Before the fall: American chestnuts in the Great Smokey Mountains of North Carolina in 1910.

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The chestnut resurrection

Once king of eastern forests, the American chestnut was wiped out by blight. Now it is poised to rise again.

BY HELEN THOMPSON

"They're hard to breed and easy to kill," says plant pathologist Fred Hebard as he attacks a 2-metre-tall chestnut tree in southwest Virginia. Hebard bores a hole in the bark and squeezes a mash of orange fungus into the wood. The tree is a hybrid of the Chinese and American chestnut species, and Hebard hopes that it has enough resistance genes to keep the fungus — called chestnut blight — at bay. If so, the hybrid could help to resurrect a long-gone icon.

Until a century ago, the American chestnut (*Castanea dentata*) was the cornerstone tree species of eastern North America. With long, straight trunks and bushy crowns, it carpeted the forest floor each autumn with prickly brown nuts. But the arrival of chestnut blight (*Cryphonectria parasitica)* from Asia wiped out almost all the stately trees, leaving only a few, isolated stands. Since then, a faithful fan club of scientists and citizens has sought to tame the blight.

As chief scientist of the American Chestnut Foundation (ACF), a group of chestnut enthusiasts and scientists, Hebard has bred thousands of hybrids at the organization's research farm in Meadowview, Virginia. He crosses descendants of the original American chestnut with the much smaller Chinese variety (*Castanea mollissima*), which has some natural immunity to the Asian fungus. And after decades of work, he is within reach of his goal, a tall American tree with enough Chinese traits to keep it healthy.

Other researchers are trying to attack the blight with viruses or are creating trees that are genetically modified (GM) to resist the fungus, and could be the first GM forest trees released in the wild in the United States. Progress with all three approaches is raising hopes that chestnuts will soon start to flourish again in the forests of the American east. "We're starting to pull the American chestnut tree back from the brink of extinction," says Hebard.

The work is also offering lessons that could help to save other trees, such as elm and ash, which face similar threats in America and abroad.

FROM GIANTS TO STUMPS

Once known as the sequoia of the east, the American chestnut was one of the tallest trees in the forest, and dominated a range of 800,000 square kilometres, from Mississippi to Maine (see 'Felled by a fungus'). It made up 25% of the forest, and its annual nut crop was a major source of food for both animals and humans. The decay-resistant wood was also used to make telephone poles, roofs, fence posts and parts of railway lines.

The first warning signs came in 1904, when rust-coloured cankers developed on chestnuts at the Bronx Zoo in New York. Zoo forester Hermann Merkel took a sample across the street to the New York Botanical Garden, where mycologist William Murrill soon identified the spores as chestnut blight. The blight probably hitched a ride on nursery imports of Japanese chestnuts beginning in 1876. Spreading through rain and air, fungal spores infected trees through bark wounds and breaks. Cankers developed, quickly encircling a branch or trunk and cutting off the supply of water and nutrients from the soil. Within 50 years, the blight had laid waste to nearly the entire population of some 4 billion trees.

Other hardwoods, mainly oak, eventually filled the void but they do not produce a consistent crop of nuts every year. "You had a really dominant species that the wildlife depended on which was then replaced with a species that then didn't produce as much," says Douglass Jacobs, a forest ecologist at Purdue University in West Lafayette, Indiana. Reports from the period suggest that squirrel populations initially collapsed, and that five moth species dependent on chestnuts went extinct (D. A. Orwig *J. Biogeogr.* **29,** 1471–1474; 2002).

As the chestnut succumbed to the blight, scientists raced to find solutions. They started breeding hybrids of American and Asian chestnuts, which have evolved alongside the blight. But the attempts produced no trees that were sufficiently resistant yet still had enough

American traits to make them a popular replacement. Asian chestnuts are shorter and less sturdy than their American counterparts.

Over the years, chestnut lovers have tried a range of remedies, including fungicides, sulphur fumes and radiation. They even conducted religious ceremonies, assuming that the sins of the people had brought on the blight. But all these strategies failed.

RESTORATION CHESTNUT

In 1983, a group of plant scientists joined together to form the ACF with the goal of creating a resistant tree. The foundation has since grown to 6,000 volunteer members, ranging from retired physicists to farmers, and it maintains 486 regional breeding orchards and 120,000 experimental trees.

At Meadowview Research Farm, where Hebard is based, a refrigerator holds prized pollen from American chestnuts and between 600 and 700 strains of the fungus. The valley and hillsides are lined with rows of hybrid chestnuts as well as unadulterated American, Chinese and Japanese chestnuts (*Castanea crenata*). Some have plastic bags covering their flowers to prevent unwanted natural pollination from ruining the experiments. "It's a chastity belt for chestnuts," jokes Hebard. "You bag it if you want to know who the daddy is."

Nearby are rows of chestnuts that stand about a metre tall, the most promising hybrid yet to emerge from nearly 30 years of breeding experiments. This 'restoration chestnut' is 94% American and 6% Chinese and seems to show strong resistance to the blight. But the Virginia trees may not thrive in other locations, so the researchers are working to adapt the restoration chestnut to other climates. At the same time, they are breeding new hybrids at other experimental farms to preserve a healthy degree of genetic diversity.

More than 1,100 kilometres to the north, researchers are experimenting with chestnuts that contain genes thought to provide resistance, which were taken from Chinese chestnuts as well as plants such as wheat, peppers and grapes. At the State University of New York in Syracuse, plant pathologist Bill Powell and forest biologist Chuck Maynard have planted some 600 transgenic trees for field trials of disease resistance. A transgenic variety with a wheat gene for the enzyme oxalate oxidase, which disarms the fungus, has already shown resistance in the field.

Maynard, Powell and their colleagues are collaborating with a non-profit organization called the Forest Health Initiative in Research Triangle Park, North Carolina, to develop a GM version of an American chestnut with strong resistance based on genes from Asian chestnuts. Because such 'cisgenic' trees contain only chestnut genes, researchers hope that the trees won't provoke strong public objection. The Forest Health Initiative is testing the cisgenic chestnuts now and aims to obtain federal approval to conduct larger plantings. "Chestnut may be the first case of a genetically engineered tree that's planted out. If that happens it can probably pave the way for other trees," says Jacobs. Along the way, the transgenic work will help researchers to determine which resistance genes are the most useful.

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Other researchers are enlisting viruses that attack the chestnut fungus. Such viruses spread most easily among closely related fungi and are effective at controlling the blight in Europe. But American fungal strains are more diverse, so the virus cannot spread as effectively. To get around that problem, virologist Don Nuss at the University of Maryland in College Park has developed a transgenic fungus that is designed to spread the virus more easily. Other researchers are conducting field trials to test whether the engineered fungus can compete with — and replace — wild-type fungal strains. If so, the viruses could be used in targeted ways to protect American chestnuts.

Most researchers agree that restoring the American chestnut will require a combination of fungal viruses and resistant trees, whether hybrid, GM or a mixture of both. And the trees will need to defend themselves against more than just the blight. Researchers are also concerned about the threat of diseases already attacking other trees, such as root rot mould (*Phytophthora cinnamomi*) and invasive insects, including ambrosia beetles and gall wasps. Hybridization and transgenic programmes are beginning to target some of these threats.

Lessons learned from the battle to restore the chestnut could help save other threatened trees. The American elm (*Ulmus americana*) and its European counterpart (*Ulmus laevis*) have been hit hard by Dutch elm disease, and ash and hemlock are succumbing to invasive insects. In the United Kingdom, the beloved horse chestnut (*Aesculus hippocastanum*) is in danger of being wiped out by a combination of a bacterium and a moth. Researchers are developing hybrid and transgenic programmes to defend against many of those threats.

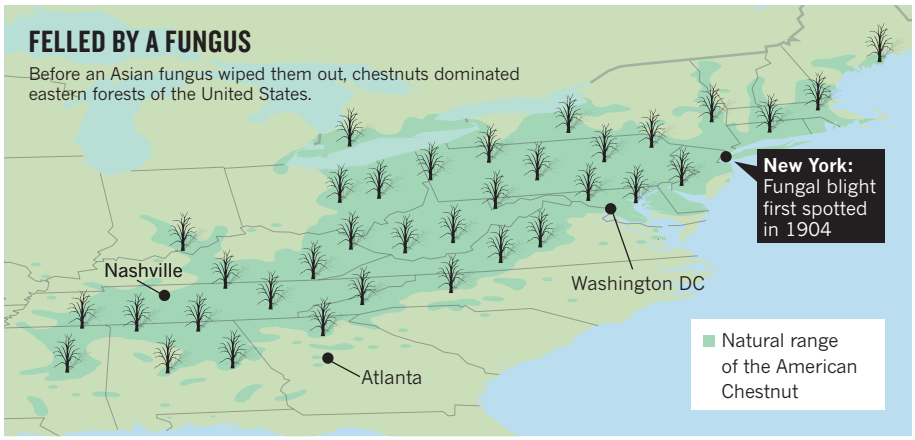
NEVER-ENDING BATTLE

"It's the pest of the month club. We've lost chestnut and lost elm. Now, it's almost a new species or pest is being identified and a new tree or forest is being threatened almost on a monthly basis," says Carlton Owen, a forester and wildlife biologist who chairs the Forest Health Initiative's governing board. Owen says that the results of the chestnut work will help researchers to protect other species by showing which strategies work best in different situations and by testing public acceptance of GM trees.

Once researchers have a resistant chestnut, the question is where to plant it. Forest ecosystems have transformed in the past century, and reintroducing the chestnut could upset the new ecological balance. "You can't assume that a forest with chestnut is better than a forest without. You can't roll the clock back," says Steve Hamburg, chief scientist at the Environmental Defense Fund in Boston.

"Reintroduction is going to be kind of a gradual thing," says Jacobs. In 2009, the ACF began planting restoration chestnuts on US Forest Service land in Virginia, Tennessee and North Carolina. In April 2012, it also started planting hybrid chestnuts and other hardwood saplings at a former mining site.

For chestnut lovers, other signs of hope stand alongside a path at the New York Botanical Garden. In a corner of the garden, a transgenic chestnut and a restoration hybrid both reach about a metre high. Although their leaves have shrivelled a bit, the scrappy saplings have managed to survive one of the warmest summers on record. And their bark is smooth, with no sign of the cankers that claimed their ancestors. ■



FELLED BY A FUNGUS

Before an Asian fungus wiped them out, chestnuts dominated eastern forests of the United States.

Helen Thompson *recently completed an internship at* Nature *in Washington DC.*