LITERATURE CITED

1. Brutsch, M. O., P. Allan and B. N. Wolstenholme. 1976. The anatomy of adventitious root formation of adult-phase pecan (*Carya illinoensis* (Wang.) K. Koch) stem cuttings. *Hort. Res.* 17:23-31.
2. Knox, C. A. 1980. Histological and physiological aspects of growth responses and differentiation of pecan, *Carya illinoensis* (Wang.) Koch. tissues *in vitro*. Ph.D. Dissertation, Texas A & M University, College Station.
3. McEachern, G. R. and J. B. Storey. 1972. Pecan clonal rootstock propagation techniques. *Pecan Quart.* 6(3):5-7.
4. McEachern, G. R. 1973. The influence of propagating technique, the rest period phenomenon, and juvenility on the propagation of pecans, *Carya illinoensis*, stem cuttings. Ph.D. Dissertation, Texas A & M University, College Station.
5. Smith, M. W. 1977. Shoot meristem and callus tissue culture of pecans, *Carya illinoensis* (Wang.) K. Koch. Ph.D. Dissertation, Texas A & M University, College Station.
6. Wolstenholme, B. N., and P. Allan. 1975. Progress and problems in pecan clonal propagation by stem cuttings. *Gewasproduksie/Crop Production* IV:29-32.

ESTABLISHING TISSUE-CULTURED PLANTS IN SOIL

JOHN L GRIFFIS, JR., GARY HENNEN, AND RAYMOND P. OGLESBY

Oglesby Plant Laboratories, Inc.

Hollywood, Florida 33023

It has been almost 9 years since Oglesby Nursery, Inc. ventured into the plant tissue culture business. In that time, our facility has grown from a small laboratory with one technician and 120 ft^2 of culture space to a modern production laboratory with over 3500 ft^2 of culture space and about 40 employees, plus a separate research and development facility with 250 ft^2 of culture space and 2 employees. The demand for tissue-cultured plants is such that our laboratory is in continuous operation, 24 hours a day, Monday through Friday. An additional shift also operates on Saturday. We have, over the years, successfully propagated through tissue culture more than 400 kinds of plants including bananas, pineapples, plantains, gerbera daisies, spathiphyllums, daylilies, caladiums, and many other ornamental species (2). Included among current research projects are tissue-culture propagation of avocados, nandinas, heliconias, araucarias, various spices, and numerous other plants.

Because of our considerable expertise in tissue culture propagation of plants, we are often asked many questions concerning all stages of the process. One of the most common

questions asked is "How do you successfully establish tissue-cultured plants in soil?"

Since our liner division, which is one of our laboratory's largest customers, successfully establishes thousands of tissue-cultured plants per month in soil, we would like to share with you the factors we consider and the procedures we follow at Oglesby Nursery, Inc. to accomplish this task.

For those of you not completely familiar with tissue culture propagation systems, there are 4 steps or stages involved in the process. Stage I involves selection of plant materials to be cultured, disinfestation of the plant tissues, and establishment in the tissue culture medium. Stage II basically involves the multiplication of the plants. Stage III involves the rooting of the plantlets from Stage II. Stage IV involves the return of the rooted plantlets from Stage III to the soil and the natural environment. All plant tissue culture systems require Stages I, II, and IV. Some plants require Stage III while others may be returned to the environment, i.e. Stage IV, directly from Stage II and are treated as unrooted microcuttings (1). Many factors are involved in successfully returning tissue-cultured plants to the environment.

The single most important factor affecting plantlet establishment is plant quality. Whether the plantlets are Stage II microcuttings or Stage III rooted plantlets, plantlet health is very important. Some factors which influence plant health include light availability, media components, and bacterial or fungal contaminations. We know that low light intensity will produce weak, spindly plantlets, but high light intensity may produce burned, chlorotic foliage. The ideal amount of light varies with the plant species involved; however, at Oglesby we generally use 200 to 300 f.c. for Stage II cultures and 350 to 600 f.c. for Stage III culture areas. Although all media components may have some affect on the tissue-cultured plantlets, we have found that selection of cytokinin used in Stage II greatly affects the survivability of plantlets in Stage IV. The use of benzyladenine in Stage II reduces the number of plantlets of liriope, schefflera, or philodendron that survive transplanting to as low as 10%. The use of kinetin or 2iP instead of BA gives us a survival rate in excess of 90%. The reason for this is not yet clear, although it appears that BA has some adverse effects on stomatal regulation. Other plants, however, are not adversely affected by BA. Additionally, plantlets which become contaminated during Stage II or III may also show some loss of transplant survivability. These three factors are all dealt with in the laboratory and are out of the purchaser's control. However, there are many factors which you, as a

purchaser of Stage II microcuttings, or Stage III rooted plantlets, do control.

Factors in Stage IV which may directly affect transplant survivability of tissue-cultured plantlets include soil mix selection, pot selection, humidity control, watering techniques, light availability, and pesticide application.

Although there are several important factors involved in soil mix selection, the main requirement is that the mix must be pasteurized or sanitized. Drainage is also an important factor, as is the addition of fertilizer to the mix. Other components that may be added to the soil mix include perlite, vermiculite, polystyrene, bark, sand, and peat. The mix must provide adequate aeration yet also hold enough moisture to stimulate root development. As an example, we use Metromix 300, supplemented with Osmocote 13:13:13, Micromax, and additional perlite for drainage and aeration for the transplanting of gerbera daisies and spathiphyllum out of Stage III.

Selection of the correct pot or tray size may also affect survivability of tissue-cultured plantlets (not to mention your labor and costs). Again, the main requirement here is cleanliness. The pots or trays should either be new or sanitized if previously used. The size of the cells or pots varies with the space requirements and the ease of handling required. There are a large variety of trays and systems available such as cell packs, peat pellets or cubes, Todd Planters, single pots, and larger cell trays. At Oglesby spathiphyllum and gerbera daisies are planted in Grow System 73 cell trays with 1¼-in. cells. These plants can grow in such cells for 2 to 3 months before they are shipped as liners or potted up into larger containers. Bananas, on the other hand, are sometimes planted directly into 4-in. cells where they attain a height of about 1 ft. in 2 to 3 months and are then directly planted into the field. We are also currently investigating the use of newer systems that may make better use of available space, such as the Castle & Cook trays, which hold 400 plantlets in an area only slightly larger than 1 ft^2.

Another very important factor in transplant survivability of Stage II microcuttings or Stage III rooted plantlets is humidity and moisture control. Since the plantlets are coming from an environment that provided them with 100% humidity, they need to be given a similar environment and gradually hardened-off. We generally transfer the plantlets into trays, water them in, and place them in one of several structures that help us maintain a high-humidity environment. These various structures all have advantages and disadvantages, as listed below:

STRUCTURE	ADVANTAGE	DISADVANTAGE
1. Humidity tent (clear or shaded plastic enclosure with or without mist system).	Relatively inexpensive; Relatively easy to construct; Does excellent job of maintaining high humidity.	Heat build up; may be difficult to control temperature; Must be monitored often for misting or watering; "Permanent" structure required.
2. Automatic mist system (with or without plastic coverings).	Automatically mists plantlets and increases humidity, therefore requires very little labor to monitor; Variable controls allow variation in amount of mist and timing of mist (e.g. 5 seconds every 10 min. to 3 min. every 4 hours).	Leaches nutrients from soil and plantlets; Soil may be too wet; System may result in serious fungal or algal buildup — should be cleaned often; "Permanent" structure required.
3. Plastic covers (covers single tray)	Allows greater flexibility with different crops since each tray is maintained separately; Does excellent job of maintaining high humidity; Does not require any structural changes to existing facilities; Quick and easy to use, portable system.	Heat build up can be very rapid since air space provided is small; Requires a great deal of labor to examine each individual tray several times a day.
4. Fog systems (high pressure systems).	Maintains 100% humidity automatically; Does not leach soil or plant nutrients; Lowers heat buildup significantly; Provides pleasant conditions for labor force to work in; May also help lower light intensities.	Very expensive; "Permanent" structure required; High maintenance requirement if water is not very pure.

With any system used, the plantlets will require the highest possible humidity levels in the beginning and a gradual reduction in humidity over 1 to 4 weeks as the plantlets become established. Some of these systems lend themselves better than others to this gradual hardening-off process.

Other important factors involved in survivability of tissue-cultured plantlets include light intensity and pesticide application. Plantlets must be protected from high light intensity

since they are coming from a low-light environment. The available light should be gradually increased as the plantlets become established. Our greenhouse fog system has 90% shade. After about 2 to 4 weeks in this structure plantlets are moved to other greenhouses with 73% to 80% shade. Some plants, such as spathiphyllum, will remain at 73% shade until they are sold. Other plants, such as gerbera daisies, will be transferred to structures with 30% shade after several weeks at 73% shade. We discourage application of any pesticide during the first 2 weeks after transplanting. Strict adherence to correct sanitation procedures is much more desirable. However, plantlets should be monitored closely after transplanting from the sterile tissue-culture environment and pesticides may be used cautiously if any problems do arise. Be sure a pest is involved before you use chemicals.

As you can see, numerous factors are involved in establishing tissue-cultured plantlets in soil. By paying close attention to them, one should have few problems in establishing tissue-cultured plantlets and in producing an outstanding crop.

LITERATURE CITED

1. Jones, Jeanne B. 1982. How can we get microcuttings out of the lab? *Proc. Inter. Plant Prop. Soc.* 32:322-327.
2. Oglesby, Raymond P. 1978. Tissue culture of ornamentals and flowers: problems and perspectives. In, Propagation of Higher Plants Through Tissue Culture, 1978. Hughes, K. W., *et al*, eds., Technical Information Center, United States Department of Energy, Oak Ridge, Tenn., 59-61.

ASEXUAL *MAGNOLIA GRANDIFLORA* PROPAGATION AT SHADY GROVE NURSERY

WILLIAM M. BRAILSFORD

Shady Grove Plantation and Nursery, Inc.
3030 Charleston Road, SW
Orangeburg, South Carolina 29115

Our nursery was established in 1939 by John F. Brailsford, Sr., with retail sales, container yard, and a garden shop in town. Shady Grove Nursery now has 350 acres under cultivation with 150 acres of new ground; 60 acres of this will come into production this year, 25 to 30 acres a year later. Hopefully, we will then be able to rotate fields in the old nursery and top out at 500 acres. We are wholesale growers, now serving landscape contractors, architects, and other nurseries.

Around 20 years ago we realized people deserved better than seedling-grown magnolias. We were also interested in