LITERATURE CITED

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Success with Stage 3 Tissue Culture Plantlets©

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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.

The single most important factor affecting plantlet establishment is plant quality. Plants received from the laboratory must be in good condition, free from infection, clean, have a good root system, and have been kept cool during shipping. Other factors that influence plant health and survival include light, humidity, and media.

As you can see numerous factors are involved in establishing micropropagated rooted plantlets in media. Even by paying close attention to detail problems can still occur in establishing them.

Due to the very precise conditions under which plantlets are cultured in the lab, carefully controlled hardening off procedures are necessary for survival when transplanting.

HOW WE DO IT

We have been purchasing Stage 3 rooted plantlets for a number of years from labs in Oregon and New Jersey. Most of the TC material we work with now is *H. quercifolia*, oakleaf hydrangea.

The method we have perfected is really very simple and that's the best way to keep it. It's the old KISS principle, "keep it simple stupid".

We try to schedule deliveries in early to late spring and late summer and early fall. We prefer to work with material during periods when temperatures are not extreme.

Material arrives from the lab usually in small plastic containers The material is usually well rooted and needs to be handled as soon as possible as it has already been in transit for 1 or 2 days.

The rooted Stage 3 rooted plantlets are small and can be difficult to handle. The plantlets are carefully removed form the container and transplanted into either a 72 or 98 cell plug tray. We use this type of tray as it saves a lot of space. It is important to grade the material at this time, smaller rooted plantlets being separated from larger material and planted in separate trays. The plug trays are filled with 510 Metro mix, which provides a well-aerated and well-drained media.

The plug trays are placed in a tent erected within a propagation mist house. The mist lines are within the tent and help provide the required humidity. The key to establishing these plants is high humidy without applying too much water. Excessive amounts of water can drown a plant very easily.

The rooted plantlets are watered in and sprayed with fungicide as *Botrytis* can be a problem.

It can't be stressed enough that one of the most important factors in transplant survivability of Stage 3 is humidity and moisture control. Rooted plantlets are coming from an environment that provided them with 100% humidity, so they need a similar environment.

The greenhouse is ventilated and shaded and depending on the time of year, the humidity tent may need shading as well. Temperature control is important in the greenhouse and tent as very high temperatures can occur if additional shading is not added.

The rooted plantlets stay in the humidity tent for 7 to10 days by which time they have started to produce new root and shoot growth.

Acclimation of rooted plantlets is accomplished by reducing humidity by raising the sides of the tent and increasing light intensity. After another 7 to 10 days, the plants can be removed from the tent and placed in a more normal greenhouse environment. The advantage of using a tent within the propagation house is the tent

can be removed rather than moving the flats. At this time we start to fertilize the crop with liquid fertilizer.

If we receive Stage 3 in the spring or summer, the established plugs will be potted into a 3-inch pot as soon as they have adequate roots to be moved. If we are establishing material in the late summer or fall, the plugs will be kept in a frost-free environment for the winter, then shifted in the spring.

One of our latest challenges is *C. avellana* 'Contorta'. A very difficult plant to root using traditional methods, and a miserable plant to grow if it is grafted, due to the suckering problem.

In the lab this is a difficult and slow plant to multiply. In the acclimation stage this plant has proved to be very difficult, with heavy losses. What we don't understand is if we can get 60% to live and grow why won't the other 40% survive. They are all grown under identical conditions, why so many losses. We are experimenting with taking material from the lab at different times of the year to see if that will improve the survival rate.

Stage 3 can present a challenge to the grower, but with an understanding of the needs of the plant and the environment from which it has come, the fear associated with using this type of material should be diminished.