



ScienceWatch – Resurrection of the American Chestnut

“It’s taken 26 years of research involving a team of more than 100 university scientists and students ..., but we’ve finally developed a non-patented, blight-resistant American chestnut tree.” – W. Powell

The American chestnut (*Castanea dentate*) was once an essential component of the eastern U.S. forest ecosystem from Maine to Louisiana, accounting for 25% of the hardwood forest. Mature trees grew to more than 100 feet tall and lived 300 years or more. The nuts were an important source of food for wildlife, livestock and people. The rot-resistant wood was used in home construction and fine

furniture.

Then in 1904 came a report from the Bronx Zoo of trees dying from a fungus infection. The fungus, known as chestnut blight (*Cryphonectria parasitica*), was imported on trees from an Asian nursery. It spread so rapidly that within 40 years four billion trees were gone and the American chestnut was nearly wiped out.

The blight fungus creates an orange canker on the bark and secretes toxic oxalic acid that kills the cambium layer. Once the tree is girdled everything above the infection site dies. The roots survive and send up new sprouts, but these too eventually are killed by the fungus. Today the American chestnut in the wild has been reduced from a dominant canopy species to a small shrub, where it exists at all.



The Brooklyn Botanic Garden and the USDA began a breeding program 100 years ago to restore the species to the wild. Plant breeders tried hybridizing *C. dentate* with an Asian chestnut species resistant to the pathogen. But traditional breeding programs are slow and unpredictable—it takes five years for *C. dentate* to flower—and the hybrids could not compete in the forest. After many years of failing to obtain fully resistant trees with all the defining traits of *C. dentate*, that program ended in the 1960’s.

But the desire to restore this keystone species to its original habitat was enormous. In 1983 the American Chestnut Foundation was created to continue traditional breeding by backcrossing *C. dentate* with the Chinese species (*C. mollissima*). Since then four successive crosses of a *C. mollissima* and its descendents have been made to *C. dentate*. Each backcross halves the Chinese gene contribution so only 6% (1/16) of the Chinese genome remains in the current generation. But the trees are only partially resistant. To enhance resistance breeders are crossing these trees among themselves hoping to combine multiple resistant genes within individuals in the next generation.

Genetic engineering is a quicker and more reliable means to yield a blight-resistant tree. In 1990 forest biotechnologists William Powell and Charles Maynard, SUNY College of Environmental

Science and Forestry, Syracuse, NY, used a newly-developed method for gene transfer in plants to create resistant *C. dentate*. Oxalic acid made by the fungus kills the surrounding plant tissue, paving the way for fungus growth. Using a soil bacterium (*Agrobacterium**) as the gene transfer vector, they inserted a gene (OxO) from bread wheat that makes an enzyme, oxalate oxidase, which destroys oxalic acid.



OxO is a common defense gene found in cereals and many food crops like strawberries, beets and peanuts. Lacking oxalic acid, the fungus is disarmed. Cankers can no longer form and the tree's natural defenses kick in. "We are basically taking the weapon away from the fungus," says Powell. By 2014 Powell's team had obtained transgenic trees expressing high oxalate oxidase levels. These blight-resistant trees** are still 99.999% genetically identical to wild-type *C. dentate* and should be well adapted to its original habitat. A summary of that research appears in the August 29, 2018 issue of *Science*.

The OxO gene does not naturally exist in *C. dentate* so the team faces the regulatory hurdle of getting a genetically modified plant released into the wild, a process that Powell expects will take 3-5 years. The USDA will determine if the trees can threaten other plants by becoming weeds. The FDA will determine whether the nuts are safe to eat, and the EPA will consider whether the OxO enzyme should be regulated as a fungicide.

Nevertheless, Powell is hopeful. He says, "The American chestnut was once one of the most important hardwood tree species in the eastern forests of North America and it can be again."

Saul Scheinbach

**Agrobacterium tumefaciens* is a naturally-occurring vector that genetically modifies plants.

** See photo of transgenic vs. wild type trees after 30 days infection with chestnut blight.