

Cuttings are taken early in the morning to prevent wilting. They are soaked in a mixture of Diazanone and Kelthane at a rate of 2 tbs of each in 25 gal water. Cuttings are made and a rooting hormone is used in the usual manner. The flats of medium are watered lightly before the cuttings are placed into them. Flats are placed under mist immediately after sticking. The bed temperature is kept between 70° and 80°F. The greenhouse is kept between 85° and 90°F. Initially, the mist is held at a heavy rate, running this way for approximately one week so the foliage never dries. Mist is then decreased slowly as roots develop and plant growth begins. Mist is discontinued after 2 to 3 weeks when no wilting occurs.

Most material will be held over through the winter for transplanting the following spring. *Potentilla* that has been started in May may be transplanted by August or September. *Magnolias* are transplanted into 4-in pots by fall to allow for root growth. Cell packs that are held over are thoroughly drenched every 2 to 3 weeks to prevent disease, using Captan and Benlate, or Benlate and Truban. The rate is 4 oz of each to 100 gal water.

Liners are sheared often in order to have a well-branched plant. They then require less attention after transplanting. Most all of our summer cuttings have rooted and grown well in the medium described. We have experienced problems with only mock orange and heathers.

PROGRESS AND NEW IDEAS IN TISSUE CULTURE PROPAGATION

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There have been no recent discoveries of new substances which affect plant material and it is now a matter of adjusting formulas of the known ones to suit the needs of different plants and of refining our techniques for handling. This paper will chiefly consider the physical aspects of a tissue culture laboratory, with a brief overview of media and plant material handling. B & B Laboratories, like most plant enterprises, is interested in producing plant material in the most efficient way. The actual physical lab and the handling processes can be very costly and I will discuss ways in which costs have been held down at our lab. We grow a wide variety of plant

material, ranging from woody shrubs (rhododendrons, azalea, kalmia, etc.) to ornamental trees, perennials, foliage plants, and bulbs.

Lab Construction and Operation. Our physical building is of a modular construction and built within a large open warehouse of 3,500 sq ft. The present lab size is 1,400 sq ft but is now being expanded. The walls are of 4×8 ft panelled sections and the ceiling is made of 4×12 ft sections. Because these sections are bolted together rooms can be added or changed in size or configuration easily to accommodate changing needs.

To keep the air cleaned and the temperature controlled, we use an air filtration system which recycles warmed air in winter or brings in cooler outside air in summer. This can be tied to a heat pump system to add heating or cooling as necessary. The use of outside or recycled air greatly reduces the cost of operating the heat pump or air conditioning unit. (This has also been done with good results at the Briggs Nursery tissue culture lab in Olympia, Washington.) The air is filtered before entering the lab with a hepa type filter. We have a separate air system for each room so that we can maintain different temperatures in separate areas as our plant and employee needs require.

The media preparation area is U-shaped to facilitate an even work flow, starting on the right side with a household refrigerator and counter top, with shelves above which hold all chemicals, stock solutions, and the scales. In the center is the sink with the distilled water unit and the dishwasher. The left side has the pH meter, counter-top stove, and the stirrer for cooking, followed by counter space for filling the tubes and containers, then the autoclave and cooling shelves which connect to the cutting area.

In the cutting area we use simple air filtration stations based on a design from Dr. Wilbur Anderson of the Northwest Washington Research Station, Mount Vernon, Washington. The stations use only one ½ in thick filter-down filter because the air going into the room has already been cleaned. We have been using the stations for more than 4 years and they have proven adequate and are much cheaper to build and maintain than the commercially produced laminar air stations. All air entering the rooms passes through a 12 in. hepa filter.

We find it advantageous to use several culture rooms rather than one large one. This way we can create different temperatures and lighting (intensity and photoperiods) for different kinds of plants; for example, we use a dark warm room for lily bulbs, a lighted and warm room for foliage plants, and a lighted and cool room for trees, shrubs, and perennials.

Sometimes, in order to avoid unnecessary handling, we store plant material for use at a later date. For example, we store lily bulbs, rhododendrons, and perennials by sealing the culture tubes with Celons (to eliminate contamination) and keep them for up to six months at 34°F in an unlit 38 cu ft refrigerator which was made for the food industry and which we bought used at a fraction of the cost of a new one.

Containers, Space and Handling. We still grow mostly in 25 mm culture tubes because we started with them and have them on hand. They are placed on slant trays we made ourselves. In the future we will stand the tubes upright as we can afford the changeover. This alone could double the capacity of the culture rooms, delaying the need for more rooms. We are considering the two types of Majenta trays for this. One holds 30 tubes and the other 36. The latter would require use of clear caps for the tubes, while with the 30-tube holder, we could still use our opaque caps.

We have also begun to use the Magenta GA-7 container for plant material which grows fairly large and we are considering baby food jars with the Magenta lid as a possible growing container which would also save space in the culture rooms.

We identify our containers and tubes with stamps giving date, cultivar, and cutter initials on round or oblong labels meant for office use. They are easily put on and removed.

Bill Brown of B & B Laboratories has designed and built a media-dispensing machine which fills 40 tubes at a time and can fill 480 tubes in 3 to 4 minutes. The concept and design has been turned over to Bellco, Inc. in New Jersey for development and marketing. It has been a valuable time saver for us because we use so many tubes. He will doubtless also design a similar machine for dispensing into baby food jars and the GA-7s.

We find that by reducing the agar amount slightly we are able to avoid emptying the tubes of the medium before running them through our household dishwasher, which holds 200 tubes in five racks and cycles in 30 minutes. There are commercial dishwashers which cycle in 2 minutes and do a little better job. They use more electricity but make up for that cost in efficiency.

Media. We use three basic salt formulations: Murashige-Skoog at various concentrations, Anderson's rhododendron, and Lepoivre; we keep stock solutions of these on hand at all times.

We use the inexpensive gum agar from Sigma Chemical, St. Louis, Missouri, and have had no problems with the plants. Different agars and gelling agents work differently and I feel

that it is important to use one exclusively in order to get consistent results.

As with all other aspects of our operation, we try to keep media preparation as simple as possible and often eliminate the many kinds of media addenda which appear in the published papers without apparent harm to plant performance. We do use i-inositol, thiamine HCl, and adenine sulfate-H₂O where appropriate.

Shipping. We ship nearly all of our products in vitro in a rooting medium using two types of containers, both of which are made for the food industry where mass production brings the unit price down. One type is a clear plastic food tray 4½×4½× 2 in. with a heat-sealed Mylar covering which makes it air-tight. These are sterilized by soaking in a 10% household bleach solution for 10 minutes. The second type is a slightly larger aluminum tray with a clear plastic lid which is crimped on. We sterilize the trays in the autoclave in a turkey roasting plastic bag along with the medium to be dispensed and the lids are soaked in the bleach solution. We sterilize the rooting media in the autoclave in quart jars and dispense into the trays in a special pouring hood built at the lab. We make 6 liters at a time which fills 60 trays.

Both trays are lightweight and disposable and therefore good for shipping. The plastic trays cost about \$0.06 each and the aluminum ones cost more than twice as much. The reason we use the more expensive one is that some plants need the breathability of its less-tight closure. We are experimenting with various ways to ventilate the plastic trays to alleviate any gas build-up problems.

We ship 72 to 75 trays per 22×14×12 in. box in which 1800 to 3750 plants, depending on the species. The box can be packed in 10 minutes or less. If we were to remove the plants from their trays and put them in plastic bags, we could ship tens of thousands per box, but that would require several hours of labor, increasing our cost, and the grower would have to handle the plants immediately upon their arrival.

In conclusion, we feel that in order to have an efficient plant propagation unit we need to look around, sometimes outside the industry, for ideas to use for the production, handling, and delivery of tissue-cultured plant material and not be afraid to try something different. We need to improvise, experiment, and keep asking these questions: Why are we doing it this way? Could it be done faster or easier? Is it cost effective?