New, high-intensity tree plantations are setting the stage for rapid biotechnological change in forestry. But the novel methods may never be used if the ecological risks and economic obstacles cannot be overcome

Forest Biotech Edges Out of the Lab

BOARDMAN, OREGON—"Warning," says the sign on the interstate. "Blowing Dust Area Next 45 Miles."

The drylands of northeast Oregon, an almost treeless region with an annual rainfall of just 20 cm, is one of the last places one would expect to see the future of forestry. But just outside Boardman, next to a Navy bombing range, sits a harbinger of things to come: 7200 hectares of cloned hybrid poplars, planted in square blocks 400 meters to a side. Grown by Potlatch, a Spokane, Washington–based forest-products company, the trees receive fertilizer, pest treatments, and water from a computer-controlled "fertigation" system that pumps water from the Columbia River, 8 km away, through 24,000 km of plastic pipes that crisscross the plantation. "We control what the trees get almost as precisely as if they were on a petri dish in a lab," says research manager Jake Eaton. In this way, he says, Potlatch can grow 20-meter trees in just 6 years, achieving wood production rates 10 times the global average.

Yet these huge, mechanized plantations, which are now sprouting in countries from New Zealand to Brazil, are just the beginning, say many forestry researchers. The next, and far more controversial, step in forest biotech will be to stock these high-intensity plantations with genetically altered trees that scientists say will grow faster, require fewer chemicals to pulp, or have wood with special properties. Already, researchers have inserted genes for traits such as pesticide resistance, herbicide tolerance, and delayed flowering into several types of trees, and the U.S. Department of Agriculture has received applications to field-test 138 types of transformed trees, 52 of them in the last 2 years.

Farther down the road, biotech supporters imagine extraordinarily fast-growing trees that can not only reduce the pressure on natural forests but help combat climate change as well. The ultimate goal, says botanist Toby Bradshaw of the University of Washington, Seattle, is to redesign trees altogether, creating superproductive organisms that in many ways will not resemble today's trees at all. Not only will the forest-products industry gain but so will the environment, says Eaton, who calls forest biotech "win-win."

The future? At its high-tech plantation in Boardman, Oregon, Potlatch carefully controls agricultural inputs to create poplars that grow at 10 times the global average rate.

Not everyone embraces this high-tech, bioengineered vision, however. Some forestry research leaders—notably Weyerhaeuser in Federal Way, Washington—have decided not to pursue some of the most advanced techniques, especially genetic engineering. Forest-biotech research may well pay off in the long term, these companies believe, but the short-term scientific, economic, and political hurdles are so high that they cannot justify embracing it all.

And even some scientists who endorse superintense tree plantations worry about ecological risks of genetically engineering the trees in them. Introduced traits, they argue, could have unintended consequences if transferred to natural trees. And outside the research community, activists have already vandalized research plots and burned down a laboratory in an effort to rid the world of "Frankentrees" (*Science*, 6 April 2001, p. 34). In November, police found two bombs outside a forestry lab in Michigan. So intense is the opposition, in fact, that even some of forest biotech's strongest scientific supporters acknowledge that their research may not make it out of the lab for years, or even decades.

"Rearchitecting" trees

On a table in Steve Strauss's laboratory at Oregon State University in Corvallis, a leading center of arboreal genetic engineering, sits a key piece of high-tech equipment: an office hole punch. Strauss's co-workers use it to clip round, pencil-eraser–sized pieces from the leaves of a quaking aspen. They then drop the green circles into a broth thick with *Agrobacterium tumefaciens*, a common garden microorganism that inserts part of its DNA into host plants, causing tumorlike galls. Strauss's team has endowed the bacterium with genes for antibiotic resistance and delayed flowering, in the hope that it will insert those genes into the aspen DNA contained in those bits of leaf.

After exposing the leaf circles to the bacteria for 48 hours, Strauss's team dips them into an antibiotic solution that kills all the leaf cells except those that took up the antibiotic-resistance gene. In petri dishes, the transformed leaf cells grow into tiny sprouts that eventually become large enough to pot. Researchers then evaluate the emerging trees to see whether the other introduced gene, for delayed flowering, is also being expressed. (Agrobacteria insert their genes randomly into the leaf-cell

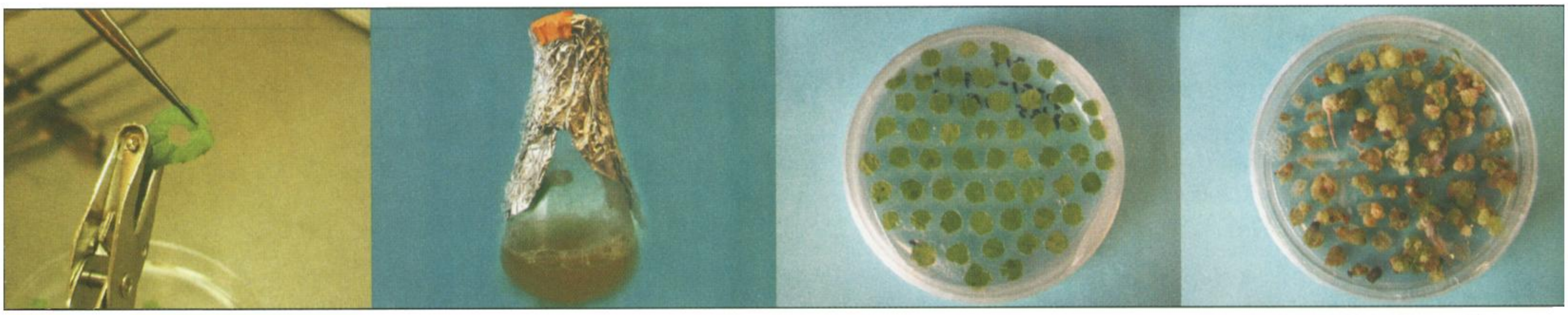
CREDIT: KAREN WATTENMAKER

DNA, and the location of the foreign genes in the genome affects their function.)

By delaying flowering past the time of harvest, Strauss hopes to reduce the likelihood that genetically modified trees will pollinate their wild relatives, an ecological safeguard he believes is essential. Not until researchers can limit the likelihood that novel genes with new properties will spread into natural forests, he argues, will industry be able to introduce transgenic trees into plantations safely.

Another major research target for forest biotech is lignin. The compound that makes tree cells stiff, lignin is desirable for sawtimber but not paper. Removing it costs the pulp and paper industry $20 billion a year, according to Jonathan Malkin of ATP Capital, a biotech investment firm that backs high-tech forestry start-ups. In July, researchers at Michigan Technological University in Houghton announced the discovery of the gene responsible for producing syringyl lignin, the type of lignin in hardwood trees; the next step, they say, is to turn down the gene's expression and, they hope, create low-lignin trees. At North Carolina State University in Raleigh, researchers have discovered a natural mutation that lowers the amount of lignin in loblolly pine. Because the mutant gene eventually harms the tree, North Carolina State botanist Ronald Sederoff and his team are trying to create heterozygous loblolly pines that grow normally but are more easily pulped.

The University of Washington's Bradshaw has a far grander goal: what he calls the "rearchitecting" of trees. "What a tree wants to do is grow its trunk as thin as possible and devote as many resources as possible to leaves and seed," he says. "What [foresters] want are as much wood as possible and as little leaves and flowering as possible." Most trees have about one-third of their biomass tied up in their root systems, a percentage foresters would like to lower. Trees today can't be packed too closely in farms because they respond to crowding by reaching for light, resulting in taller, thinner, and therefore less desirable trunks. Biotechnology, Bradshaw suggests, offers the possibility to "create the tree we want."



Bit by bit. Using an office hole punch, researchers clip pieces of aspen leaves, insert foreign genes in culture, and plate the discs to produce transgenic seedlings.

CREDITS: S. STRAUSS

In a scenario that is widely believed to be distant but feasible, scientists would create genetically modified trees for tomorrow's intense plantations: short, wide, almost branchless organisms without extensive root systems that could withstand crowding. These supertrees wouldn't "look anything like trees today," Bradshaw says, "any more than today's corn looks like its ancestor." But, as he acknowledges, turning this dream into reality will require leaping over high scientific, economic, ecological, and political hurdles.

Scientific challenges

There are three overarching scientific barriers to bioengineering trees. First, trees are so different from the annual plants used in most biotech research that scientists may have little ability to use better known genomes as guides. Second, researchers have been unable to propagate most trees clonally, an essential step in reliably disseminating new strains. (An exception is the genus *Populus*, whose members—aspens, poplars, and cottonwoods—can be easily cloned and have long been favored by researchers for that reason.) Finally, even if breeders produce trees with the desired genetic makeup, the effects would take years to evaluate, unlike wheat or maize, which can be tested in a few months. Recent work suggests that researchers are close to solving the first two difficulties; the third may be overcome with more funding and experience.

In conventional biotech, researchers studying little-known species can work with genes from better understood species, either by transplanting them directly or using them as guides to search for equivalent genes, or homologs, in the new species. The delayed-flowering gene in Strauss's lab, for example, comes from *Arabidopsis thaliana*, the model plant for molecular biology. But such techniques could be limited in silviculture. For one, long-lived trees are so unlike annuals such as *Arabidopsis* that scientists don't know whether their most important genes will be readily identifiable homologs. "A tree is essentially a mountain of poisons," Strauss notes. "Trees have to sit out there for a couple of decades and not get eaten. *Arabidopsis* doesn't, so there's no obvious reason why its genetic makeup should be comparable."

If researchers cannot rely on homologs, they will have to sequence and evaluate tree genomes, not an easy task. Many commercially important trees have unusually big genomes: Pines, with 20 billion base pairs of DNA, have a genome seven times as large as that of humans. Yet pines "are not expected to have any more genes than *Arabidopsis*," says Bradshaw. "Their genome is probably full of junk. But that doesn't make sequencing it less of a chore."

Nonetheless, genome projects are under way, focusing on spruce (a project based in Canada) and radiata pine (a commercial effort run by Genesis, a New Zealand biotech firm). In 1999, Sederoff received almost $4.5 million from the U.S. National Science Foundation to begin sequencing loblolly pine, the most important plantation tree in the southern United States. And last month the U.S. Department of Energy (DOE) launched a fast-track program to sequence a member of the *Populus* genus. Bradshaw is already growing cuttings from a black cottonwood from southern Puget Sound to send to sequencing laboratories. (At 550 million base pairs, the cottonwood's genome is of a manageable size.) The project, based at DOE's Joint Genome Institute in Walnut Creek, California, but run by an ad hoc group led by Bradshaw, should complete its first pass—sequencing each gene an average of three times to reduce the chance of error—by fall 2003.

If researchers do succeed in introducing a new trait into a tree, propagating it poses the next challenge. The ideal strategy, forest researchers agree, would be clonal propagation: the process used by breeders of annual plants when, for example, they grow violets from cuttings. But because almost all conifers and many hardwoods cannot readily reproduce in this way, scientists are investigating a process known as "somatic embryogenesis": in essence, inducing cells in nonreproductive tissues such as leaves or roots to grow embryos. Like clonal propagation, the process does not involve fertilization, so there is no risk of pollination by wild trees—which occurs frequently in conventional plantations—and the resulting embryos will be clones of the tree that produced them.

Typically, somatic embryogenesis involves knocking adult cells back to a juvenile state in which they are less firmly set on their course—often by exposing adult tree cells to dilute solutions of herbicides, especially 2,4-dichlorophenoxyacetic acid, says Scott Merkle, a tree geneticist at the University of Georgia in Athens. In the usual dosage, such herbicides "kind of stimulate plants to death," Merkle says. "At 2 parts per million, which is what we use, it simply stimulates them"—unlocking a previously hidden potential to create new clones. Creating the clone embryos is often relatively straightforward, Merkle says, "but getting them to germinate properly and make a somatic seedling is a problem, because it's difficult to get an embryo in culture to grow anywhere near in size to an actual seed embryo."

Nevertheless, using techniques developed by biologist Stephen Attree, CellFor, a forestry start-up in Vancouver, British Columbia, says it has mastered somatic embryogenesis for some of the most commercially important softwoods. (Attree did the work at the University of Saskatchewan and is now CellFor's chief of research.) According to CellFor president Christopher Worthy, next year the company will produce 8 million to 10 million embryos and deliver to forest-products companies 3 million seedlings of loblolly pine, Douglas fir, radiata pine, and

Can Genetic Engineering Help Restore 'Heritage' Trees?

In the summer of 1904 Hermann W. Merkel, a forester at the New York Zoological Park, noticed peculiar cankers on the stately chestnut trees that lined the zoo's pathways. The cankers—caused by the Asian fungus *Cryphonectria parasitica*—soon circled the trunks completely, killing the trees. Initially, Merkel's report was treated as a curiosity. But the fungus spread with astonishing speed. By the end of World War I, the American chestnut, which once dominated many eastern forests, was fast approaching oblivion.

Now, forest-biotech researchers believe genetic engineering might help restore this majestic species—and possibly other "heritage trees" menaced by disease, including elms, white pine, butternut, and several species of California oak. So promising are the new techniques that researchers from academia, industry, government, and private foundations are forming a coalition to bring back these species, starting with the American chestnut. If the effort pays off, it would put an end to decades of scientific frustration and, its backers hope, some of the negative aura of genetic engineering (see main text).

Since 1983 the American Chestnut Foundation has been trying to restore the species using conventional breeding. It has been crossing American chestnuts (*Castanea dentata*) with blight-resistant Chinese chestnuts (*Castanea mollissima*), then repeatedly "back-crossing" hybrids that showed resistance, to obtain resistant trees that look like pure American chestnuts. Under the best of circumstances, back-crossing takes decades, and the end product would still have many unwanted Asian genes. But the problem has proven even harder to solve than the foundation initially anticipated.

Blight resistance in the Chinese chestnut is largely due to three genes located on widely separated portions of the plant's genome. Because the genes are inherited independently, the only way to pass on the trait is to mate resistant hybrids with other resistant hybrids, and that entails creating many resistant hybrid lines—"really a difficult proposition," says William Powell of the State University of New York (SUNY) College of Environmental Science and Forestry in Syracuse.

Raised from the dead? Using genetic manipulation, scientists hope to restore the American chestnut, which once dominated eastern forests.

To several researchers, including Powell and Charles Maynard of SUNY and Scott Merkle of the University of Georgia in Athens, genetic engineering offers a clear shortcut. But it, too, has proven tough. "The chestnut hates genetic manipulation," says Maynard. The tree is so difficult to propagate in culture, he jokes, that "it's as if it *wants* to go extinct." Indeed, scientists spent a decade devising a reliable method for propagating them in the field, a crucial first step.

The researchers are now looking for genes with antifungal properties. A leading candidate, say Powell and Maynard, is *OXY*, a wheat gene that encodes oxalate oxidase. Oxalate oxidase breaks down oxalic acid, the compound exuded by *Cryphonectria parasitica* to kill cells. By splicing in *OXY*, Powell and Maynard hope to endow chestnut cells with a weapon to fight back.

Powell, Maynard, and Merkle may soon get some much-needed help. Last November, a diverse group of academic, government, and private chestnut researchers* met at the North Carolina Biotechnology Center's Institute of Forest Biotechnology in Research Triangle Park to form a coalition to bring back the American chestnut and other heritage trees. According to institute head Edward Makowski, the parties are still working out the best legal structure for the group, which could license some patented genes from its corporate members. He hopes to resolve these issues "within the next 30 to 90 days."

But even if the coalition can design a resistant chestnut, the problem will not necessarily be solved, according to Roger Sedjo, an economist at Resources for the Future in Washington, D.C. The ecological niche formerly occupied by American chestnuts "was filled largely by oak trees," Sedjo notes. "Part of the question is, 'Could the American chestnut reestablish itself on a wide-scale basis?' Once it's been displaced, it might not get back in there" without major effort. Although he acknowledges these obstacles, Makowski notes that "the loss of the chestnut was an enormous ecological disaster. I can't imagine anything more exciting than the chance to reverse it."

–C.C.M. AND M.L.P.

* Participants included the American Chestnut Foundation, the U.S. Forest Service, the American Lands Alliance, the forest-biotech firms Arborgen and Mendel Biotechnology, and academic researchers such as Maynard and Ronald Sederoff of North Carolina State University.

CREDIT: COURTESY OF THE AMERICAN CHESTNUT FOUNDATION spruce; ultimately it intends to scale up to more than 250 million seedlings a year.

Even if propagation can be achieved, researchers will still face the inherent difficulty of evaluating the results of forest-biotech experiments. "If you insert a new gene into a tree," explains William Baughman, forest-research manager of the MeadWestvaco timber company in Stamford, Connecticut, "you have to grow that tree long enough to show that after a generation or so the only change that occurred is what you expected and that it has not mutated into something strange." Because trees may not mature for years or even decades, testing is costly and slow.

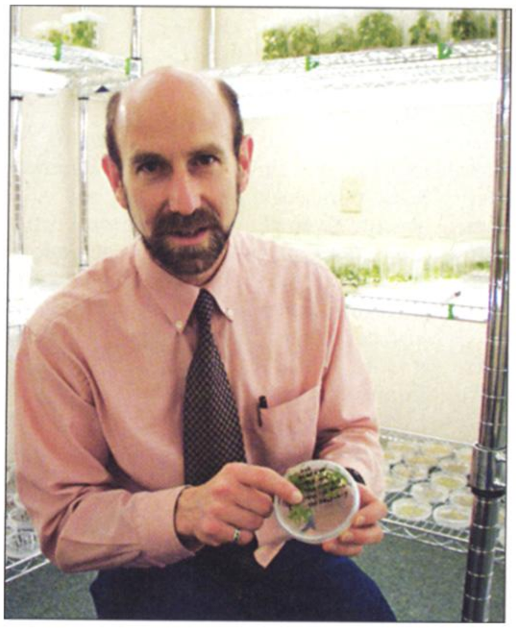
Examining faster growing species may at least help speed early research. Sederoff notes, for example, that Simcha Lev-Yadun, a plant geneticist at the University of Haifa, Israel, has discovered that "if you prune *Arabidopsis* in the right way and raise it in the right conditions, it grows to 10 times its normal size and makes woody stems." *Arabidopsis* may therefore provide some clues to the genetics of wood formation—and even, perhaps, the role of lignin.

Ecological and economic questions

If the technical hurdles for bioengineered trees can be overcome, the potential ecological payoffs could be enormous. So could the risks. According to the U.N. Food and Agriculture Organization, world demand for wood products in 2010 will be about 1.9 billion cubic meters, almost 20% higher than it is now. To meet that demand without laying waste to the world's remaining forests, economist Roger Sedjo of Resources for the Future in Washington, D.C., and ecologist Daniel Botkin of George Mason University in Fairfax, Virginia, suggested in a widely read 1997 paper that forest-products companies devote small areas "to intensive timber production and large areas to other uses, including biological conservation." This, they said, could drastically reduce the pressure on natural forests.

And if logging were almost entirely confined to high-intensity plantations, speculates economist David G. Victor, director of Stanford University's Program on Energy and Sustainable Development, the tropical forests that now release carbon as they are cleared might instead become a carbon sink. Simply using techniques such as somatic embryogenesis to put the best, fastest growing lines of conifers in the field, says Baughman of MeadWestvaco, "would let companies use a third less land to grow the same amount of wood. For a company like International Paper [the largest private forestland owner in the United States], that's 3.5 million acres [1.4 million hectares] you don't have to cut. And that's without transgenics. Add in transgenics, and you're talking about completely transforming the industry."

Don Doering, a senior associate at the World Resources Institute (WRI), a think tank in Washington, D.C., is not convinced. "A transgenic pine in Georgia will no more save the forests of Indonesia than an improved soybean grown in Iowa benefits the food-insecure peoples of Africa and Asia," he said at a forest-biotechnology conference last summer. Even researchers such as Botkin who favor intensive plantations have strong reservations about transgenic trees. He likens the environmental dangers to introducing exotic species into an ecosystem—a practice that has produced "good-willed disasters." Plenty of benefits can be achieved without genetic modification, he insists. Potlatch-style plantations, he says, "have side effects that are better understood and less of a risk. … Why not do the simple thing first?"



Tree doctor. If transgenic trees can be designed to be sterile, says Steven Strauss, they will pose fewer ecological risks.

CREDIT: JACE CARSON AND S. STRAUSS/OREGON STATE UNIVERSITY

Strauss thinks that his and others' work on producing sterile trees can reduce the likelihood of gene flow from genetically altered trees to their wild relatives. He also notes that rearchitected supertrees will have traits—short stature, small branches—that make them unlikely to survive outside carefully controlled tree farms.

Aside from safety concerns, the basic economics of forestry will make costly research programs such as tree genetic engineering a tough call. "When you have to wait 20 to 30 years to get payback," says Todd Jones, director of Weyerhaeuser forest biotechnology, "you have to have something that looks like it's going to have some real economic potential. If we look at economic models for some of the genes that do appear to be out there, there aren't that many that make that hurdle." Take herbicide resistance. Applying herbicides "is not that large of an expense" in the forest industry, Jones says.

Competition from conventional tree breeding poses another economic barrier. Because most breeding programs are now in only their second or third generations, traditional methods can still yield sizable gains. The approach may not be cutting-edge, but its more predictable returns make it attractive to a fiscally conservative industry. Finally, uncertainty over how bioengineered trees will be regulated adds to their economic risk. For ordinary crops that have been genetically engineered, running this regulatory gauntlet can cost years and "millions of dollars," says Nancy Bryson, a Washington, D.C., attorney who works on biotech regulation issues. The rules for trees are just beginning to evolve, she points out, and companies can't predict how burdensome they are likely to be.

All dressed up with no place to go

To WRI's Doering, the slow emergence of forest biotech has a positive side. Unlike transgenic crops, which were deployed in a frenzy, "there's a real chance of getting [tree engineering] right," he says. "There isn't overwhelming pressure, everyone can be cautious, and no one's going to make a fast buck on this. Society has the chance to make some good choices." He suggests that the forest-products industry demonstrate biotech's societal benefits rather than concentrating on economic gain. Genetically transforming the American chestnut to confer resistance to the blight that has ravaged this beloved tree in the eastern United States, he says, would be something that "speaks directly" to the public (see sidebar).

Potlatch, though, is moving away from genetic engineering, a decision that highlights forest biotech's uncertain future. In 2000, the company decided to seek certification of its environmental practices from the Forest Stewardship Council, a nonprofit organization that issues a kind of ecological Good Housekeeping seal to qualified timber companies. Potlatch's intensive, high-technology tree farm passed muster with the council last summer, but with an important condition: It had to remove any genetically modified organisms from its Boardman plantation—a decision that permanently shut down a 1.2-hectare plot that the company was hosting as part of Strauss's research.

Potlatch still supports Strauss's work at Oregon State University, says Eaton: "We just can't do it on our farm."

—CHARLES C. MANN AND MARK L. PLUMMER

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