomplishing what you desire. What works for one person may not work for another.

Talking with Bruce Briggs the other day, I heard another new idea on tissue culture. If you get too many tubes of one rhododendron cultivar you can put them on refrigeration until you need them again. This opens a whole new field of questions. But at what stage of the multiplication do you put the tubes in storage and, roughly, how long will they hold in storage, and at what point will they be ready to use when you do return them to the growing room? I have started refrigerating several kinds in three different stages of the multiplication. After 2, 4, and 6 weeks I will take these tubes out of refrigeration, so later I will know which group of the tubes will stand refrigeration the best and how long they may be kept in refrigeration. But it takes a long time to get the answers to questions like these, possibly 1 to 2 years or even longer.

MODERATOR PARVIN: We have time for a few questions. Yes — John Hart.

JOHN HART: Question for Arie van Vliet. On your blue spruce grafting, how does the temperature for the August date

in Boskoop, Holland, correspond with temperature in the Pacific Northwest at that time of year; is it somewhat the same? And when you place the grafts in sawdust, what is the advantage of laying them on their side? Do you get roots growing into the sawdust outside of the pots the grafts are in?

ARIE VAN VLIET: First of all, it is not sawdust, it is peat moss. In the cold frames, the inner layer is clear glass; we don't use any cover on it. Now you might think that will burn, but as soon as the sun comes out, we will cover it. We cover extra heavy; otherwise the grafts will burn very fast. But we need the light the whole day, to get that cambium growth. As soon as the sun comes out, we run out real fast and cover it. Our average temperature in July is 17°C (63°F).

JOHN HART: The other thing in which I am interested is when you have such a high concentration of nurseries in Boskoop, do you have any problems with chemicals being sprayed by another nursery coming down on yours?

ARIE VAN VLIET: There have been a few claims, yes.

JUDY GARLOCK: I have a question for Ed Bunker. You were talking about *Grevillea* nutrition in relation to rooting. Our nursery has had a problem this past year with rooting *Grevillea* cuttings. I was wondering about a couple of things you said; one of them was about the superphosphate, to which you said they are sensitive. Another one is fertilization — if they are fertilized too heavily maybe the cuttings wouldn't root as well. Do you know whether that might extend to all *Grevillea* species?

ED BUNKER: As far as I know, it applies to all *Grevilleas*. I have talked to quite a few people around Australia about this. Everyone has had similar experiences; as soon as the fertilizer levels get out of balance, the plants won't grow properly. Consequently, you don't get good cutting material and you don't get good rooting.

MODERATOR BRUCE MORTON: The last panel of the meeting will deal with the topic of "Hard-to-Root Cultivars." Dr. Wilbur Anderson will be the lead-off speaker: