confined to the rootball could aid plant establishment under conditions in which the surrounding field soil remains too wet for good root growth between irrigations.

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Ed. Note: Dr. Tsai Ying Cheng, Oregon Graduate Center, Beaverton, Oregon, discussed her work on mass clonal propagation of fruit and shade trees.

A SIMPLIFIED ENTRY INTO TISSUE CULTURE PRODUCTION OF RHODODENDRONS

LYDIANE KYTE and BRUCE BRIGGS

Briggs Nursery
Olympia, Washington 98501

Some growers are asking if tissue culture is a tool they should try. There is no single answer but with a few guidelines and a modest investment answers are soon evident. In the past two years Briggs Nursery has ventured into rhododendron tissue culture production. This effort is backed up by 10 years of interest and research support. A number of cultivars are now beginning to come out of test tubes and into pots in significant quantities. At this stage of production we feel it appropriate to share some of our beginning experiences including a brief review of starting rhododendrons in tissue culture and some of the systems that have worked for us.

Growers looking for information on how to get started can find help through many sources (5). Among these are agricultural extension agents, colleges, experiment stations, libraries, tissue culture and horticultural organizations, companies that sell tissue culture supplies, and from nurseries engaged in plant tissue culture. Courses in plant tissue culture are available at the W. Alton Jones Cell Science Center in Lake Placid, New York and many universities in the United States. The basic re-

search for rhododendron tissue culture was accomplished by Anderson (1).

Rhododendrons in tissue culture. Our original cultures were started either by ourselves or other laboratories. There is no particular way to tell when a rhododendron is ready to be cut for starting in tissue culture. Usually the cuttings are taken in a similar state to that for normal propagation by cuttings. Two-inch cuttings are stripped of leaves and terminal bud then washed in water with detergent. They are raised then placed in a 10% solution of household bleach (5% sodium hypochlorite) with a few drops of detergent for 15 minutes. While in this solution they can be agitated by hand, by a magnetic stirrer or in an ultrasonic cleaner. Next they are rinsed in a 1% solution of bleach. Using sterile technique the basal end is trimmed and each cutting is placed in a test tube containing sterile media.

If successful, the cuttings will prove to be uncontaminated and start growing. Growth first takes place by development of one or more axial buds. If it is going to happen at all such growth usually occurs from two to eight weeks after starting. The leaves produced in these breaks are very small. In addition to the nodal breaks a green, granular tissue mass may form. Each granule of this mass is a potential shoot.

The axial shoots are weaned from the original cutting in two or three transfers by removing more of the original stem in each successive transfer. These early transfers may be required every two weeks as determined by the size or the deterioration in color of the explant. As the shoots grow they may be cut into 1 inch sections and laid on fresh agar where they will produce more shoots. Any tissue mass that has grown can also be divided. In this way the multiplication stage is reached in a few months from when the cutting was first taken. Later transfer intervals should be six to eight weeks apart. It was of experimental interest that some cultures which were held in a dimly lighted refrigerator for a year suffered no ill effects.

Rooting. At anytime in the multiplication stage one may wish to remove shoots for rooting and growing on. Shoots are transferred to a sterile root-inducing, agar medium or removed from sterile culture and placed in a growing mix in a covered or misted container. In either case roots appear in about two months. In six months to a year they produce normal size foliage and are ready for gallon cans. The few cultivars we have observed through blooming stage appear true to form. More experience is needed to evaluate genetic stability both in general and on specific cultivars.

To root rhododendrons while still in sterile culture, we maintain the sugar concentrations (30 g/l), eliminate 2IP, IAA, and

KI, reduce the remaining constituents to ⅓ strength, and add activated charcoal (Gibco, 600 mg/l) (2,4). Our work indicates that rooting occurs soon, (one month in some) in agar containing only sugar (30 gm), IBA (5 mg), and activated charcoal (800 mg/l).

Greenhouse rooting of tissue cultured rhododendrons. Anderson has discussed some of the problems of rooting tissue cultured rhododendrons in soil mixes (3). We use peat, perlite, decomposed bark (1-1-1) with a covering of screened sphagnum moss. Flats, trays or 4″ pots are satisfactory for rooting. PLANTCON covers fit over 4″ pots to make a desirable covered container. The constant temperature and light of a controlled growth room are ideal for this demanding stage of development (6). As soon as plants are rooted, then hardened to greenhouse conditions, they are transplanted to individual cells.

Facilities. The first laboratory at Briggs Nursery was an old kitchen adjacent to the office. The first growth room was a back closet with lighted shelves. A homemade transfer chamber with an ultraviolet light and a small (1′ × 2′) HEPA (high efficiency particulate air) filter was satisfactory for transferring (5). This chamber was located in a closed off corner of an existing greenhouse. More lighted shelves for tubes were added in another corner of the same greenhouse. With these simple facilities, we multipled the cultures to about 3000 tubes.

In a few months we outgrew our original facilities. A three room laboratory was built within a new greenhouse with room for expansion. The 15′ × 15′ media preparation room is basically a home kitchen with storage, stove, dishwasher, refrigerator, and sink. A faucet with deionized water leads from a treatment tank in an adjacent restroom. Next to the media preparation room a small corridor has sliding glass doorways which open into the transfer room or the tube room. The 10′ × 10′ transfer room contains an eight foot commercial laminar flow hood (transfer chamber). Air is blown through the HEPA filter providing sterile air in which to work when transferring. The tube room is 12′ × 18′. It has a total of 400 square feet of shelf space which can support approximately 20,000 cultures or 200,000 or more potential plants. The cultures are 18″ from the fluorescent lights and receive 100 to 250 foot candles of light 16 hours a day. Most of the fluorescent tube ballasts have been removed and placed in an adjoining room to eliminate excessive heat in the tube room. Pass-through windows allow material flow between the preparation room and the transfer room and between the transfer room and the tube room. Temperature is controlled between 70° and 80°F with air conditioners and electric wall heaters.

As we expanded, the plastic racks which hold ten test tubes required too much room and handling. An excellent off-the-shelf tube holder was found in the 128 hole, 2½" deep SPEEDLING plastic foam tray commonly used for seedlings. These shallow, square-holed trays hold the tubes on a slant, are lightweight and easily moved. In our search for economy of room we also use 25 mm deep plastic petri dishes and PLANTCONS, a much taller dish we use for rooting.

Media Preparation. The agar media we use are Murashige and Skoog formulae as modified by Anderson (1) as given in Table 1. Nine liters of medium are mixed at one time in an enamel pan. As the chemicals are added to deionized water to mix the formula they are checked off on a list of ingredients. An adequate balance for weighing chemicals can be a major expense. We bought two used balances, a ROLLER SMITH, which weighs up to 500 milligrams, and a HARVARD balance for larger quantities. No weighing is required if a grower buys pre-mixed media. We use an inexpensive pH meter to bring the media to a pH of 4.5. Some commercial mixes have the pH pre-adjusted.

Table 1. Rhododendron medium (Multiplication).

Chemical	Mg/l	Chemical	Mg/l
Sucrose	30000.00	Magnesium sulphate	370.00
Inositol	100.00	Manganous sulphate	16.90
Adenine sulphate	80.00	Zinc sulphate	8.60
Ammonium nitrate	400.00	Copper sulphate	
Potassium nitrate	480.00	EDTA	74.50
Sodium phosphate (monobasic)	380.00	Ferrous sulphate	55.7
Boric acid	6.20	Thiamine hydrochloride	0.40
Sodium Molybdate	0.25	IAA	1.00
Calcium chloride		2IP	5.00
Potassium iodide	0.83	Agar	6000.00
Cobalt chloride	0.03	(pH 4.5)	

The medium must be heated to dissolve the agar (which is a gelatinous extract from seaweed) in it. We heat the medium in three-liter batches in an Erlenmeyer flask on a combination hot plate magnetic stirrer. When heated, the medium is poured from a pitcher into test tubes (18 ml per tube) which are held in 40-hole test tube holders. The test tubes we use are the disposable type made of borosilicate glass. We reuse these tubes indefinitely. For sterilizing we use a household type pressure cooker canner. The canner basket is lined with screen so it will hold the test tubes. Before processing for 15 minutes at 15 lbs pressure the tubes are capped with permeable membrane closures or caps which have been stuffed with a little cotton to help insure against contaminants. After the basket of tubes is processed, it is slanted until the agar is cooled and solidified. The medium is first sterilized in quart jars if it is to be poured into sterile plastic petri dishes or PLANTCONS which cannot be heat sterilized. The dishes are poured with medium inside the transfer

chamber. The medium should be stored in a dust-free area until it is used.

Procedures. Sterile technique is basic to the whole tissue culture operation. It requires an imagination to realize sources of contamination. Particularly in the transfer chamber it is necessary to be aware of what is sterile and what is not. If chamber air flow is obstructed contaminated air may enter the chamber. Tube racks may have contaminants blown from them onto exposed sterile cultures. Solutions of household bleach (10% and 1%) are used for disinfecting gloved hands, implements, petri dishes, and counter tops. Implements must be of stainless steel so they will not corrode in the bleach. Frequent dips into the strong and then the weak solutions are essential. The weaker bleach solution does not harm most cultures. Some laboratories prefer to use alcohol and flame but we find the bleach solutions simple and effective.

General cleanliness is essential but hospital sterility is not practical. No vacuum cleaner or broom should be used in the area due to the dust they may circulate. Floors should be wet mopped every day and counter tops scrubbed. No moldy culture tubes should be opened before sterilizing them. Moldy petri dishes should be handled so as not to disperse the spores into the lab. Good soil catching mats at the door will help keep out nursery soil.

Several housekeeping chores are done in the transfer room. Tubes with freshly divided cultures are labeled with cultivar number, date, and kind of medium. When a transfer is made there is often some dark basal debris which is cut off and discarded. To make this disposal easier we cut up commercial paper toweling into 2½" squares and sterilize them in a covered beaker. A clean paper is placed in a petri dish each time a culture is removed from a tube and placed in the dish for cleaning and dividing. At this time tube caps removed from the used tubes are dropped into a box. The caps are reused later without washing because they are chemically clean and will be sterilized after capping fresh tubes. Plastic petri dishes with cultures are taped shut with PARAFILM to reduce contamination potential, to prevent the extended shoots from forcing the lid off, and to permit easy handling.

The used tubes, still with media but without plants or debris, are returned to the media preparation room. There they are placed in racks which hold 40 upright tubes. Hardware cloth (coarse wire screen) is secured over the tops of the uncapped tubes in the racks. The wire covered racks holding the tubes are inverted and placed in this dishwasher which melts and removes the medium and cleans the tubes. The plastic petri dish-

es are hand washed then dipped in bleach solutions in the transfer chamber before pouring with fresh agar.

Conclusions. At Briggs Nursery we have worked out some of the fine points of getting started in tissue culture. There are several advantages to this method of propagation. From a single cutting there is potential for an infinite number of plants providing no mutations occur. Plants in tissue culture reproduce regardless of season. There is no watering requirement until plants are removed from sterile culture. Thousands of plants can be started in a comparatively small area. We believe we can produce rhododendrons at equal or less cost in the same time frame as by traditional means.

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VIRUS ELIMINATION AND RAPID PROPAGATION OF GRAPES *IN VITRO*

ROBERT E. HARRIS and JOHN H. STEVENSON

Agriculture Canada, Saanichton Research Station
Sidney, B.C. V8L 1H3, Canada

Abstract. Heat treatment and *in vitro* culture of shoot tips were used to free *Vitis vinifera* 'Liemberger' of leafroll virus and 'Forta' and 'Auxerrois' ('Cl-21') of fanleaf virus. Rapid propagation of the French hybrid 'Baco' was obtained on full-strength MS medium plus adenine sulfate (80 mg/l), $NaH_2PO_4 \cdot H_2O$ (170 mg/l), i-inositol (100 mg/l), thiamine-HCl (0.4 mg/l), and BAP (3 to 4 mg/l). Rooting of proliferated shoots was most rapid on ¼-strength MS with 0.08 mg/l IBA. The method appears suitable for the rapid propagation of other cultivars.

INTRODUCTION

British Columbia grape growers and wineries are continually seeking new cultivars for increasing hardiness and wine