Leila Pinchot

Leila is a research ecologist with the US Forest Service Northern Research Station and a member of the Forest Guild Membership Policy Council.

Above: Transgenic American chestnut seedling, developed at the American Chestnut Research and Restoration Project at SUNY College of Environmental Science and Forestry.

Photo courtesy of Bill Powell.

American Chestnut: A Test Case for Genetic Engineering?

by Leila Pinchot

The thought of genetically engineered (GE) trees might conjure images of mutant trees with unnatural and invasive tendencies, but there is much more to the story. GE trees are a new reality that, like it or not, will probably be part of the future of forestry. The basic inclination of most Forest Guild stewards is to reject GE trees as violating our principle to imitate nature, but are there cases where GE trees should be used? The American chestnut (*Castanea dentate*) may be the most compelling case thus far for the use of genetic engineering. Bill Powell and Chuck Maynard, both from the American Chestnut Research and Restoration Project at SUNY College of Environmental Science and Forestry would tell you that the GE American chestnut trees they have developed look and act very similarly to non-GE American chestnuts, except for the simple fact that they seem to be resistant to the chestnut blight fungus. While the long-term blight resistance of these trees needs to be extensively tested, early results offer the hope of a blight-resistant American chestnut in the not so distant future.

Forest biotechnologists are the new kids in town when it comes to American chestnut restoration. Breeders have been working to develop a blight-resistant American chestnut since the 1920s, when it became clear the species would be lost to the non-native chestnut blight fungus. Though well accepted, breeding is a form, perhaps the oldest, of biotechnology, i.e., the use of a living organism to make products for a specific use. Compared side by side, traditional breeding and genetic engineering each have their pros and cons. In some ways, breeding is much simpler – it involves crossing together individual trees with desired characteristics; in the case of the chestnut, six crosses are necessary to develop a tree with the blight-resistance of a Chinese (*Castanea crenata*) or Japanese (*Castanea mollissima*) chestnut, with the growth characteristics of the American chestnut. Alternatively, GE chestnuts are the product of no crosses, only genetic manipulation. Scientists make use of a natural genetic engineer — *Agrobacterium tumefaciens*, a widely spread soil bacterium that naturally inserts genes into plants to cause the development of galls. Scientists transfer genes of interest into a plasmid, a small circular DNA strand, which is then inserted into the agrobacterium. Finally, the agrobacterium containing the desired genes is injected into chestnut embryos. If all goes right, this process will transfer the selected genes (in this case genes that confer resistance) into the chestnut embryos, which are then grown into seedlings. While this process is expensive (though becoming less so), it offers some benefits not afforded by traditional breeding. For example, using genetic modification scientists can introduce several genes conferring desired traits into the species of interest, while breeding introduces thousands of genes, with very little control. In the chestnut, researchers have found that the backcross hybrid still shows some signs of its Asian progenitor, including earlier bud-break, which may have ecological consequences, particularly in the northern extents of the tree's range. On the other hand, trying to confer long-term blight resistance through the introduction of only a small number of genes may not replicate the complexity of blight resistance found in Chinese chestnuts.

But it is not the case of one strategy against another. In fact, combining forest biotechnology with traditional breeding may provide the most effective route to securing stable blight resistance. Scientists from various institutions working together as part of the Forest Health Initiative (FHI), described below, have nearly completed sequencing the genome of the Chinese chestnut. The hope is to locate the genes in Chinese chestnut responsible for blight resistance. Breeders will then be able to identify which of their progeny contain the desired genes, and cull those that do not. This technique, termed marker-assisted selection, allows breeders to ensure the genes that confer desired traits – such as blight resistance or good timber-form – are present in the individuals chosen for breeding.

Genetic modification may offer modern solutions to modern ecological challenges; however, the technology may also pose ecological threats. Perhaps the threat that incites the most concern is gene flow from transgenic trees to sexually compatible wild trees. For example, if a transgenic poplar tree modified for increased insect resistance pollinated a compatible wild poplar tree, the transgene may be present in the resulting progeny. This would be particularly worrisome if the escaped gene gave its host a competitive advantage over other trees, which also raises concerns about the potential for GE trees to become new invasive species. In the case of chestnut, it will actually be the goal for the transgenic tree to reproduce with the wild American chestnut, to increase genetic diversity of the transgenic trees, while also disseminating the transgenes that confer blight resistance. Other potential risks of GE trees include unintended impacts on other organisms. To address this particular concern, researchers, including Powell and Maynard, are studying potential impacts of the transgenic chestnut on mycorrhizal fungi. Early results suggest no difference in mycorrhizae on transgenic and wild American chestnuts. Long-term testing, of course, is imperative to evaluate the potential ecological threats.

Using genetic engineering to promote forest health is a relatively new practice. For the first several decades of forest biotechnology research, the primary focus of the technology was to increase the production of high-yield forest plantations. For example, much of the research focused on modifying plantation tree species, like poplar and eucalyptus, for increased wood production, herbicide resistance, and decreased lignin production, among other modifications. These uses of biotechnology have been controversial among the forestry community, as well as among the general public, both because of possible ecological threats, described above, and because of proprietary issues related to transgenic plants – that is, who will own, control, and regulate transgenic trees. While this research is still continuing, the focus of forest biotechnology has expanded dramatically to include the restoration of threatened three species, as well as climate change mitigation and adaptation.

This change in focus is exemplified by the FHI– a collaborative effort with representatives from the federal government, academia, industry, and the non-profit sector– all working together "to advance the country's understanding and the role of biotechnology to address some of today's most pressing forest health challenges," using the American chestnut as a test case. As John Heissenbuttle, one of the FHI's original stakeholders, put it, "We saw potential for saving an icon of U.S. forests – American chestnut – through transgenics." What made the FHI unique was that, from the very beginning, the group understood the importance of involving multiple stakeholders through the entire process of developing the GE chestnut— it couldn't just be industry biotechnicians working behind closed doors. To encourage a productive conversation about the potential uses, threats, and benefits of this technology, a transparent conversation is absolutely imperative.

Because the Forest Guild's position statement (available online at www.forestguild.org) opposed the use of genetic engineering of trees for any

Below: Jennifer DeRosa taking leaf samples from a transgenic chestnut seedling developed by Powell and Maynard.

Photo courtesy of Kathleen Baier.

purpose, FHI invited the Forest Guild to participate in the conversation. Professional Member and forest manager Kim LaDuke, skeptical of genetic engineering, agreed to join the conversations. "I felt like a juror foreman on a murder trial. My job was to ask all the hard questions I could ask them." LaDuke was concerned with genetic modification, in part because trees have such long generations: "We're talking hundreds of years before you find out what you've done."

In 2012, three years into the project, the FHI successfully reached one of their primary research goals– to develop a putatively blight-resistant transgenic American chestnut.

While early results indicate a geneically engineered chestnut may resist the deadly blight fungus, not everybody is comfortable with this approach. Martha Crouch is a molecular biologist who consults for non-profits, and helped write a report critical of genetically engineered trees for the Center for Food Safety, a group that generally opposes genetic modification in agriculture. She is concerned with potential unpredictable long-term consequences of the release of GE chestnut trees. Additionally, she questions the ability of scientists to develop a transgenic chestnut with durable blight resistance, given the complicated pathways that confer fungal resistance in plants. In traditional breeding, she argues, "The breeder has a goal and facilitates

crossing and screening, but the trees provide the genetic information that they have fine-tuned over many environments. With something like chestnut trees that are long-lived, and poorly known ecologically, can the theories of [genetic] engineers come close to the 'intelligence' of the trees themselves?" And should breeding fail, Crouch finds hope for American chestnut in the resilience of nature and the healing power of deep time, citing the example of the near extinction of hemlock. Some 5,000 years ago, Eastern hemlock (*Tsuga Canadensis*) experienced a sudden and drastic decline in abundance, likely caused by a pest or pathogen, a similar scenario to the impact of chestnut blight. Over the time span of 1,000 years or more, hemlock gradually recovered in abundance. "Our time frame for success," she suggests "may simply be too short".

We are in a geologic era, called the Anthropocene, defined by the action of humans, as opposed to naturally occurring forces. As articulated by the title of Bill McKibben's 1989 book, in some ways we are experiencing "the end of nature", in a world where ecosystems can no longer be thought of as independent of humans. Forest management in the Anthropocene is very complex, as it requires that we make management decisions today that may or may not reflect the ecological conditions of the future. It is in this context, in which we may lose the American chestnut, the eastern hemlock, the American beech, the butternut, the black walnut, the Port-Orford cedar, the flowering dogwood, the American elm, and the ashes— all species threatened with functional extinction and all candidates for protection or restoration via GE techniques, that we ask what tools are appropriate for forest management in the Anthropocene. Should we count on traditional breeding, should we wait for the hope of natural recovery, or do we need every tool to bring back this keystone species to hold together threatened forests? ∎

Editor's note: A copy of the complete article including references and endnotes may be downloaded from the Forest Guild website at www.forestguild.org/FW21.html