Micropropagation Technique
May Speed Reforestation Efforts

Reforestation efforts may get a lift thanks to a new advance in plant-tissue culturing at ARS's National Center for Agricultural Utilization Research, Peoria, Illinois. Plant physiologist Brent Tisserat has devised an automated plant culture system (APCS), coupled with enhanced CO_2 treatments, that bolsters yield and survival of delicate shoots.

In reforestry operations, budding shoots are cultured inside small glass tubes or in Magenta vessels and nourished on an agar gel. Tissue culturists treat these shoots, originally derived from leaves, with growth regulators to coax them into multiplying secondary shoots. Transplanted to soil, these shoots eventually root and become whole, free-living plantlets that can then be put in the field.

With this technique, known as micropropagation, forest-product companies can restock plantations with millions of genetically identical tree plantlets. The yield of trees clonally derived from these plantlets is much more predictable than that from fertilized seed, says Tisserat.

Even so, not all micropropagated shoots survive transplanting—especially "vitrified" shoots, the source of axillary branches that do not readily root. In contrast, nonvitrified shoots readily form roots but produce few axillary branches.

Tisserat's tree micropropagation involvement began in 1996, when Union Camp contacted him about tackling the yield problem in sweetgum trees. The Savannah, Georgia-based company, since merged with International Paper, grew sweetgum for making bags and other paper products. Like other forest-product companies, Union Camp used traditional micropropagation techniques to produce shoots and plantlets. The company wanted a method to mass-produce clonal sweetgum shoots and a way to root these shoots at high percentages. Tisserat observed that only 40-60 percent of Union's vitrified shoots survived transplantation.

The standard approach to solving the problem would have involved tweaking the agar medium's nutritional composition. But Tisserat changed the way nutrient medium is applied to the shoots, modified the physical environment in which they are grown, and switched from Magenta vessels to larger growth chambers, which provide the tender shoots with much more space and media.

Tisserat's APCS uses an automated pump to microirrigate the shoots with liquid medium piped in from a separate tank. The medium is applied and removed several times over a 24-hour period. In trials, this resulted in a 10-fold increase in shoot yields compared to traditional culture methods and a 14-fold increase in fresh weight.

To root these sweetgum shoots into soil, Tisserat subjected them to high CO_2 levels under high humidity, and they were able to survive, photosynthesize, and make roots. By pumping the gas directly into the chamber, ultra-high CO_2 levels can be achieved. For example, instead of an ambient CO_2 concentration of 350 ppm of CO_2 (0.03 percent), Tisserat's system uses 10,000 ppm (1.0 percent).

The CO_2 treatments resulted in a 94-percent survival rate for transplanted sweetgum shoots (including vitrified ones). The APCS also speeds seedling growth of loblolly pine, a chief lumber resource in the southeastern United States. Tisserat and co-investigators had earlier success micropropagating and rooting peas, lettuce, tomatoes, beans, and spearmint.—By **Jan Suszkiw,** ARS.

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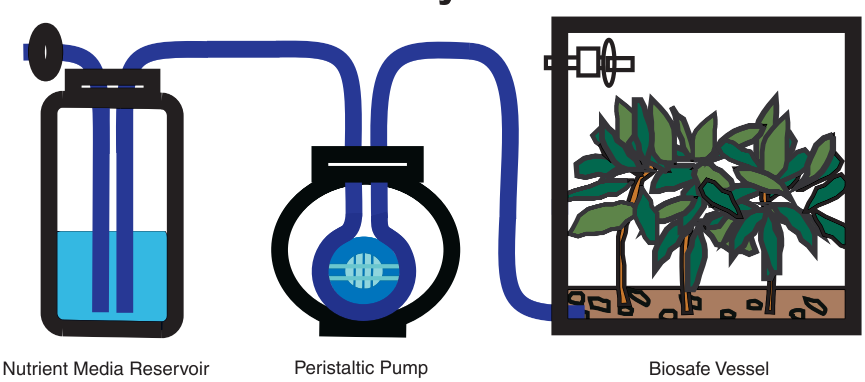
Automated Plant Culture System



CO_2 Treatments