American chestnut restoration effort getting a boost from molecular geneticists

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Last spring, in The Arboretum at Penn State's chestnut orchard, researchers performed controlled pollination, bagging flowers on selected trees to keep unwanted pollen away and then introducing pollen from trees known to have a high level of blight resistance. Credit: Penn State

Efforts to restore American chestnut trees to their rightful place in the

North American forest ecosystem are progressing, although progress has come at a slower pace than once expected, according to researchers in Penn State's College of Agricultural Sciences, who explain they have reached a pivotal point.

The 27-year-old traditional breeding program, which has attempted to infuse blight resistance from the Chinese chestnut tree into American chestnuts, is receiving a boost from tree molecular geneticists at Penn State and five other universities working collaboratively in a bid to improve the process. While traditional breeding has been taking place, so have parallel lines of research into genetic modification and also bio-control of the fungus that causes the blight.

"Teams of researchers are now at a crossroads where all three methodologies may be combined to provide a more robust product," said Sara Fitzsimmons, a research technologist who is also director of restoration for The American Chestnut Foundation, the group leading the chestnut-restoration effort. "By merging successes in genetic modification, hypovirulence and traditional breeding, restoration of a disease-resistant American chestnut tree is closer."

The chestnut blight—which wiped out the American chestnut species across its 180-million-acre range in the first half of the 20th century—is caused by a fungus inadvertently introduced from Asia. Some view the loss of the chestnuts, which produced untold tons of food for wildlife and food and lumber for humans, as one of the worst U.S. ecological disasters.

"We didn't account in our time estimates for how long it would take after we got nuts with blight resistance to plant out orchards and select progeny with the strongest resistance and eliminate material susceptible to the blight," said Fitzsimmons. "When we plant these trees with nuts generated by our latest generation of backcrossed trees, only 1 percent

have the resistance that we are looking for. So you can imagine, if we're planting 27,000 trees, only about 270 have the combination of blight resistance and American chestnut characteristics we need."

The chestnut orchard in The Arboretum at Penn State and the Chestnut Foundation's Meadowview Research Farms in Virginia contains the latest generation of traditionally bred plant material with the most chestnut blight resistance and American character. At the Penn State orchard last spring, Fitzsimmons and her colleagues conducted controlled pollination of selected trees. The tactic underlines challenges faced by researchers trying to bring back the American chestnut.

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disasters. Credit: Penn State

"Because we still haven't finished planting out the orchard and we still haven't finished selecting and culling highly blight-susceptible trees we planted a few years ago, the blight-susceptible trees are pollinating trees that are selected and resistant," she explained. "So, when we collect nuts in this orchard, they have a wide variety of resistance and very few have full resistance, because there is so much pollen at this location."

Fitzsimmons and other researchers bagged flowers on selected trees to keep unwanted pollen away and introduced pollen from trees known to have a high level of blight resistance. Blight resistance is measured after the fungus that causes the disease is applied to wounds made in the young chestnuts' trunks or branches.

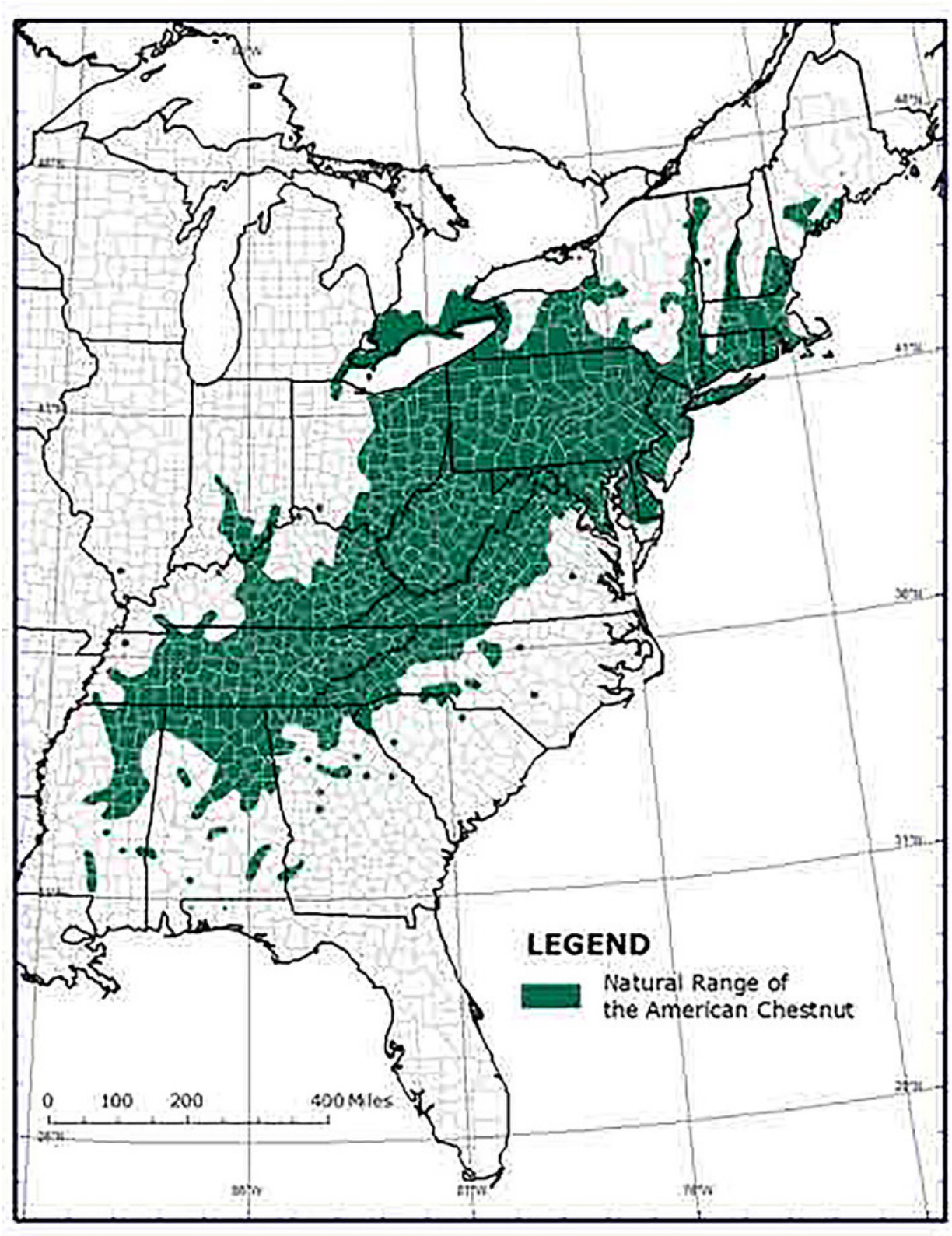
"When we were collecting open-pollination nuts, we were hoping that they wouldn't have this much susceptibility in the progeny, but because there is so much susceptibility in the pollen cloud, we were not able to get rid of it," Fitzsimmons explained. "So this year, we took the best of the best, and we performed controlled pollination. Controlled pollination, however, yields about 50 percent or fewer nuts than open pollination does.

"These control-pollinated nuts will be a true test of levels of resistance possible in this population."

The American Chestnut Foundation started its cross-breeding program in 1989, and Penn State got involved in 1997. The first orchard was planted by Professor Emeritus of Forest Genetics Henry Gerhold on State Game Land 176, not far from the University Park campus, as part of Christmas tree improvement research he was conducting. Kim

Steiner, professor of forest biology and now arboretum director—and also the senior science adviser to The American Chestnut Foundation—then conducted silvicultural trials with chestnuts at the University's Stone Valley Recreation Area, starting in 1997.

The chestnut orchard in The Arboretum at Penn State was started in 2002. In 2004, Professor of Molecular Genetics John Carlson started to examine the underlying molecular components of blight resistance. In a project funded by the National Science Foundation from 2006 to 2009, his laboratory identified several potential blight-resistance genes by painstakingly comparing genes expressed in cankers of susceptible American and resistant Chinese chestnut plants.



The American chestnut's 180-million-acre range once stretched all the way from

Maine to Florida. Credit: Penn State

With funding from The Forest Health Initiative, Carlson since 2009 has led a project to sequence and characterize the entire genome of one of the blight-resistant donor Chinese chestnut trees in the chestnut foundation's breeding program, with an eye toward identifying all of the resistance genes.

Carlson, director of Penn State's Schatz Center for Tree Molecular Genetics, is now collaborating with tree geneticists and researchers at the University of Kentucky, Clemson University, Virginia Tech, the University of Tennessee at Knoxville, the State University of New York at Syracuse and the foundation to unravel the mystery of blight resistance. The group also is testing a genome-sequence-based system to accelerate the selection of blight-resistant plants that now are genetically American, using nuts harvested this fall from the trees that underwent controlled pollination in the Penn State orchard.

Developing blight resistance in American chestnut is complex and challenging, concedes Carlson, who also has applied molecular-genetics techniques to modify poplar trees for bioprocessing and biofuels. "Our aspirations are to move the resistance genes from Chinese chestnuts into American chestnuts and find out which combinations of genes would give the best resistance," he said.

"That has proven to be extremely complicated because more than a few genes are involved, and we haven't yet pinned down which ones are the most important. Genetic engineering groups have been testing about a dozen blight-resistance genes that have been identified. We have to test them in combination because we know that blight resistance is not a single-gene trait, so we have to test multiple combinations of genes,

which is very difficult to do and takes time."

If biotechnology researchers do develop a genetically modified, blight-free American chestnut, current federal regulations would limit distribution of the plants, Fitzsimmons noted. An estimated five years will be required to have the product deregulated by governmental agencies before it is available for widespread distribution and planting.

"We expect to have research plantings of GMO backcross trees within the next two years," she said. "While GMO cannot be allowed to open pollinate under current regulations, GMO American chestnuts appear to offer an excellent chance of creating a blight-resistant American chestnut."

Sara Fitzsimmons, a research technologist in Penn State's college of Agricultural Sciences and director of restoration for The American Chestnut Foundation, shows a canker that formed where a young chestnut tree was inoculated with fungus to see whether it was resistant to the blight. Credit: Penn State

The Chestnut Foundation also is planning to soon deploy a biocontrol, developed by pathologists from West Virginia University and the University of Maryland, to weaken the fungus that causes chestnut blight. The biocontrol involves infecting the fungus that causes chestnut blight with a virus that makes the fungus sick and reduces its virulence.

"We are now focusing on the three Bs in concert to restore the American chestnut—breeding, biocontrols and biotechnology," Fitzsimmons said. "This gives us a bigger suite of tools to fight off this fungus and blight."

But even if all of these initiatives to restore the American chestnut come off without a hitch, it may take a century or more to see chestnuts again across their former range, from Maine to Florida, she concedes. Based on research she is conducting in Maine and Vermont on naturally regenerating sites, it looks like it takes at least 20 years for a plot of chestnuts just to become established beneath a forest canopy.

"Tree breeding, especially hardwoods, takes extraordinary patience because results often aren't seen over a lifetime—we knew that," she said. "To see a naturally regenerating American chestnut population regaining its reproductive niche in the ecological landscape will take a long time—50 years at least after we plant nuts from truly blight-resistant trees."

No matter how long it takes, the chestnut reintroduction effort is monumental, Steiner stressed, because it is likely the most complex and

long-term attempt to rescue a plant species ever pursued. Breeding trees to develop blight resistance is difficult enough, but in this particular case the rescue requires transferring genes from one species to another while still maintaining the genetic diversity of the original species.

"A 'horticultural' solution—where a successful product is commonly a single, clonally propagated genotype—is not sufficient because we are attempting to restore a species to the wild where it must survive, reproduce and eventually evolve on its own," Steiner said. "A remarkable feature of this project is that it is being performed by a small non-profit with the help of thousands of volunteers. Fortunately, the work of the foundation has catalyzed millions of dollars in research by collaborators such as John Carlson, and this has greatly assisted progress."

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