The Tree Urchin

Newsletter of the Maine Chapter of The American Chestnut Foundation

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2023 was a year of change

"Learn to expect the unexpected." As a wildlife biologist, I learned this tenet of ecology early in my career. Nature is full of surprises from the elegant workings of the DNA molecule to the complex cogs and wheels of the clockwork of a forest ecosystem. In 2023, rapid advancements in genetic science provided us with both disappointment and hope for restoring the American chestnut. This issue of The Tree Urchin newsletter explains unanticipated discoveries in the world of chestnut science, how the new science affects chestnut restoration, and provides a peek at the year ahead.

The hybrid chestnut program

What is happening with Maine's hybrid chestnut breeding program? When the program began in the 1980s, scientist believed that 2 or 3 genes in the Chinese chestnut controlled blight resistance. The Maine chapter planted over 35,000 hybrid seedlings in six seed orchards; 5th generation of B3F2 trees. Many volunteers, perhaps including you, spent days on hands and knees carefully planting, nurturing, inoculating, or culling these young chestnut trees. After a rigorous selection process, we expected a few of the offspring of these trees (B3F3) to be blight resistant and suitable for restoration. However, a recent genetic analysis showed these trees were likely to have insufficient resistant to the blight. Why?

Scientists from several institutions are unraveling the mysteries of the genetic code of the American and Chinese chestnut. They recently revealed some unexpected findings. There are over 200 genes responsible for blight resistance in the Chinese chestnut (not 2 or 3)! These critical genes are scattered across all the chromosomes of the chestnut. Some genes have more influence than others. Some genes work in concert to battle the blight, and others act independently. Different groups of genes "turn on" hours after the blight attacks a chestnut. Some genes fight the blight days or even months after infection.

Tom Fox, Mark McCollough, and Al Faust pose with the forestry mulcher.

Given this reality, the odds are slim that random pollination in our seed orchards, inoculation, and selection will yield a blight-resistant restoration chestnut. This year the Maine Chapter rapidly adapted to these new findings. Following the advice of chestnut scientists from TACF, this summer we thinned about 40-60% of the trees in our seed orchards. We hired Fox Forestry from Orland to help us remove thousands of young trees that are redundant or show little to no blight resistance. In keeping with our annual, long-practiced procedure, several thousand trees in our orchards were intentionally inoculated with the blight fungus in June. Those that show any sign of resisting infection are

retained, while underperformers are culled. As a result of this selective, our collection of 35,000 trees has been whittled to just a few thousand. In the same plots where volunteers once planted 150 trees a decade ago, just 5 to 15 remain. TACF's Meadowview research center in Virginia and other TACF chapters are doing the same. We will continue to genetically test and evaluate the remaining trees, winnowing further selections to find that "needle in a haystack" that demonstrates a hybrid chestnut can tolerate blight.

Maine TACF volunteers bagging chestnut flowers at the Bok breeding orchard in Hope, Maine. This tree may not look pretty, but it has a better than average combination of blight-resistant genes.

The hybrid program still has much value. Field trials by the U.S. Forest Service from GA to WV shows that hybrid chestnuts from the TACF breeding program have twice the survival of American chestnuts in the forest. These trees are producing nuts in the wild, which American chestnuts in the south rarely do. A few hybrid trees in our seed orchards have successfully walled off blight cankers from our inoculations. This gives us reason for hope. Furthermore, the chestnuts remaining in our seed orchards are a goldmine of chestnut genetics that will be of great utility in the future. The original hybrid pollen brought from Meadowview about 15 years ago was used to pollinate many of Maine's last pure American chestnut trees. Many of those wild trees have since died from the blight, but their unique genes live on in our orchards in the form of B3F2 hybrids. This summer we hope you can join us for orchard tours to show what we have accomplished and how the remaining trees, freed from competition, are flourishing and are beginning to produce chestnuts.

"Best by Best" Cross-Pollination: Applying the best genetic science

We are rapidly adapting to the new genetic discoveries. TACF scientists have been genetically testing and evaluating chestnut trees in breeding orchards from ME to GA. Several hundred of our "best" trees that have more of the blight-tolerant genes have been located. A new "best x best" breeding program was launched to hand-pollinate these trees with each other to yield trees with an improved collection of blight-tolerant genes. Modeling shows that "best x best" pollination and rigorous selection of the offspring could result in trees with substantially improved blight tolerance within a generation or two. Many of the trees in Maine's 10 breeding orchards are worse for the wear having been twice inoculated with blight fungus. Despite their ragtag appearance, recent genetic tests show about a dozen trees have a higher level of blight resistance. This summer Maine chapter breeding program coordinator, Eric Evans, led volunteers to bag the flowers at these "best" trees in the Bok orchard in Hope and hand-pollinated them with pollen from our "best" trees from the nearby Rowe orchard. Eric harvested over 400 seeds this fall from these crosses. The seeds will be used to establish a new best x best orchard in New Hampshire. Pollen from Maine's best trees was sent to the TACF research station in Meadowview in Virginia to pollinate other "best" trees in other states.

TACF withdraws support for the Darling 58 transgenic chestnut

About a decade ago, Dr. William Powell's research team at the State University of New York - Environmental Science and Forestry (SUNY-ESF) in Syracuse, NY developed a transgenic American chestnut by inserting the oxalic oxidase gene (OxO) from the wheat plant into the DNA of an American chestnut. The hypothesis was that the OxO gene would produce an enzyme that would neutralize the lethal oxalic acid produced by the blight fungus. SUNY-ESF submitted a proposal to the U.S. Department of Agriculture and other federal agencies to allow the use of the Darling 58 tree as a restoration tree. Following TACF's lead, the Maine Chapter Board of Directors voted to support the transgenic

approach to chestnut restoration and wrote letters of support to USDA last December.

Dr. Tom Klak, at the University of New England in Biddeford, ME worked closely with SUNY-ESF to “speed-breed” the Darling 58 and a variant called “DarWin” under high light conditions in the laboratory. Dr. Klak produced pollen on trees that were just nine months old. He produced female flowers in seedlings just over a year old. Under special permit from the USDA, transgenic pollen from “speed bred” seedlings was used to pollinate a few wild chestnuts in Cape Elizabeth and hybrids in our Phippsburg seed orchard. Seeds were collected to grow seedlings for field trials in Maine of the new transgenic chestnuts. Similar field trials of transgenic chestnuts were initiated at Meadowview and Purdue Universities to confirm the blight resistance of these trees and to assess whether the OxO gene had any detrimental effects.

At the Searsport seed orchard board members Al Faust, Eric Evans, and Bob Dueitt review thinning recommendations from national TACF scientist Kendra Collins.

Data emerged from field trials that the trees were exhibiting variable response to chestnut blight and may grow more slowly because of the metabolic demands of the OxO gene always being activated. This means that it is not likely to be a good candidate as a restoration tree. Furthermore, research in Maine by Tom Klak at UNE and Han Tan at UMaine discovered that the OxO gene on the alleged D58 test trees was not on the correct chromosome and was in an inferior "D54" configuration. Errors were made at SUNY-ESF as far back as 2016, and all subsequent graduate student projects and tests of the alleged D58 tree mistakenly used D54 trees. In early December, The American Chestnut Foundation made the difficult decision to discontinue support for the transgenic Darling 58 American chestnut. More information on the decision to withdraw support for the Darling 58 transgenic chestnut can be found at the TACF website at https://tacf.org/darling-58/

Peering into the crystal ball

What does the future hold for chestnut restoration? As someone who has worked on species restorations my entire career, setbacks are not unusual. Scientists are human and sometimes we make mistakes. We learn, document, challenge hypotheses, experiment, adapt, and move forward. Restoring a species is difficult work. TACF continues to support the transgenic approach to developing a blight resistant chestnut. Research is ongoing at SUNY-ESF, University of Georgia, Virginia Polytech and several other institutions to employ state-of-the art genetic science to evaluate other options using the OxO gene. Researchers are also evaluating the possibility of inserting the genes responsible for blight resistance from other chestnut species. “Stacked resistance” options are being evaluated by crossing transgenic trees with our best hybrids. Genetic science is accelerating at a lightning pace, and even more alternatives to develop transgenic tolerance to chestnut blight will be developed and evaluated as we move forward.

The Maine chapter is well-positioned to adapt to these findings. Tom and Han will continue to evaluate options for transgenic trees and make valuable research contributions in Maine. Our 20 breeding, seed, and germplasm conservation orchards now take on added importance as reservoirs of Maine's chestnut diversity. We will continue our hybrid program and selection of trees with blight tolerant qualities in our extensive orchard collection. We plan additional “best x best” crosses in 2024 informed by TACF’s genetic evaluation. We made several important discoveries of wild chestnut trees this year and have harvested nuts for our germplasm conservation orchards and seedling sales.

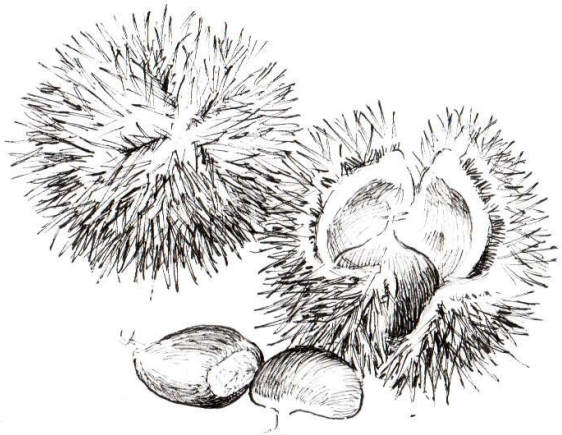
When will a blight resistant chestnut be available? Our goal is to develop a tree of American character that has blight resistance at least as good, and preferably better, than 50/50 Chinese-American hybrids. TACF is engaged in a multi-faceted, science-driven effort to meet this high standard. Anticipating a day when a restoration chestnut is a reality, the Maine Chapter will develop partnerships with forest industry, land trusts, state and federal agencies and conservation partners in restoration trials. We are busy growing the relationships that will support restoration well into the future.

With science advancing at such a fast pace, tea leaves, crystal balls, soothsayers, and druids may guess where chestnut restoration will be a decade or two hence. What we know for certain is that the outcome and timeline of any scientific process is unpredictable. We’ve certainly learned to “expect the unexpected.” TACF is committed to a science-based approach, and does not support the restoration of inferior chestnut trees. We are confident that the latest science and discoveries yet to come will produce an American chestnut tree that we can use to restore Maine’s forests.

Maine chapter breeding orchard coordinator, Eric Evans, marks trees for removal at the Huff Hill seed orchard in Hartland. Thinning the plots will release these trees and promote flowering for future best X best crosses of hybrid trees.

An invitation to active optimism

Recent scientific revelations do not deter our optimism to restore the American chestnut; it may take just a little longer than we hoped. Just a blink of the eye in tree years. A few more growth rings on a tree. We invite you to be involved with this next phase of chestnut restoration. When you volunteer to help chestnuts, you’ll discover how a tree better connects us with one another. It’s as if the chestnut knows it will need a vibrant community to restore its place in the forest. For over 40 years the chestnut has been sowing seeds to create a connected, caring community. We call it TACF but I imagine the chestnut calls it hope.



Mark McCollough, President of the Maine Chapter TACF