Land Manager's Corner

New Hope for Restoring an Old Forest Giant

While very few of us have ever gotten to see the chestnut-filled forests that were the norm a century ago, we've certainly heard plenty about them—and for good reason. The American chestnut tree played a huge role in the economy and the ecosystem. As a prevalent canopy tree, with a tall straight trunk of rot-resistant wood, it was used for everything from log cabins and furniture to telephone poles and railroad ties. Its ability to quickly re-grow from cut stumps further increased its value and it is thought that it may have been the most commonly cut tree species in American in the early 1900's. Ecologically speaking, the American chestnut provided a large and dependable food source for many species of wildlife. Unlike oaks, chestnuts produced mast every year and the fact that they didn't flower until June meant that their buds were not in danger of being impacted by a late-season frost which results in diminished fruit production in some other native species that flower earlier in the year (***https://www.americanforests.org/magazine/article/revival-of-the-american-chestnut/***). Of course humans ate the plentiful nut as well and it was an important source of income for farmers in the region who could collect and sell them or use them to fatten their hogs (Popkin, 2020).

Before the introduction of C*ryphonectria parasitica*, the fungus that causes chestnut blight, it was estimated that there were 4 billion mature chestnut trees in the forests of the eastern U.S. (Detwiler, 1915). After surviving for 40 million years, the entire species was functionally extinct within just 40 years of the disease being noticed in the U.S. in 1904, though research suggests the fungus may have been brought over on Japanese chestnut trees as early as 1876 (Anagnostakis, 1987; Anagnostakis and Hillman, 1992). Knowing the important role the American chestnut played in the economy and the environment, it's no wonder there is so much interest in efforts to restore this impressive forest giant. Work to create blight resistant American chestnuts have been underway for decades, but could restoring the chestnut-dominated forests of our ancestors be a real possibility in the not-so-distant future?

Today, there are two main methods that are being used to develop potentially blight-resistant trees. The American Chestnut Foundation's (TACF) breeding program is probably the most well-known. For 30 years, TACF has selectively bred American chestnuts with Chinese chestnuts to generate a hybrid tree species that retains the growth form and ecological function of the American chestnut but contains the blight resistance of the Chinese chestnut. The goal is to dilute the gene pool so that ultimately, trees contain as much of the American chestnut genome as possible while still exhibiting blight resistance. TACF increases resistance with each generation by breeding trees with the most resistance and then identifying the most blight resistant progeny. According the TACF's website, they have completed three generations and

Map of the historic American chestnut range. Photo from The American Chestnut Foundation, Carolinas chapter website: ***https://www.acf.org/nc-sc/photos/american-chestnut-blight/***

"selected hybrids have inherited between 60% and 90% of their genome from American chestnut and exhibit blight resistance on a spectrum that is intermediate between American chestnut and Chinese chestnut" (***https://www.acf.org/science-strategies/tree-breeding/***).

A perhaps lesser-known effort that has been underway for almost the same amount of time is the transgenic work being completed by two tree geneticists at the State University of New York's College of Environmental Science and Forestry. Bill Powell and Chuck Maynard have been working on a separate but parallel effort to genetically engineer an American chestnut tree that is resistant to the chestnut blight. While equally challenging and time consuming, genetic engineering allows for more control over selecting for blight resistance rather than relying on the random mixing of genes that occurs during tradition breeding programs like the one being undertaken at TACF. One of the first thoughts was to simply take the gene that expressed resistance to the blight in Chinese chestnut trees and insert this gene into the American chestnut genome. Of course, the answer wasn't that simple as they found that at least 6 different genes were involved in creating blight resistance in Chinese chestnuts (Popkin, 2020).

According to a recent New York Times article, Powell also spent a few years researching an antimicrobial compound based on a frog gene, but ultimately decided to abandon that path because he feared a negative response from the public over a tree that had been altered to include animal genes. Finally, Powell learned of a gene in wheat that produces the enzyme oxalate oxidase (OxO), an enzyme that would prove very useful in allowing chestnut trees to survive after being infected by chestnut blight (Popkin, 2020).

The *Cryphonectria parasitica* fungus enters a tree through wounds in the tree's outer bark. Once the fungus becomes established in the tree, it generates oxalic acid which results in an acidic environment that weakens plant cell walls by decreasing lignin content and increasing cellulose content within the cells and makes them more vulnerable to being infected and killed by other enzymes associated with the blight fungus. As the fungal infection progresses, living cells in the cambium are killed, eventually girdling the tree and preventing the flow of water and nutrients which ultimately results in the death of the above-ground portion of the tree (Anagnostakis, 2000; Dutton and Evans 1996; Welch et al., 2007). The OxO enzyme catalyzes the degradation of the oxalic acid that is caused by the chestnut blight infection and breaks it down into carbon dioxide and hydrogen peroxide, allowing the tree cells to survive despite a fungal infection and enabling the tree to show resistance to the disease (Welch et al., 2007).

Image of Chestnut blight on an American chestnut tree. Image from the National Park Service: ***https://www.nps.gov/articles/american-chestnuts-in-the-capital-region.htm***

The level of resistance shown by American chestnut trees with the wheat gene that produces the OxO gene has made these transgenic trees the most promising hope for the possibility of a blight resistant American chestnut tree. However, it may be a few years still until there is a chance of them being planted in forests that they once dominated. Because the OxO gene was transported into the American chestnut genome using an Agrobacterium, the new transgenic tree is regulated by the USDA. Additionally, the EPA interprets the enzyme to be acting as a pesticide because it is impacting the spread of a fungal disease, so it is also under their regulatory review. And in case review by two federal agencies wasn't enough, it is also be voluntarily submitted for review by the FDA since its nuts will likely be consumed by humans (National Academies of Sciences, Engineering, and Medicine, 2019; Popkin, 2020). Navigating the regulatory pathway for 3 federal agencies will be yet another hurdle in the long and challenging process of trying to restore the American chestnut tree.

The trees resulting from the traditional breeding efforts undertaken by TACF will not have to go through review by any of these federal agencies (National Academies of Sciences, Engineering, and Medicine, 2019). If the transgenic trees from the SUNY are approved for use by the USDA, EPA, and FDA, they will be integrated into TACF's breeding program to combine the resistance mechanisms achieved by both programs and to increase the native gene pool of chestnut trees that carry the wheat gene and will ultimately be planted in the wild.

The fungus that causes chestnut blight affects the above ground portion of the tree but cannot survive in the soil and therefore does not affect the health of the roots themselves. This enables infected chestnut trees to re-sprout after the above ground portion of the tree is killed by the blight. The C*ryphonectria parasitica* fungus cannot survive in the soil because microorganisms found in the soil compete with the fungus. Unfortunately, one of these microorganisms that can be found in the soil is *Phytophthora cinnamomi*, which causes root rot (also known as ink rot disease) in chestnut trees in warmer climates. *P. cinnamomi* historically impacted chestnut trees in the southern portion of the U.S., but as the climate changes and temperatures rise, the areas where the pathogen can survive are expanding northward and are expected to reach New England by 2080. So, while *C. parasitica* kills the aboveground portion of the tree, *P. cinnamomi* kills the below ground portion of the tree. To address this, TACF has incorporated breeding to select for *P. cinnamomi* resistance into its breeding program and aims to breed trees that show resistance to the root rot pathogen with transgenic or blight-resistant hybrids to create trees that exhibit resistance to both diseases (***https://www.acf.org/science-strategies/tree-breeding/***).

While the soil may host the root rot pathogen, it can successfully fight off the fungus that causes chestnut blight. Due to this, a technique called mudpacking was developed by TACF's pathologist Dr. Fred Hebard and has been used to increase the lifespan of chestnut trees that have been infected by the chestnut blight. Mudpacking involves gathering soil from within 10 feet of the tree and adding enough water to turn it into a sticky mud. The mud is then applied around the entire stem or trunk wherever a chestnut canker is present. The mud should extend at least one foot beyond the canker in both directions to ensure the canker can't spread beyond the mud before the soil microorganisms have a chance to fight the fungus. The entire area should then be wrapped with shrink wrap to keep the soil moist and hold the mixture on the tree. The wrapped area should be checked monthly to ensure the canker has not spread beyond the wrapped area and to ensure the mud is still moist (***https://www.acf.org/ma-ri/the-project/mudpacking-cankers/***).

While this will not cure the chestnut tree, it will allow the tree to fight the blight at the location of each canker and reduce the chances of the blight girdling the tree. In order for this method to keep the tree alive, it is important to treat each canker (***https://www.acf.org/ma-ri/the-project/mudpacking-cankers/***). This is hard to do on large trees where some cankers may be inaccessible; however, we are going to attempt to use mudpacking on cankers on the few remaining chestnut trees on ACLT property when possible to prolong the life of these few specimen trees that have far outlasted all of their relatives.

It seems that a blight-resistant American chestnut may finally be within reach, but this brings about the question of whether this new American chestnut will regain its role as a dominant canopy species. In its absence, this niche has been filled by oaks, hickories, and maples throughout much of the Appalachian region and also by tulip poplars and beeches in the Southern Maryland region. Oaks can also harbor the chestnut blight fungus and while it has much less of a detrimental effect on them as a whole, oaks have helped sustain the fungus while the American chestnut has largely been absent from our forests. With the persistent *C. parasitica* still present in the ecosystem, a resistant American chestnut is the only way to bring back this forest giant. Now, there is more hope than ever, that someday soon we will see transgenic and hybrid American chestnut trees that are able to fight off the fungus and survive in the complex forest ecosystems that they once dominated.

Namesake American Chestnut tree with the main trunk lying on ground on the right and smaller trunk still standing.

Autumn Phillips-Lewis
Land Manager

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