DOUG SABIN: It is a selected seedling of Myrobalan plum — *Prunus cerasifera* — which has been maintained as a clone and used as a clonal, vegetatively propagated, rootstock. It was probably originally selected for its vigor and ease of propagation by hardwood cuttings.

VOICE: What is a good control for mildew on sugar maple seedlings?

DON POND: We use Captan-Benlate sprays.

MICROPROPAGATION OF DECIDUOUS TREES

GAYLE R. L. SUTTLE

Microplant Nurseries, Inc.
13357 Portland Road, N.E.
Gervais, Oregon 97026

Microplant Nurseries was established in 1980, with our first crop going to the field in the spring of 1981. We have specialized in the micropropagation of new and improved fruit tree rootstocks of apple, pear, plum, and cherry, as well as self-rooted ornamental and shade trees such as flowering plum, flowering crabapple, birch, and Norway maples. We presently have 26 cultivars in production. These are produced in quantities of 5,000 or more per year. An additional 42 subjects are in various stages of research. Such items as red maple, sugar maple, ornamental pear, filbert, apple, and cherry cultivars are all part of our research program.

Our facility consists of a 1600 sq. ft. building divided into 4 separate areas: an outer office and storage area; a media preparation room complete with an autoclave, water purification system, pH meter, weighing machines and dishwasher; a transfer room with three laminar flow hoods, where all sterile sub-culturing takes place; and a culture room with 1,024 sq. ft. of shelf area. The culture room is maintained at 25°C with a 16-hour photoperiod.

There are three *in vitro* stages of growth in the micropropagation process: culture initiation, multiplication, and rooting. Each stage requires a different medium formulation. We have found that there are differences in nutrient requirements almost on a cultivar by cultivar basis, so that we now start with Murashige and Skoog's (MS) basic formula (1) and make systematic changes as needed. While much work has been done in the past on changing the type and concentration of plant growth regulators, we have found that the inorganic salts also play an extremely important role in promoting or inhibiting

optimal culture growth. For example, we have found that *Acer platanoides* 'Crimson Sentry' requires roughly 3 times as much phosphate as MS recommends, while needing only ½ the sulfate level during Stage II. The result of these changes has been a 3-fold increase in multiplication rate and better rooting as well.

THE PROCESS

Stage I: Culture Initiation. Cuttings are sterilized to rid them of bacteria and fungi and then planted on a sterile nutriet medium. Then the plant must grow or "activate" in culture. While we have successfully started cultures during all seasons of the year by taking dormant buds in the fall and winter and tiny softwood cuttings in spring and summer, we have had the best results with softwood cuttings taken during early spring. The young shoot tips are cut 2 to 5 cm long and, after the larger leaves are removed, the tips are placed in a solution of 10% household bleach, plus a pinch of surfactant, for 10 min. The tips are then rinsed once in sterile water and placed on ½-strength MS medium, with no growth regulators, and allowed to incubate for 10 days. If the shoot tips are clean and active after this period of time, the material is transferred to fresh Stage II medium.

Proper stock plant care greatly improves our success during Stage I. We select the healthiest, youngest, cleanest stock available and maintain it in the greenhouse using an intense pesticide and fertilization program along with bottom watering. We also prune the stock trees frequently.

Stage II. Multiplication. By adjusting plant growth regulators and nutrients in the medium we can promote axillary branching. The branches are harvested every 10 to 21 days and planted into a fresh Stage II medium which, in turn, allows these new shoots to branch. This step is repeated every 2 to 3 weeks until a sufficient quantity of shoots are generated to satisfy our needs. Timing is critical during Stage II as it is important to catch the cultures when they are at the peak of the growth cycle. A delay of 5 to 10 days results in reduced yields and a lower multiplication rate during the next cycle.

Stage III: Rooting. There are two ways of rooting Stage II plantlets: 1) ripe shoots 1 to 3 cm tall may be rooted *in vitro* in media with reduced salts and no cytokinin, or 2) the microcuttings may be rooted directly into a potting mix under greenhouse conditions. The finished product at Microplant is either the rooted plantlet, or the microcutting.

Often we find ourselves working with plant materials which are difficult to root by conventional methods. If our

standard formulation of ½ strength MS medium, with 0.2 ppm indole-3-butyric acid (IBA) does not promote roots, we look at a number of factors such as auxin type and concentration, timing, brand of agar, the salt level and composition during Stages II and III, carbon to nitrogen ratio, etiolation, or additives such as charcoal, ancymidol, vitamins, and phenolic compounds.

GREENHOUSE ACCLIMATIZATION

The rooted plantlets or microcuttings must be placed under a high humidity environment in the greenhouse for several days and then the humidity is gradually lowered as the plants become adjusted to the greenhouse atmosphere. It takes 6 to 12 weeks of greenhouse growing for the plants to reach the 15 cm height desired for field planting. Most of our material is field-planted in the same season it is brought out of culture. Material that is brought out in late summer is either fall-planted or held over until the following spring.

COLD STORAGE

While it is possible to produce micropropagated material on a year around basis, the high cost of heating and lighting greenhouses during the winter months of October through February has led us to the use of cold storage during these months. The rooted plantlets, still in sterile containers, are placed under refrigeration at 2°C and total darkness. We are able to store material in this manner for up to 6 months without significant losses. We also use cold storage to maintain stock cultures of items that are produced on a seasonal basis. We can maintain material indefinitely this way if subcultured at least once a year. This greatly aids in smoothing out our production peaks, allows much more efficient use of personnel, equipment and, most important, allows the grower much more flexibility as to when to plant in the greenhouse.

Another important lesson we have learned is that many deciduous trees have a chilling requirement which must be satisfied in culture in order to achieve optimum growth. We have seen a remarkable increase in greenhouse growth when *Pyrus communis* 'Old Home × Farmingdale #333' Stage III plantlets are given a chilling treatment prior to greenhouse planting. We have also seen this in several crabapple and apple selections, but not in any of the *Prunus* species. We are now pre-chilling all material 1000 hours as a standard practice prior to greenhouse planting.

THE FUTURE

Every propagator has at least one plant — be it a shrub, a perennial, or a tree — that they wish they had more of. Perhaps it is a new cultivar, or a plant that does not root well, or maybe it would be advantageous to get the plant on its own roots to avoid graft incompatibility or low bud stands. These are the types of situations where micropropagation can and will be a big benefit to the industry. In the short time that micropropagation has been applied to deciduous trees, much progress has been made on developing formulations for new plants and streamlining the process to make it economically competitive. It is a tool which all propagators should consider as part of their arsenal when considering their propagation options.

LITERATURE CITED

1. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.

VOICE: Gayle, what is the sterilizing solution you use in preparing your birch explants?

GAYLE SUTTLE: We use 10% Clorox household bleach plus a surfactant, such as Tween 20, for 5 to 20 minutes.

RANDY BURR: On your direct stick micro-cutting birch are you coming right from the multiplication medium or do you use a rooting medium also?

GAYLE SUTTLE: We come straight from the multiplication medium for the cutleaf birch.

RANDY BURR: Have you found any seasonal differences when you bring the materials out?

GAYLE SUTTLE: Yes, the best time to bring the micro-cuttings out in the Oregon climate is in March-April so as to fit in with the growing season, so we use cold storage quite a bit.

VOICE: Dr. Anderson, what components are is your rooting medium?

WILBUR ANDERSON: We use ½ strength inorganics, drop out the cytokinins, and use 0.5 mg/liter indoleacetic acid.

LES CLAY: This for Gayle Suttle. When there is a callus at the base of your sub-cultures do you or do you not throw them away?

GAYLE SUTTLE: There is no set time that we sub-culture. If there is a lot of callus build-up we want to get rid of it for two reasons: for genetic stability — and lots of callus means that the clump is over the hill anyway.