

Biotechnology and Genetic Engineering in Forest Trees

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As with agricultural plants and animals, technical innovations in genetics, genomics, and related disciplines are also being developed for forest trees. However, the nature of trees and forests, and the wider range of products that we expect from them compared to crops, creates new challenges and opportunities.

In addition to wood and fiber products, forest managers must also balance trade-offs in producing ecological and social services. Inevitably, controversy develops over management goals and technologies. This includes where, how, and *whether* genetic technology and breeding in any form is appropriate. The goal of this essay is to review forest biotechnology, with a focus on its most controversial form, genetic engineering (GE).

Question: What is forest biotechnology?

Answer: Forest tree biotechnology emerged during the 1980s and encompasses a developing collection of tools for modifying tree physiology and genetics to aid breeding, propagation, and research (Burdon and Libby 2006). As is described elsewhere in this series, biotechnology is not a single approach, but instead encompasses tissue culture, micropropagation, genetic engineering, and genetic markers. The same is true for biotechnology as applied to forest trees. Over the past two decades, these various methods have become increasingly sophisticated, but all are still considered under the larger umbrella of forest tree biotechnology (FAO 2004).

Of all biotechnology methods, genetic engineering has received the most attention and scrutiny by regulators and the general public. At least part of this is due to the nature of the technology itself — artificially recombining genes from different organisms and bypassing natural barriers to sexual reproduction. In addition, new biochemical or signaling pathways to increase stress tolerance, or new products such as novel bioproducts, can be engineered. The movement of genes using conventional breeding techniques is limited to sexually compatible species, usually close relatives, and new biochemical or signaling pathways cannot normally be bred, as these require very long periods of evolution to develop.

Question: How does forest biotechnology differ from traditional breeding?

Answer: Traditional breeding and biotechnology share many common goals, principles, and practices. Practitioners of both methods are working to enhance the overall health and adaptability of forest populations or to improve production of desired goods and services.

Rather than representing distinct approaches, traditional breeding and biotechnology are better described as encompassing an overlapping collection of tools. In general, traditional breeding relies more heavily on sexual crosses and observations of trait phenotypes, whereas biotechnology tends to encompass methods that require one or more laboratory- or greenhouse-intensive steps to provide more precision, or a wider range of outcomes, than could be obtained using phenotypic selection alone.

Forest biologists are applying biotechnology in forest trees because these methods can help save time, reduce costs, or accomplish new goals. For example, genetic markers are beginning to be integrated into traditional breeding programs to enhance genetic diversity, speed the notoriously slow rate of progress over generations, and to reduce the costs of selection. Other approaches, such as embryogenesis as a means of multiplication and amplification of the best performing clones, is seeing increasing use in conifer forestry.

Question: To what extent is genetic engineering of forest trees underway?

Answer: Genetic engineering of trees, like that of other plants and animals, involves isolating genes from one individual, asexually inserting them into another individual's cell, and then coaxing that modified cell to regenerate into a new individual. In most cases, gene segments from different species have been manipulated and spliced together in the laboratory before inserting them into a recipient cell. However, as methods improve, genomic knowledge of a diversity of species grows, and GE may increasingly employ genes, with or without further modification, that have been obtained from the same or closely related species (Schouten et al. 2006).

The pace of gene discovery in many forest tree species has increased substantially due to technical advances in high-throughput genomic tools, including genome sequences (e.g., Tuskan et al. 2006, Grattapaglia et al. 2009). A key feature is that the asexual insertion process typically involves a small number of well-defined genes (one to a dozen). This contrasts with sexual reproduction in which copies of all genes, typically tens of thousands, are combined together following fertilization.

The first genetically engineered tree, reported by Fillatti et al. (1987), was developed by a team of scientists from the University of Wisconsin, the U.S. Forest Service, and the biotechnology company Calgene (now part of Monsanto). Since then, dozens of other forest tree species have been genetically engineered for research purposes, though none have seen commercial use in the U.S. Traits such as herbicide tolerance and insect resistance that have been widely used in commercial agriculture in the U.S. were also shown to be highly effective in field-grown forest trees. In China, genetically engineered poplar trees containing insect resistance (Bt) genes have been deployed that are very similar to those used in agricultural crops.

Wood-specific genes are of particular interest (Groover 2005). For example, early results altering lignin production (an important constituent of wood) demonstrated potential to reduce environmental impacts of pulp production (Pilate et al. 2002). There have also been a number

of studies demonstrating modified wood properties useful for making pulp or ethanol, among other traits.

The only commercialized tree in the U.S. to date is papaya, a horticultural tree which was made virus resistant via GE methods; no genetically engineered forest trees have yet been commercialized. A virus-resistant plum tree has been authorized by the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) and is awaiting final approval by the U.S. Environmental Protection Agency (EPA), and a cold-tolerant eucalyptus is now under consideration for commercial authorization at USDA. Both of these might not see widespread use for a number of years. The same general regulatory framework as applies for other crops also applies to genetically engineered trees; depending on the trait, one or all of the USDA, EPA, and FDA may be involved.

Question: What are the expected environmental and economic benefits for genetic engineering of forest trees?

Answer: The use of GE is often motivated by both economic and environmental goals. Herbicide tolerance should provide lower cost, more efficient, and less energy intensive means for weed control in plantations. Pest tolerance should improve yields, reduce product degradation, and in some cases reduce the use of pesticides. In other cases, GE can help protect or restore native trees in wild forests, such as following invasion of an introduced pest. Modified wood should reduce the energy and chemical requirements for processing wood into pulp and/or biofuels. Salt tolerance should allow trees to be established on poor, degraded lands. Trees engineered to take up or break down chemicals in the soil (bioremediation), may provide lower cost and less environmentally damaging ways to reduce toxicity of former industrial sites.

Question: Are there environmental concerns associated with the genetic engineering of forest trees?

Answer: More than ever before, forest practices are evaluated in the context of ecosystems, yet as the world's population grows, forest lands will be increasingly asked to provide more from less (Salwasser 2004). One way to meet some of these demands is through intensively managed industrial forest plantations, where genetically engineered trees could play a large role (Sedjo 2003). Plantation forests often have low diversity in tree species and low overall biodiversity at some life stages, both of which are sometimes considered undesirable. To avoid confusion, it is desirable to discuss the direct effects of genetic engineering separately from the indirect effects of plantation systems. Here we focus on direct effects.

As with other kinds of tree breeding, genetic engineering introduces novel or modified traits that could have unintended effects. For example, herbicide tolerance may create problems in control of trees when they are considered weeds. Because most trees can spread in the environment, mostly through pollen and seeds, this can create problems outside the original target area. Because of the undomesticated state of most forest trees compared to most agricultural and horticultural species, they can spread and establish more readily.

Forest trees can send pollen or seeds over considerable distances, often several miles (Smouse et al. 2007). Such undesired long-distance gene flow has already caused legal problems in other genetically engineered crops, such as bentgrass, alfalfa, and sugar beets. In recent litigation involving alfalfa and sugar beets, courts have ruled that failure to intensively consider economic impacts associated with gene dispersal violates the National Environmental Policy Act (e.g., Endres and Redick 2008). Thus, a precedent exists for similar controversies due to gene flow in forest biotechnology.

This propensity for gene dispersal in trees has prompted considerable effort to use genetic engineering to produce trees that flower poorly or not at all (Brunner et al. 2007). Whereas some field-grown poplars and eucalypti have shown dramatically reduced male fertility, most efforts to reduce fertility are still at an early stage. With proven technologies available today, fertility can be drastically reduced but not eliminated entirely. Thus, some gene flow is likely to occur, and the persistence and effect of introduced genes over time will depend on their initial frequency and how they affect the viability or competitive ability of progeny (Ellstrand 2006).

The uncertainty of future evolutionary and ecological effects creates enormous challenges for risk assessment and thus regulatory decisions. Though similar uncertainties exist for other kinds of breeding as well, these are unregulated due to their long-standing public and legal acceptance.

Other potential concerns include:

1. Persistence in the field. Whereas most agricultural crops are annuals, trees are typically long-lived, and species such as poplar and eucalyptus often vigorously resprout after they are cut. This longevity can be problematic if plants need to be removed, whether they are genetically engineered or not.
2. New traits such as modified wood can also have unforeseen side effects, such as reduced vigor under stress. In conventional breeding, the alleles under selection have already undergone some degree of natural selection, and thus are less likely to have large deleterious effects on tree health than are the new alleles produced by genetic engineering. However, it must also be realized that just because an effect is unintended doesn't mean there is a safety concern.
3. Genes that enhance stress and pest tolerance could be advantageous for trees outside of plantations, helping them to establish in the wild. So although these traits might provide environmental benefits by helping forests thrive, some scientists are concerned that such increased vigor or new forms of pest resistance might also have undesired effects, such as by reducing populations of nontarget insects valued for biodiversity.

Question: Does genetic engineering of forest trees offer unique advantages for improving forest health?

Answer: Genetic engineering can offer a unique tool to manipulate how plants grow, what they might produce, or how they respond to stress. Because GE circumvents sexual barriers, novel genes can be introduced from virtually any species, or they can be newly created or modified based on fundamental scientific principles. In some instances, such as resistance to certain introduced pests, it may be impossible — or extremely slow and difficult — to find a source of innate genetic resistance within a species or its sexually compatible relatives. It may be that genetic engineering is the only practical way to introduce resistance genes in a useful time frame.

With pressures from factors such as an increasing global economy and climate change, the threats of exotic pests and climate stresses are growing ever more significant; there are literally dozens of major forest tree species under serious threat throughout or in some parts of their ranges (Strauss et al. 2009a). Seriously threatened species include elms, ashes, dogwoods, beeches, oaks, maples, and firs.

The chestnut blight in North America is a striking example. No resistant American chestnut trees have been found since the disease was introduced to America in the early 1900s. Even after many decades of interbreeding with Asian species, resistant hybrids are only now starting to be released, and are unlikely to have the full set of resistances and environmental adaptation needed for long term survival over American chestnut's former wide range. A combination of approaches, including genomics, cloning of the best trees, and genetic engineering is providing renewed hope that fully resistant trees can be developed (Wheeler and Sederoff, 2009).

Question: Apart from its use for breeding, is genetic engineering a powerful scientific tool?

Answer: Genetic engineering is the most powerful tool for studying gene function in biology today, including forest trees. For example, GE can be used to manipulate the level or timing of gene expression with specificity and precision that is not provided by any other approach. Armed with a more thorough understanding of gene function, scientists can modify the frequency of different natural gene variants (alleles) with conventional breeding that is augmented by genetic markers. Thus, genetic engineering, used only as a tool for research, can substantially augment breeding by the insights it can provide.

Question: Despite their value, why are there no commercialized genetically engineered forest trees?

Answer: Several factors have contributed to delays. First, in many tree species the methods for gene insertion and regeneration of healthy trees do not exist, or are too slow and costly to develop for each desired variety. For many desired traits, the causative genes are unknown, or the traits are too complex to modify with one or a few genes, given current knowledge.

Moving beyond a greenhouse to outdoor studies is essential to understand the ecological impact and value of newly inserted genes, but securing approval for such studies from USDA's Animal and Plant Health Inspection Service (APHIS) has become increasingly costly and difficult, especially for university and other public sector scientists (Strauss et al. 2009b). Complete containment of all pollen and seeds from large trees during such studies is especially problematic.

Finally, for many companies the economic benefits are unclear when all costs and the long time frame for trees are considered. There is also real concern that marketplace restrictions, such as the Forest Stewardship Council certification scheme that excludes all genetically engineered trees, even when used in contained and environmentally motivated field research, will prevent sale of the products in desired markets (Strauss et al. 2001).

Question: What is the future of forest biotechnology?

Answer: There is a great deal of progress being made on the use of non-genetically engineered techniques in commercial breeding, especially cloning methods and genetic markers. The main limits appear to be economic rather than biological. Reduced costs of sequencing and genotyping, coupled with dramatic increases in throughput and efficiency, have resulted in rapid progress in non-genetically engineered applications in biomedicine as well as in plant and animal agriculture. We also expect expanded application of these techniques to forest trees.

There is currently limited investment in genetic engineering applications outside of a small number of companies and a few public researchers, primarily because of the regulatory and market acceptance issues discussed above. Likewise, regulatory issues are also causing large problems in agriculture. Existing regulatory processes are being reexamined, and are expected to change considerably in upcoming years. What should arise from this is a balance that stresses the actual benefits as well as actual risks. Still, the nature of change and its effects on research investment and commercial uptake are likely to be the main drivers of GE development in forestry. Increasing food, water, and fiber shortages associated with population growth and climate change, and the consequent stresses on ecological and social systems, may compel greater acceptance and less strident forms of regulation.



A row of non-transgenic and transgenic cottonwoods in a research trial in Oregon. The transgenic trees were highly resistant to the cottonwood leaf beetle due to expression of a cry3a type of *Bacillus thuringiensis* endotoxin gene.



Transgenic poplars in plant tissue culture undergoing propagation. These plants are ready for transplanting to soil for further growth and outplanting.

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