ppm, so we have to inject sulfuric acid. Once we do, the pH drops to about 6.2.

DICK AMMON: Question for Curtis Wilkins: Do you have any problem getting your phosphates through your mix?

CURTIS WILKINS: No.

BOB KNECHT: Question for Tom Dodd. How do you avoid leaching minor elements from the pot?

CURTIS WILKINS: With the injector system, everytime they are fed, which is daily during the summer, they are getting minor elements. Out in the fields we use the fritted trace element mix. In addition we may use foliar trace element mix.

COMMERCIAL TISSUE CULTURING AT OGLESBY NURSERY

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Ornamental plant tissue culture successes in California have stimulated Florida growers' interest. Because of refinements in laboratory technique and the availability of commercially produced and packaged tissue culture media, tissue culturing commercially has become practical and profitable. This report will describe steps in rapid propagation of certain ornamental plants, including *Ficus elastica* 'Decora Burgandy.' Methods, technique, conditions and duration are described in detail from the initial shoot tip explant to outdoor planting and growing in 6-inch containers.

The tissue culture lab at Oglesby Nursery is just into its fourth year. Our present facility occupies 2,000 square feet and has a capacity of over 60,000 culture tubes, or a production level of over four million plants per year. We employ 13 people. High school students are used to wash the glassware. The development of the lab has been a tremendous expense; its operation is also expensive. Its function is to utilize *in vitro* culture procedures to these ends: (1) mass produce several desired cultivars for Oglesby Nursery, (2) provide tissue culture service for other nurseries, (3) produce retail consumer products *in vitro* and, (4) establish plant stocks that can be certified for freedom from certain pathogens and pests.

The starting tissue (explant): A shoot tip is cut with a pen knife from the parent plant. The outermost tissue, including all leaves, is removed. After a 10 mm by 20 mm piece of tissue is

excised, it is washed in soapy water for several minutes to remove any dirt.

The shoot tip is then cut further to about 5 mm by 12 mm. At this point the explant would include the meristem plus many tiny leaf primordia and subadjacent stem tissue. Approximately 20 shoot tips are cut and put in a 50 ml beaker for disinfectation. We use a disinfectant solution of 10 percent Clorox (laundry bleach) and a drop or two of liquid Joy detergent per 50 ml of autoclaved water. The shoot tips are agitated in the solution for 10 minutes by a slowly revolving magnet and are then rinsed 3 times with autoclaved water.

The shoot tips are then cut further using aseptic techniques under a laminar-flow clean air hood. The laminar flow hood is essential for preventing contamination. This final cutting is done under a dissecting microscope on a sterile petri dish using sterile forceps and a scalpel. The finished explant is approximately 1 mm by 3 mm in size and is inserted into the sterile culture tube containing the growing medium. The most expensive part of the entire operation is obtaining sterile first explants.

Nutrient medium. The Murashige-Skoog salt mix with minimal organics is used for the basic medium. It can be purchased in packages sized to make either 1 or 5 liters of medium.[1] The composition of the Murashige-Skoog Salt Mix is given in Table 1. Various recommended amendments are added to this. Table 2 lists the other constituents of the medium for the first and second stages. During stage 1 the plant becomes established and starts growing in sterile culture; in stage 2 it begins to multiply rapidly. Table 3 lists those constituents used for the rooting of *Ficus elastica*. This period is referred to as stage 3.

Table 1. The Murashige-Skoog salt mix with minimal organics.

Compound	mg/l	Compound	mg/l
NH_4NO_3	1650.0	i-inositol	100.0
KNO_3	1900.0	Thiamine HCl	0.4
$CaCl_2 \cdot H_2O$	440.0	H_3BO_3	6.2
$MgSO_4 \cdot 7H_2O$	370.0	$MnSO_4 \cdot H_2O$	16.9
KH_2PO_4	170.0	$ZnSO_4 \cdot 7H_2$	8.6
$Na_2 \cdot EDTA$	37.3	KI	0.83
$FeSO_4 \cdot 7H_2O$	27.8	$Na_2MoO_4 \cdot 2H_2O$	0.25
minimal organics	mg/l	$CuSO_4 \cdot 5H_2O$	0.025
Sucrose	30,000.0	$CoCl_2 \cdot 6H_2O$	0.025

The medium is mixed in 1 to 5 liter portions. The pH is adjusted to 5.6 with drops of 1 molar solution of NaOH or HCl. The media for stages 1 and 2 are dispensed into tubes 25 mm

[1] The source is Gibco-Invenex, 250-3, 17800 Chillicothe Road, Chagrin Falls, Ohio 44022.

Table 2. Nutrient addenda used for stages 1 and 2.

Addendum	mg/l	Addendum	mg/l
NaH_2PO_4	170.0	IAA (indole-3-acetic acid)	0.1
Adenine sulfate·H_2O	80.0	2iP	30.0

by 150 mm, 10 ml per tube, by means of a funnel, rubber tube, clamp and a plastic tube (40 cm) bored with 4 holes. This arrangement makes it possible to fill 4 culture tubes with the same volume at the same time. The tubes are capped with Bellco Kaputs caps and moved about in stainless steel racks of 36.

Table 3. Nutrient addenda used for rooting or stage 3.

Addendum	mg/l	Addendum	mg/l
NaH_2PO_4	170.0	IAA (indole-3-acetic acid)	1.0

The stage 3 medium is prepared in 5 liter batches in a large stainless steel stock pot. It is then transferred to two 4,000 ml Kimax bottles, which have an outlet at the bottom (#14605). Aluminum foil is placed over the opening at the top and 1 foot of amber tubing is attached to the bottom. A pinch clamp is placed at the dispensing end of the tube. Aluminum trays (EKCO Products, Inc. #705-30) are placed in a Mylar bag and autoclaved. Both the medium and trays are autoclaved for 15 minutes at 250° F, 15 lbs. pressure.

Clear polystyrene lids (EKCO Products, Inc. #9105-19) for the trays are soaked for 30 minutes in a bucket containing a 10 percent Clorox solution. They are then rinsed with hot sterile water. This procedure is done under a laminar flow hood. The medium is then dispensed into the sterile trays under a laminar flow hood and the sterile lid put on. The third stage container, now complete, is placed on an enclosed cart to cool.

The culture room. During stages 1 and 2 a constant temperature of 28°C is provided in a culture room illuminated 16 hours daily with 1,000 lux (circa 100 foot-candles) light from cool white fluorescent lamps. Stage 3, the pretransplant step, is illuminated with 10,000 lux (1,000 footcandles) cool white power groove fluorescent lamps.

The multiplication process: After 4 to 6 weeks the explants have increased in size to about 10 to 20 mm and at this point are put into fresh medium. Within 6 weeks divisions should be evident. Again a transfer is made into fresh medium. Once they are apparent, divisions are separated and put into fresh medium every 6 weeks until we have reached the number of stage 2 culture tubes we need to maintain a given output from the lab.

In our program we started with 6 groups of 100 tubes so

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 1/3 sand, 1/3 peat and 1/3 loam. The cavity is 2 inches square and 2¼ 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