Getting Stage III to Nurseries . . . Proper Handling by Everyone©

Deborah D. McCown
Knight Hollow Nursery, Inc., 3333 Atom Road Middleton, Wisconsin 53562 U.S.A.

INTRODUCTION

What is Stage III? The "stage" terminology used in micropropagation is something I've never really been comfortable with and avoid using. But since Dick Bir assigned the topic to me, I'll try my best to muddle through. Checking with a Plant Propagation textbook (Hartmann et al., 1997) gives this scenario:

Stage I. Establishment. Basically this is putting the plant into culture. It includes sterilizing the tissue and getting it to grow at a predictable rate. This is the tricky phase we call "stabilization". But this is not really relevant to the assigned topic.

Stage II. Multiplication. The text says, "After approximately 1 month or when the culture has grown sufficiently, cut into segments approximately 1 cm long...and transfer to fresh medium". I don't know what crops they're talking about but this is not the way it works with woody plants. It can take months or years to get some woody plants into the multiplication phase because they take so long in the stabilization phase. Commercial multiplication requires shoot-tip cultures grow at a defined rate. Again this is not directly relevant to our topic.

Stage III. Rooting. The text says, "Transfer microcuttings to a medium without kinetin or NAA. Roots form easily in vitro. Ex vitro rooting is an alternative."

Stage IV. Acclimatization. Again the text says, "Remove rooted microshoots from container. Wash away solid media if present. Transplant into growing medium and hold under mist or under high-humidity tent. New functional roots need to form to replace the older roots....Once established, microcuttings are gradually exposed to lower humidity and higher light intensity."

Every micropropagation laboratory does it differently and it is important for each customer to know and be prepared for the microcuttings they are going to receive. Alan Jones follows me on this program and is going to talk about success buying plants from a competitor of KHN who does it very differently than we do it.

WHAT DOES KNIGHT HOLLOW NURSERY (KHN) SUPPLY TO OUR CUSTOMERS?

We supply three different levels of micropropagated plants depending on the facilities and skills of the receiving nursery.

- Unrooted cultured cuttings.
- Rooted cultured cuttings.
- Cultured cutting liners.

Unrooted Cultured Cuttings. We do ship microshoots directly from Stage II. These unrooted microcuttings are growing on the agar multiplication medium. Generally, these are blueberry and lilac microcuttings, which are relatively easy to

root. They are harvested directly from the culture jars, rinsed in tap water and packed in Zip-Lock bags with damp paper towels. If the weather is inclement (very hot or cold), we put them in Styrofoam coolers but in most cases they go in a standard cardboard box. These plants are always shipped UPS overnight. They leave our lab at 3:30 P.M. in the afternoon and are delivered anywhere in the USA by 10:30 A.M. the next morning. Our customers do exactly what we would do — cuttings are stuck in a propagation medium and provided with some type of high humidity. This could be mist or some sort of tent system. For most crops, rooting generally takes 6 to 8 weeks and acclimation another week to 10 days.

Rooted Cultured Cuttings. The majority of our business is Stage III rooted and acclimated microcuttings. We do not root in culture for several reasons. Firstly, our lab space is too valuable to dedicate to rooting. All of our lighted culture shelves are devoted to multiplication. Secondly, Mary McClelland (1988) presented a paper here at I.P.P.S. looking at in vitro versus ex vitro root development and found that in vitro root systems were essentially replaced when cuttings were planted in a traditional rooting mix. My staff takes the cultures and sticks them in peat moss for Ericaceous crops or ProMix BX for *Syringa*, *Amelanchier*, *Betula*, and most other woody crops. We use 1020 flats with clear plastic domes and root under artificial light. Again, it takes 6 to 8 weeks to root these microcuttings and another week to 10 days to acclimate them to ambient humidity.

Since these microcuttings are essentially "bed-rooted", we lift them and shake off the excess rooting medium. We pack them in strips of plastic with a damp paper towel, 50 microcuttings at a time, and jellyroll the cuttings in the paper towel/plastic strip. We have specially made boxes that are 14 inches × 14 inches × 14 inches and hold 1600 to 2000 microcuttings per box. The jelly-rolled plants are packed in two layers with separations to help protect the microcuttings. Void spaces are filled with Styrofoam "peanuts". All rooted microcuttings are shipped next day air, which is highly cost effective at 10% or less of the value of the plants. The receiving customers immediately transplant into a variety of containers, usually some sort of cell tray. Some customers put under mist for a week or so to help overcome the transplant shock. All of our microcuttings need to be protected from high light since we do root them under artificial light.

Cultured Cutting Liners. We do have a small number of customers who cannot handle our Stage III so we grow a limited number of liners. We are doing what our customers do. The rooted flats of microcuttings are taken to our greenhouse and generally given 2 to 3 weeks to acclimate to the higher light and harsher conditions. Ericaceous crops are transplanted into an acid mix in Nu-Pots while our other woody crops are transplanted into 2¼-inch × 5-inch deep Anderson Treepots using Fafard 3B mix. As a nursery buying Stage III plants, you need to be clear just exactly what your supplying laboratory is sending you — rooted but unacclimated or rooted and acclimated. I always recommend nurseries buy a small number (couple hundred rather than thousands) initially so they can get a feel of how to handle the microcuttings.

If you're having trouble, talk with the lab. They do want your business and most will be more than happy to walk through the "how-to-succeed" protocol. Also, it is important to remember that some crops are just innately more difficult than others and may require significant TLC.

LITERATURE CITED

Hartmann, H.Y., D.E. Kester, F.T. Davies, Jr., and **R.L. Geneve.** 1997. Plant Propagation: Principles and practices. 6th ed., Prentice Hall, Englewood Cliffs, New Jersey.

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Success with Stage 3 Tissue Culture Plantlets©

Alan Jones

Manor View Farm, Inc., 15601 Manor Road Monkton, Maryland 21111 U.S.A.

INTRODUCTION

To have success with something usually means you have been unsuccessful at some point. Often what you have been unsuccessful with is more interesting than what you are now doing successfully.

What I intend to present in this paper is an outline of how Manor View Farm has moved from being unsuccessful with establishing Stage 3 to being mostly successful with Stage 3. However, we are still having problems with *Corylus*.

The use of tissue culture or should it be called micropropagation has proved to have many benefits for propagation of certain plants in our industry. It is used not only for hard to propagate species, but is being used more often for easier-to-produce subjects. The key issue with many species is the cost effectiveness of using this type of propagation when compared to more traditional methods.

We have had success with establishing a range of material using basically the same technique. *Hydrangea quercifolia* and *Betula* are two groups we have had great success with, *C. avellana* 'Contorta' is a different story. I'm going to describe the technique we use for *H. quercifolia* cultivars. We started to use rooted microcuttings produced in a lab as a way to increase numbers more quickly. *Hydrangea quercifolia* roots easily enough from traditional cuttings, but building up numbers of newer cultivars like *H. quercifolia* 'Pee Wee' takes time using the traditional method.

Micropropagated liners are often embarrassingly vigorous and we like the high level of bud breaks at the crown of the plant. We also like the shorter internodes in shrubs that contribute to a bushier and more compact liner.

However, it should be realized that plants propagated via tissue culture/ micropropagation differ from conventional nursery practices in several key areas; an understanding of these differences is necessary to maximize survival rates. The rooted plantlets are usually much smaller and have been held in more precise controlled environments than cuttings. The rooted plantlets are cultured under sterile conditions and the material is usually grown in high humidity conditions.

Since we are dealing with Stage 3 you may wonder what happens in Stage I and II.

Stage 1: The selection of plant material to be cultured — culture initiation.

Stage 2: The multiplication of plants

Stage 3: The rooting of plantlets

Stage 4: Transplanting of rooted plantlets into media.