

that we have 100 tubes for each week of 6 weeks. On 3 square feet of shelf holding the 100 tubes we obtain 6.3 divisions per tube. One division is used for replacement into fresh stage 2 medium. These numbers give us a production of 500 plus plantlets per week.

The Pretransplant Step and Establishment in Soil. The plantlets are rooted in stage 3 containers in 2 to 3 weeks and then moved into the outside world. The plantlets are placed in trays containing 72 cavities holding medium of $\frac{1}{3}$ sand, $\frac{1}{3}$ peat and $\frac{1}{3}$ loam. The cavity is 2 inches square and $2\frac{1}{4}$ inches deep. After placing in the moist soil the plantlet is watered well and covered for 5 to 6 days with a near clear plastic dome. After the dome is removed, they remain in the glasshouse under 10,000 lux (1,000 foot-candles) for 3 weeks. These plants develop a superior root system in this period of time and are then transferred to a 6 inch container for growing onto a finished product.

Six months after the plantlets come out of the lab (5 months from the liner stage), we have 18-inch finished plants.

SETTING UP A TISSUE CULTURE SYSTEM

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Plant tissue culture is the placing of excised plant cells, tissues or organs in an artificial environment for the purpose of controlling the development of the explant. Plant tissue culture is pertinent to those in commercial horticulture as a method of achieving rapid vegetative multiplication. Shoot tip or shoot apex culture is the usual method; however, other tissues such as bulb scales, leaf parts, petioles, and embryos are also often used.

Plant tissue culture is not a new science as Haberlandt was the first to place leaf tissues into a nutrient solution for observation in 1902. Successful embryo cultures were achieved in 1904 by Manning. In 1934 White succeeded in culturing tomato roots, which display unlimited growth. These cultures are still being maintained; 1934 was also important due to the discovery of the auxin, indoleacetic acid. Gautheret and Nobecourt in France, and White in the United States, all reported the indefinite culture of callus on an artificial medium. Van Overbeek in 1941 reported the control of differentiation into embryos or callus with coconut milk treatments, and in 1946 Ball obtained

complete plants of *Lupinus* and *Tropaeolum* through shoot tip cultures.

A fundamental principle in plant tissue culture was introduced in 1957 by Skoog and Miller when they found differentiation of plant organs in tobacco callus was controlled by the interactions between auxins and cytokinins.

In 1960 Morel described the first commercially applicable methods of tissue culture for rapid vegetative production of orchids. Many orchid tissue cultures are erroneously termed meristem cultures, or mericlones, but it wasn't until 1970 that Smith achieved the first true apical meristem cultures.

Basic principles and procedures were made available to commercial horticulturists through the research of Murashige on the rapid multiplication of ornamental plants. This has led to the development of commercial tissue culture laboratories for the purpose of propagating plants. These labs are able to take advantage of the potentially high multiplication rates. For example: given a single explant of plant tissue and conditions which yield a 5-fold multiplication rate at the end of the subculture period, with subculturing occurring every four weeks (if the first culture period yields no multiplication, which is common), at the end of a 40 week period two million plants can be obtained.

My first introduction to tissue culture was in the mid-1960s when the California Association of Nurserymen (CAN) Research Committee contributed \$1,000 to the University of California, Riverside, Plant Science Department to foster and encourage research in tissue culture. This research at UCR and subsequent events convinced me that while tissue culture was only commercially feasible for a few specific plants, it could be an invaluable form of propagation.

In 1967 the California Association of Nurserymen made its first horticultural tour to Hawaii. The most impressive part of the trip was the visit to the University of Hawaii Plant Science Department with its numerous growth chambers and extensive research in plant tissue culture. I couldn't believe what I was seeing. It is of no wonder the University of Hawaii was so advanced in this field as Dr. Tosh Murashige completed his doctorate program here and is recognized today as one of the foremost plant tissue culture experts of the world.

Orchids, bromeliads, and anthuriums, together with other plant genera, were being successfully propagated by this method. I was convinced that now I must seek and find a commercial tissue culture laboratory upon my return. I found one in South San Francisco less than a hundred miles from our nursery.

During a visit to Rod McClellan's Nursery in South San Francisco, a company known for its orchids and other greenhouse products, I witnessed orchids in commercial propagation for the first time. Though "primitive" by laboratory standards, nonetheless seemingly "millions" of orchid plantlets were in production. There seemed to be test tubes everywhere, on rotating racks and stationery racks, with plantlets in all different stages of maturity. Mayonnaise jars for the final stages appeared to have been collected in abundance. I was impressed that a clone could be reproduced in these numbers in such a concentrated area.

There are two areas of importance for successful tissue culture: (1) a knowledgeable person, and (2) the lab facilities. Fortunately, both of my two sons expressed interest in horticulture in general, and it took little persuasion to have my number 2 son, Loren, specialize in tissue culture. Now the timetable was critical, for it would be nearly six years before we had full time access to his newly acquired skill and knowledge. The timetable was near perfect.

The tissue culture laboratories existed only in Universities and at Rod McClellan's. A thorough evaluation was made and a list of equipment necessary was compiled. It was not long before we recognized that this was not an ordinary project. Even though we have nearly a million square feet of greenhouses, by comparison the capital required for this venture was extensive for so small a square footage.

Our initial laboratory was less than 700 square feet and by the time it was operational the capital expenditure exceeded \$70,000. Since we were thoroughly convinced that we would be in the tissue culture business, all new equipment was purchased. Microscopes, balances, laminar-flow work areas and autoclaves, not to mention many support facilities were required to make the laboratory operational.

In the meantime we searched for a technician that could use his knowledge to coordinate our facilities and personnel. Through our contacts at various universities we found a person that wanted to move to California, and we arranged a mutually beneficial arrangement. The tissue culture venture, after a snail-paced start, was finally launched.

In 1975 an expansion program was started and the tissue culture laboratory was expanded to more than 2,000 square feet of laboratory and work area and more than 5,000 square feet of culture rooms. We are very proud of what I believe are the best in commercial facilities.

Most people who show an interest in tissue culture are already aware of its potentials. Those in commercial horticulture

are interested in the high multiplication rates, which were mentioned. Other considerations must be made in planning facilities required for tissue culture, particularly the culture rooms and greenhouses to house the quantity of cultures being produced.

To use the previous example, to produce the two million cultures mentioned, a culture room of about 2500 square feet is required to house the culture tubes (a total shelf area of 24,500 square feet is required). If the cultures are transplanted into cell packs, a greenhouse of approximately 75,000 square feet is required. Also, the greenhouse must be equipped with special facilities to be able to maintain the high humidities required for the culture-to-soil transition. The laboratory facilities, such as transfer, sterilization, and washing equipment, must also be adequate to support the work required to produce the quantities of cultures discussed.

However, primary to all of the facilities required are personnel, which are the key to the success of the lab. A supervisor who is trained in the sciences related to horticulture, with background in tissue culture, is as required as the lab itself. Procedures for each plant type are to be determined since the requirements may differ among species and perhaps even among cultivars. Special skills and imagination are necessary for obtaining good results.

Plant tissue culture, not a new science, is most important to commercial horticulture for providing rapid vegetative multiplication and is also useful for the recovery of disease-free plants. Despite the great potentials, however, there are great requirements in the utilization of these methods.

In conclusion, tissue culture is a complex scientific method of micropropagation, requiring extensive capital, knowledge and facilities. Therefore, before you launch into this venture I recommend:

1. Define your company objectives. Are crops you anticipate culturing economically feasible? Is there an alternative? What is the planned magnitude of the operation?

2. Consider your available resources. Do you have substantial cash flow to expend the capital necessary to build this facility and furnish it with the necessary equipment and technical labor? Cash flow from your products cannot be expected for nearly two to four years.

3. Determine the availability of personnel. Are they fully qualified? Are they thoroughly knowledgeable in the science of tissue culture? What is your probability of survival should your key technician decide to leave?

4. Last but extremely important, be realistic about profitability. Are we looking for that "pie in the sky", or is tissue culture profitable? Is it only a status symbol? I ask, "Is tissue culture profitable?" and I can answer, "Yes, but when?"

QUESTIONS FOLLOWING TISSUE CULTURE FORUM

CHARLES PARKERSON: Question for Raymond Oglesby. Why did you decide to get into tissue culture? Was it to grow a particular type of plant you could not obtain any other way?

RAYMOND OGLESBY: I became very interested after taking the short course that is offered at Lake Placid. Information can be obtained from the W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, New York, 12946. There is a great demand for *Hemerocallis* cv Aztec Gold for landscaping in our area. We found that we could produce 15,000 daylilies on 20 square feet in 30 weeks. We sold the 15,000 'Aztec Gold' for \$1.50 each. This inspired us to do more. I would definitely recommend taking the short course to anyone interested in tissue culture.

PROPAGATION OF LILACS

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Cultivars: At Ozark Nurseries we propagate the following lilac cultivars: *Syringa vulgaris*, produced from seed on raised beds, *Syringa rothomagensis* (Chinese lilac), *Syringa vulgaris* 'Charles Jolly', *Syringa vulgaris* 'President Grevy', *Syringa vulgaris* 'Mme. A. Buckner', *Syringa vulgaris* 'Mme. Lemoine'.

The average combined number of cuttings stuck each year is 240,000. Of this number, 160,000 are French lilacs.

Cutting beds: Our cutting beds are 60 feet by 40 inches mini-Quonset structures. Retaining walls are constructed of 2 by 6 inch lumber. A 2 by 4 inch board is used to frame the beds. In our older beds a clay drain tile is run down the center of each, and in our newer beds French drains are used. A French drain is a ditch filled with gravel and is not too satisfactory. We use a medium of native soil (light clay loam) with peat moss added each year to increase the organic matter. The amount of peat added varies with each individual bed. Osmocote 18-6-12 is then added at the rate of 15 pounds per bed and cultivated into the soil to a depth of six inches.

Methyl bromide is used to sterilize the beds, applied at a