Focusing on "Trees and the Golf Course"

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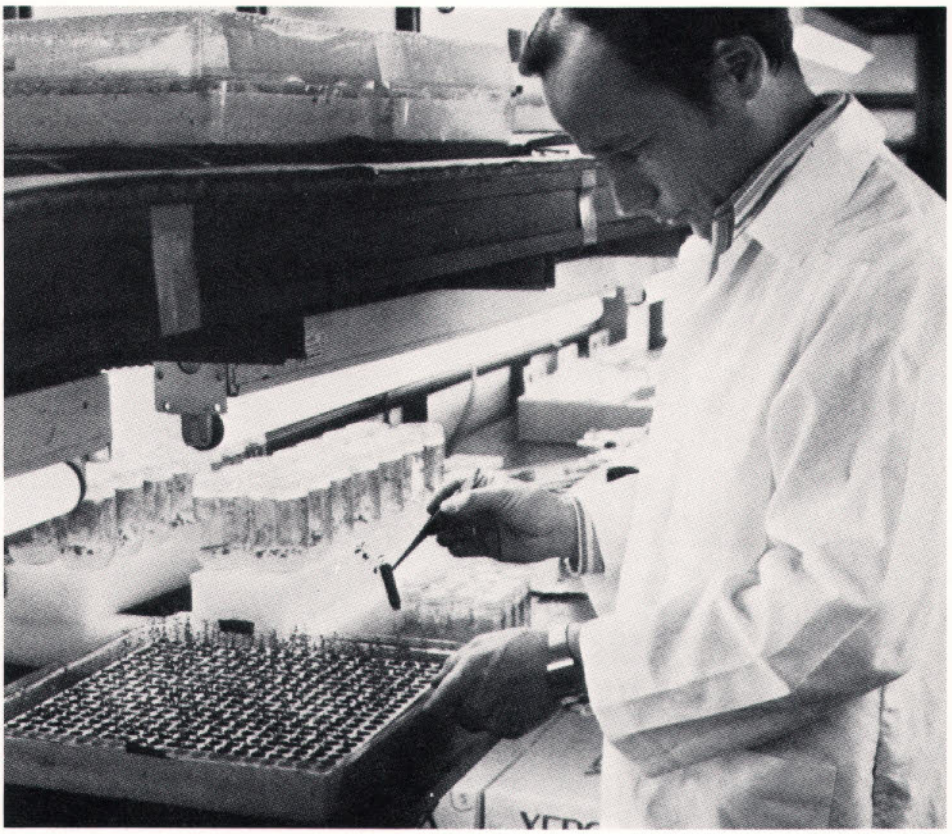
Trees from Test Tubes

Micropropagation boosts potential for breeding better trees faster

By Bill Keenan



The biggest obstacle in tree improvement programs is time. Producing tree seed takes years, often with unpredictable results. Recent advances in micropropagation—clonal propagation of plants from tissue cultured in a sterile test-tube environment—may cut both the time and uncertainty in tree improvement programs.

According to Brent McCown, a University of Wisconsin-Madison horticulturist, micropropagation has been used for years to commercially propagate houseplants. Only recently, however, has interest been aroused in using the technique on woody plants—trees and shrubs—to speed up the production of quality stock.

McCown says the number of woody species successfully propagated in culture so far is small compared with herbaceous plants. He attributes this lag to scientists' lack of knowledge of woody plants' growth regulation mechanisms—mechanisms much more complex than those of herbaceous plants.

Despite difficulties, McCown has successfully grown birch, blueberry, azalea, redwood, and other woody plant species in culture. He explains that the success of micropropagation largely depends on which tissues are selected from a particular tree or shrub. Generally, juvenile plant tissue will respond more readily to culture conditions than older tissue.

The micropropagation process begins with the removal of a small twig from a healthy plant. The twig is sterilized in a bleach solution and placed on a sterilized medium, usually a mixture of agar, inorganic salts, growth hormones, and sucrose. As lateral buds on the twig develop and elongate, the culture dishes begin to resemble small terrariums with shoots sprouting up in "bushes." The shoots are clipped off and they, in turn, are placed on fresh media where they begin to develop. Again, the lateral shoots are removed from this new growth and the process continues in the phase researchers call the "acclimatization stage." After numerous passes through the cycle, says McCown, "you have reproducible growth you can count on." These shoot cultures become the stock plants for all future operations.

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The cultures are kept in temperature-controlled growing rooms under low-intensity fluorescent lights. Eventually, technicians insert the shoots into a plug made from pasteurized peat and glue. In a matter of days or weeks, the new plants develop root systems. The end result: tiny trees in easily handled planting plugs.

McCown estimates that with this technique, 30,000 to 100,000 propagules—depending on the species—can be produced routinely every year using only one square meter of growing space. He adds that herbaceous plants are the quickest and easiest to culture; potatoes take only two weeks to go from cultured shoot to field. Deciduous woody plants such as birches and conifers are trickier to culture and to "harden off," or acclimatize to outdoor conditions, extending the time they must spend in the propagation room.

Horticulturist Brent McCown examines the developing shoots of a woody plant micropropagated via tissue culture.

Advantages of Shoot Cloning

If perfected, micropropagation of trees on a commercial scale could sidestep the costly maintenance of stock nurseries, since large numbers of new trees could be grown from existing cultured stocks. Being clones of their parents, the genetically identical new stock will produce trees of a uniform nature, which McCown lists as the procedure's biggest advantage.

He notes that micropropagation would have other advantages as well over traditional seedling propagation programs. For one thing, in a field of research whose time frame is determined by the maturation time of a tree—from a few years to hundreds of years—the time savings can be considerable. Micropropagation doesn't require the seed of mature trees; instead, tissue—the "growing stock"—is selected from immature plants with superior characteristics. This allows many "generations" of otherwise slow-maturing species to be grown in a relatively short time, says McCown.

Because micropropagated stock is also free of pathogens, pests, and unwanted chemicals and is not subject to environmental stresses such as frost and drought, its rate of growth and development is highly predictable under a given production program. McCown believes disease resistance can one day be incorporated into the culturing system. This would be a boon to tree improvement programs in the United States. One target, he says, might be scleroderris canker, a devastating disease in red pines, which is a potential threat to Wisconsin forests.

If researchers could come up with a single disease-resistant red pine, that tree's tissue could be "multiplied" in the laboratory. Since red pine is a high-priority improvement species in Wisconsin, "it really brings the nature of this research home," says McCown. However, because of the complexity of conifer life cycles, scientists have yet to commercially shoot-culture pines.

Although woody plant micropropagation brings exciting prospects to tree production programs, it's not without its drawbacks. For instance, the working gene pool is limited to the chromosomes of the parent tree. When entire crops of a monotypic, or single-parent, plant are grown (a prevalent practice in revenue-hungry developing countries), the whole crop reflects the particular weaknesses as well as strengths of the parent. The weaknesses can be an open invitation to widespread damage by pests and disease. Micropropagation of trees can be "abused" in this way as in any agricultural system. McCown notes, however, that by cloning from a variety of parent trees, "you can program diversity into the system to avoid monotypes."

Another problem, difficult to detect, is maturation in the stock culture. "This could go unnoticed for years," McCown says, "until the plants produced from it are in the field or in the customer's hands."

When Protoplasts Fuse

McCown notes that woody plant tissue culture is also moving ahead on a cellular level. Much of the work is focused on protoplasts—cells without their cell walls. Once the cell walls are removed, isolated genes can be inserted into protoplasts. Or, protoplasts from selected parents can be "fused" in a test tube, forming a "somatic hybrid."

Protoplast culture, according to McCown, is one of the most dramatic developments in plant science and could become an important research tool. "The use of isolated protoplasts may provide a key for unlocking many fundamental research problems in plant

physiology, genetics, and pathology," he says.

Protoplast fusion, a method for genetically modifying protoplasts, can conceivably produce offspring

Hormone-stimulated buds sprout from a rhododendron twig in culture.

similar to sexually-produced progeny, but in a much shorter time. Fusion begins when an enzyme solution is added to a small piece of plant tissue to dissolve its cell walls. With these barriers removed, protoplasts from the desired "parents" can be mixed together in a chemical solution. There they fuse, creating a "soup" of genetic material and cell organs within a hybrid cell. Under the right culture conditions, the hybrid cell forms a new cell wall, divides, and grows into an irregular cell mass, or callus.

The sought-after result, which occurs with limited success, is to have the cell mass develop into a new plant, genetically different from either parent. McCown says once calli develop, researchers can subject the cell masses to a selection process, exposing them, for example, to a stress such as cold or a toxic substance. Ideally, the survivors of this microscopic "selection of the fittest" will be resistant to that stress agent.

But McCown speculates that "even without a selection process we may end up with genetic variants anyway," since the cell cycle cannot be completely controlled during the culturing process. That loss of genetic stability, which is programmed into the normal reproductive "machine," can lead to new gene arrangements.

A Fusion of Species

Scientists are fusing protoplasts from different plant species and even different genuses in the hope of producing new plant varieties. Cells from unrelated species have been successfully fused, but, to date, only a few hybrid plants have resulted. McCown suspects that the nuclei may not fuse properly, chromosomes may be lost, or "a hundred other things may be going wrong."

"Plants, and especially woody plants, are governed by such complex reproductive mechanisms that it's going to be difficult to direct the fusion into a predictable end product," he says. "For instance, no one is certain whether intergeneric hybrid cells will truly retain the characteristics of both parents or whether eventually one line will completely overtake the other, resulting in a plant identical to one of the originals."

McCown says that the gene pool in plant cells, even from the same plant tissue, appears to contain so much inherent variability that the smorgasbord of possible outcomes in protoplast research raises more questions for scientists than it answers. He adds that unlocking the mystery is going to make the next decade "an exciting time in plant physiology and genetics."

Editor's Note: Bill Keenan is a Science writer for the University—Industry Research Program at the University of Wisconsin — Madison. The UIR is a program of the Graduate School of the UW—Madison and its mission is to encourage and develop university relationships with business, industry and government.

Dr. Brent McCown is a member of the Department of Horticulture faculty at the UW—Madison, a position he has held since 1972. Prior to that, he spent two years as a Visiting Professor of Plant Physiology at the Institute of Arctic Biology, University of Alaska in Fairbanks. As a Captain at the U.S. Army Cold Region Research lab in Hanover, New Hampshire he studied possible effects of the Trans-Alaska pipeline on the plant environment and vegetation of Alaska. Dr. McCown has both research and teaching responsibilities at the UW—Madison. In 1977 he received the College of Agricultural and Life Sciences "Outstanding Teaching Award." In 1980 he was awarded the International Plant Propagator's Society "Outstanding Research Paper" Award. He is author and co-author of many research papers and articles in the areas of plant physiology, horticulture and environmental resource management.

CROSS-BREEDING WITH PROTOPLASTS