

Washington College
Department of Environmental Science and Studies

American Chestnut Restoration: Strategies, Challenges, and Future

Sharon Niedringhaus

A senior thesis submitted to the
Department of Environmental Science and Studies
in partial fulfillment of the requirements for the degree of
Bachelor of Science at Washington College

April 14, 2023

Washington College Honor Code:

I pledge my word of honor that I have abided by the Washington College Honor Code while
completing this assignment.

Thesis Advisor: Dr. Rebecca Fox

Abstract

The American chestnut (*Castanea dentata*) was once widespread throughout forest ecosystems in the Eastern U.S. as a dominant canopy tree. As a foundation tree species, it was an important producer of food for other forest species and was important to humans for lumber and chestnuts. The chestnut blight (*Cryphonectria parasitica*) was introduced to the U.S. in 1904 by imported Asian chestnuts and destroyed American chestnut populations. American chestnuts became functionally extinct due to mature trees being killed by the blight and any new sprouts being infected and killed by the blight before they reached maturity to produce nuts and reproduce. Research focused on restoration of the American chestnut has been ongoing for years, but it is important to determine whether this restoration is feasible and necessary. The three main methods for restoration are: backcross breeding, bioengineering, and biocontrol. Backcross breeding confers resistance to chestnut trees by crossing American chestnuts that are not resistant to the blight with Asian species that are resistant. Bioengineering involves inserting a gene from wheat into the American chestnut's genome that will decrease the effects of the blight on them. Biocontrol involves injecting cankers caused by the blight on infected trees with a virus that will decrease the impact of the blight on the trees. In addition to threats by the blight, climate change and another disease called Phytophthora root rot could impact American chestnut populations and should be considered. Based on the current body of research, American chestnut restoration is feasible and necessary and could set a precedent for restoration of other threatened tree species in the future.

I. Introduction

The American chestnut (*Castanea dentata*) was a dominant canopy tree (Paillet, 2002; Jacobs et al., 2013; Stephenson, 1986) in forests ranging throughout the Eastern U.S. (Figure 1; Paillet, 2002; Dalglish et al., 2016; Jacobs et al., 2013). It is estimated that there were 4.2 billion chestnut trees before the introduction of the chestnut blight (*Cryphonectria parasitica*) (Gravatt, 1949). These trees had an important role in forest ecosystems as a foundation tree species (Ellison et al., 2005) with one of their major roles being a food source for mammals (Dalglish & Swihart, 2012) and macroinvertebrate species in these ecosystems (Smock & Macgregor; 1988). Diamond et al. (2000) found that pre-blight, American chestnuts made up 58% of the hard-mast-producing basal area in the Coweeta Basin in North Carolina. Hard mast production is the production of hard fruits or nuts by trees including acorns, chestnuts, and beechnuts (Bowen et al., 2019). Additionally, the American chestnut's mast production was very stable, producing similarly large masts every year, unlike other mast-producing species that have smaller, less stable mast production (Diamond et al., 2000).

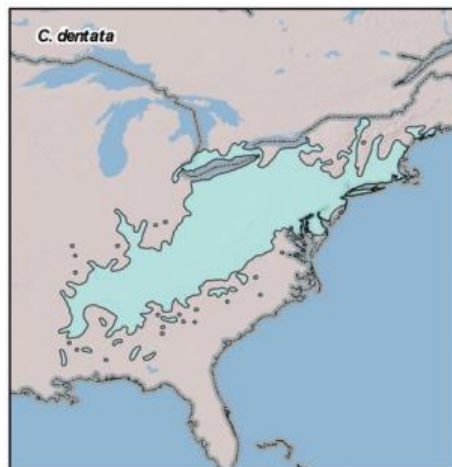


Figure 1: Natural range of the American chestnut (*C. dentata*) (Jacobs et al., 2013).

In addition to its ecological role, the American chestnut provided many ecosystem services. Chestnuts were eaten and sold, so much so that the gathering of chestnuts became a large part of the culture in the Eastern U.S. Many people relied on chestnut harvests for income (Youngs, 2000). It was also an important tree in the timber industry, demonstrated by the fact that much of the early literature on American chestnuts focused on describing how to keep American chestnut stands for timber (Paillet, 2002). American chestnut wood was desirable due to it being rot resistant, making it ideal for various uses including as railroad ties, posts, and poles (Smith, 2000).

Unfortunately, the American chestnut's population significantly declined due to the introduction of the chestnut blight (Paillet, 2002; Dalglish et al., 2016). The chestnut blight is a fungal pathogen that impacts chestnut trees (Rigling & Prospero, 2018) first introduced to New York City in 1904 (Murrill, 1906; Rigling & Prospero, 2018) by imported chestnuts from Asia that were infected with the blight (Anderson & Rankin, 1914; Rigling & Prospero, 2018). The blight enters chestnuts through wounds that cause breaks in the bark on the trees (Murrill, 1906; Rigling & Prospero, 2018). Once infected, reddish-orange cankers will appear and release spores, allowing the infection to spread (Figure 2; Murrill, 1906; Rigling & Prospero, 2018). Inside the tree, the fungus grows and ultimately girdles the tree, preventing the transport of nutrients and water, killing the tree (Murrill, 1906; Rigling & Prospero, 2018).

The chestnut blight had major impacts on American chestnuts and forest ecosystems as a whole as it spread throughout the chestnut's range. American chestnuts were no longer able to grow into tall canopy trees and were relegated to sprouting from the root collars of dead or living chestnut trees (Paillet, 2002; Dalglish et al., 2016). These sprouts continue to undergo a constant cycle of sprouting, blight infection, death and resprouting, causing them to take on the

growth form of an understory shrub (Paillet, 2002). It is rare for these sprouts to survive long enough to produce seeds, limiting the reproduction of the species (Paillet, 2002).



Figure 2: A) A canker with the red-orange chestnut blight fungus visible. B) A canker on a mature tree with the blight fungus less visible under the bark of the tree. (TACF, 2016)

The current American chestnut population is 10% of the historic population, although its range has expanded due to human planting (Dalglish et al., 2016). The loss of the American chestnut as a canopy species allowed for the expansion of other forest tree species such as oaks (Dalglish & Swihart, 2012; Elliott & Swank, 2007), eastern hemlocks (Ellison et al., 2005), and red maples (Elliott & Swank, 2007). However, these species are unable to completely fill the role of the American chestnut. Without the American chestnut, mast has decreased, and variability of masting has increased (Dalglish & Swihart, 2012; Diamond et al., 2000). Diamond et al. (2000) found that in some years, mast production post-blight was 5-27% of what it was pre-blight. This drastic decrease in mast has caused decreases in some mammal populations that depended on the American chestnut as a food source (Dalglish & Swihart, 2012). Additionally, Smock & Macgregor (1988) found that the leaves of tree species that have replaced the American chestnut do not have the same nutritional value as chestnut leaves for macroinvertebrates. The American chestnut is also unable to provide the ecosystem services it once did since its understory shrub

growth form and inability to produce seeds due to the blight (Paillet, 2002) prevent it from being useful for lumber or consumption.

Scientists and conservation organizations have been working on ways to restore the American chestnut to its former range. The American Chestnut Foundation has been trying to make blight resistant trees through backcross breeding since the 1980s based on previous crossbreeding efforts made in programs by the USDA, Brooklyn Botanical Garden, and Connecticut Agricultural Experiment Station (Burnham, 1987a, b). More recently, genome editing has shown promise as a way to make blight resistant trees and the technique developed at the College of Environmental Science and Forestry at State University of New York (SUNY-ESF) is currently under review to determine if the genetically modified chestnuts are safe for consumption and to be put in the wild as part of restoration efforts (Powell et al., 2019). Another possible restoration technique is biocontrol which entails injecting a mycovirus into infected trees in order to weaken the blight (Rigling & Prospero, 2018; Powell et al., 2019). Each of these methods has strengths and weaknesses, but they all have the potential to allow for the reintroduction of the American chestnut. However, additional threats such as climate change (Noah et al., 2021; Barnes & Delborne, 2019) and another introduced pathogen known as root rot (*Phytophthora cinnamomi*) (Gustafson et al., 2022) will further complicate reintroduction efforts.

When considering the reintroduction of a species, it is important to consider whether the restoration of the species is feasible and necessary. A significant amount of money and effort goes into the research and development of the techniques that may work to reintroduce the species, so one should carefully consider whether these efforts will succeed once they are implemented in the environment. The necessity of restoring the American chestnut is also important to consider. Will forest ecosystems benefit from its reintroduction or has another

species replaced it in its niche? Thirty percent of tree species globally are currently threatened with extinction while 11% percent of tree species in the U.S. are threatened with extinction according to a report by Botanic Gardens Conservation International (BGCI, 2021). Are there tree species that have not declined as much as the American chestnut that would benefit more from a focus of restoration efforts? Based on the current body of research, American chestnut restoration is feasible with a combination of restoration techniques and careful attention to shifts in climate and pathogens. Additionally, American chestnut restoration is necessary due to the tree's niche remaining partially open and its ability to provide a precedent for the restoration of tree species in the future.

II. Strategies for American Chestnut Restoration

Ila. Backcross Breeding

Crossbreeding between the American chestnut and non-native species began as part of a USDA program that took place between 1894 and 1911 (Van Fleet, 1914). This program consisted of crossing European chestnuts (*Castanea sativa*), Asian chestnuts, and chinquapins (*Castanea pumila*) with American chestnuts in order to try to maximize chestnut size and production (Van Fleet, 1914). However, once the blight was introduced, the American chestnut hybrids were negatively impacted by the blight, although hybrids with Asian chestnut species showed greater resistance than the other hybrids (Van Fleet, 1914). The USDA continued American chestnut breeding in 1922 using resistant trees from the previous program and other breeding programs were started at the Brooklyn Botanical Garden and Connecticut Agricultural Experiment Station in 1929 and 1936, respectively (Burnham, 1987a). A backcrossed tree, dubbed the "Clapper" hybrid after one of the scientists who worked on creating it, was made in 1946 before the USDA breeding program ended in 1960 (Burnham, 1987a). In 1986, the

American Chestnut Foundation made a plan to pursue backcross breeding using the “Clapper” hybrid trees as well as the “Graves” trees, another American chestnut hybrid (Burnham et al., 1986; Powell et al., 2019; Steiner et al., 2017).

Backcross breeding consists of one species being crossed with another species and then being repeatedly crossed with one of the two species (the recurrent parent) in future generations (Figure 3; Robbins, 2019). This allows the backcrossed hybrid to have a desired trait from one species while still sharing a majority of its genome with the other (Robbins, 2019). In the context of American chestnut restoration, an American chestnut is crossed with a Chinese (*Castanea mollissima*) or Japanese chestnut (*Castanea crenata*), as both species have blight resistance (Burnham, 1987a). The resulting resistant hybrid is then crossed again with an American chestnut (Burnham, 1987a). This crossing followed by subsequent backcrosses provides the resulting crossed tree with chestnut blight resistance while maintaining other morphological characteristics of the American chestnut (Burnham, 1987a,b; Diskin et al., 2006). The goal of the backcross breeding program for resistance was to reach three generations of backcross-bred chestnuts, as it was predicted that three generations of backcross breeding would result in resistant trees that would otherwise have the same characteristics as the American chestnut (Burnham, 1987a). These predictions have been supported by Diskin et al (2006). This study found that 8 out of the 14 morphological characteristics measured (e.g., leaf length, tooth length and depth, twig diameter, etc.) were not significantly different from those of American chestnuts in the third-generation backcrossed trees (Diskin et al., 2006). Out of the six characteristics that were significantly different, only one, bud length, was statistically similar to the Chinese chestnut (Diskin et al., 2006). This demonstrated that while not exactly the same as an uncrossed

American chestnut, the third-generation backcross-bred chestnuts were morphologically closer to the American chestnut than to the Chinese chestnut (Diskin et al., 2006).

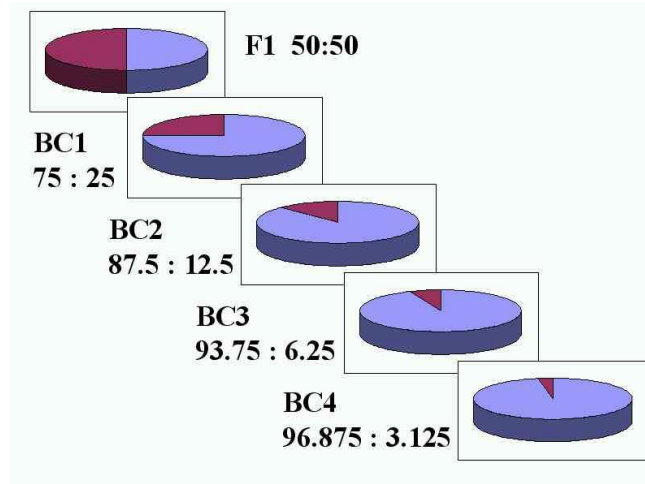


Figure 3: A visual representation of backcross breeding with F1 being the initial cross and each BC being a successive backcross. With each backcross, the percentage of the genome from the recurrent parent (light purple) increases. (Robbins, 2019)

Backcross breeding does provide backcrossed trees with resistance to the blight; however, the degree of resistance is between that of the highly impacted American chestnut and the more resistant Chinese chestnut (Steiner et al., 2017). The amount of cankering caused by the blight experienced by third-generation backcrossed trees was closer to the cankering experienced by uncrossed American chestnut trees than to that of uncrossed Chinese chestnut trees (Steiner et al., 2017). This is likely due to the fact that the blight resistance genes are controlled by three or more regions of DNA (loci) within the American chestnut genome (Powell et al., 2019; Steiner et al., 2017; Kubisiak et al., 1997), rather than the two genes that were predicted to control resistance in the original backcross breeding plan (Burnham et al., 1986). A greater number of genes controlling resistance makes it more likely for resistance to be lost between backcrossed generations (Steiner et al., 2017). However, continued research into the resistance loci in chestnut trees and alternative sources of resistance to the Chinese chestnut may help improve the

resistance provided through backcross breeding (Powell et al., 2019; Steiner et al., 2017).

Overall, while backcross breeding has produced chestnuts that are more resistant to the blight than uncrossed American chestnuts, the genetics controlling resistance is complicated, and research is continuing to improve resistance in backcrossed chestnuts (Powell et al., 2019; Diskin et al., 2006; Steiner et al., 2017).

Iib. Genome Editing

In addition to backcross breeding, work is currently being done to produce a genetically modified American chestnut with blight resistance using the oxalate oxidase (OxO) gene from wheat (Powell et al., 2019; Steiner et al., 2017). The pursuit of finding genes that would be able to confer resistance to the blight through genomic editing began in 1989 at SUNY-ESF (Powell et al., 2019). Due to the complexity of the resistance genes in resistant chestnut species, the researchers decided to look for a gene that would provide resistance from other sources based on how the blight causes disease chemically (Powell et al., 2019). The chestnut blight produces oxalate, which is the chemical that causes harm to infected trees (Powell et al., 2019; Jacobs et al., 2013; Merkle et al., 2006). This led to the selection of the oxalate oxidase (OxO) gene from wheat, which is named for the enzyme it produces that breaks down oxalate, reducing the impact of the pathogen on infected trees (Powell et al., 2019; Jacobs et al., 2013; Merkle et al., 2006).

Compared to backcross breeding, genome editing is better at providing American chestnut trees with blight resistance. Transgenic American chestnuts, or trees that have been genetically modified with the OxO gene, have demonstrated blight resistance in greenhouse (Zhang et al., 2013) and field (Newhouse et al., 2014) experiments. Zhang et al (2013) found that past a certain threshold of OxO gene expression, the lesions formed on transgenic trees were similar to those of the resistant Chinese chestnut, suggesting increased resistance to the blight

compared to wild-type, or non-genetically modified, American chestnuts. Newhouse et al. (2014) found that transgenic American chestnut trees in the “Darling 4” line had a blight resistance between that of the non-transgenic American and Chinese chestnuts (Figure 4). Furthermore, blight resistance through genome editing is passed on as a dominant trait, which means that resistance is less likely to be lost from the population through breeding in the wild than in backcross bred trees, where blight resistance is controlled by multiple loci rather than a single gene (Powell et al., 2019). Additionally, genome editing transfers the wanted resistance genes without unwanted genes that could impact the morphology of the trees because the editing process is able to transfer just the specific gene of interest, unlike backcross breeding which crosses the entire genomes of two species (Powell et al., 2019; Merkle et al., 2006; Diskin et al., 2006).

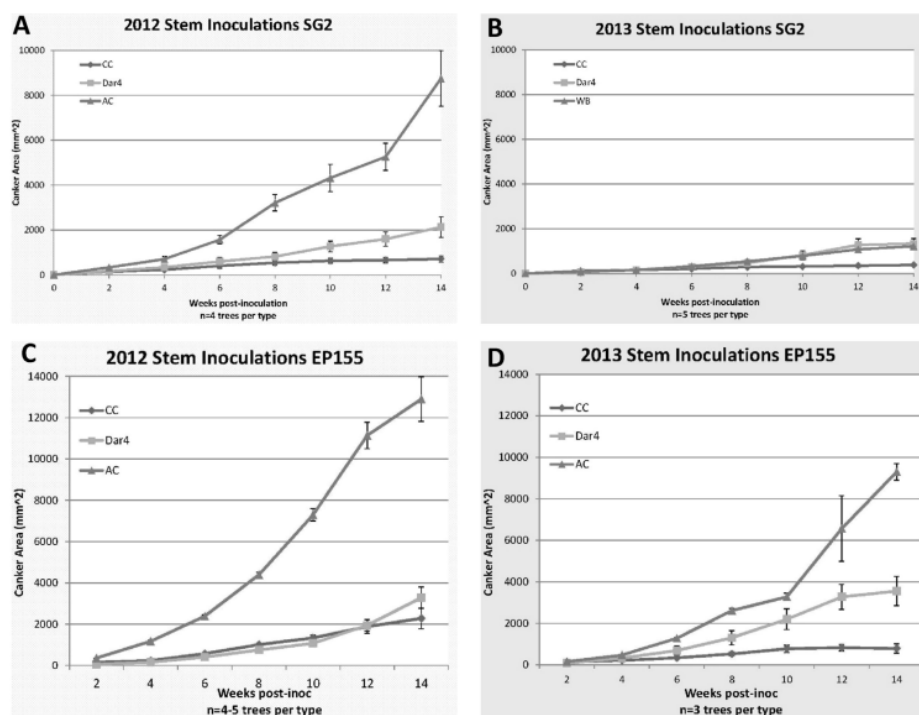


Figure 4: A comparison of canker size over time between American (AC), Chinese (CC), and transgenic (Dar4) chestnut trees in a field study as a measure of resistance to two strains of different virulence levels (SG2 – moderate (A, B); EP155 – high (C, D)) of the chestnut blight fungus in 2012 (A, C) and 2013 (B, D). The transgenic chestnuts had canker sizes between those of the

American and Chinese chestnuts except for in 2013 with the SG2 strain due to unusually high rainfall that year. (Newhouse et al., 2014)

One of the biggest concerns for the implementation of the transgenic American chestnut is the public's fear of genetically modified organisms (GMOs) (Merkle et al., 2006). Common fears surrounding GMO trees and crops in general include the fear that the altered genes would be introduced to wild populations, that these genes could have negative effects when consumed or used in products by humans, (Merkle et al., 2006), and of the possible negative impacts modified trees could have on the surrounding ecosystems (Powell et al., 2019). There is not an argument to assuage possible fears of the modified genome being introduced to the wild populations of American chestnuts because that is the goal of producing a genetically modified chestnut: to produce a tree that is resistant to the blight so it can then be introduced to the wild and be self-sustaining (Merkle et al., 2006; Powell et al., 2019). However, the OxO gene can be found in a variety of crops (e.g. peanuts (Wang et al., 2010), strawberries (Dahiya et al., 2010), and rice (Carrillo et al., 2009)), and in wild species of plants and microbes, so it is unlikely that the transgenic trees would negatively impact human or ecosystem health (Powell et al., 2019).

Additionally, various studies have been conducted looking at the effects of the transgenic tree on other species in forest ecosystems. Newhouse et al. (2021) found that the OxO gene had no significant impact on the physiology and behavior of bumble bees (*Bombus impatiens*) after exposing bee colonies to pollen containing OxO enzyme concentrations likely to be present in transgenic American chestnut pollen. Likewise, another study found that transgenic chestnut trees did not significantly impact mycorrhizal colonization or the germination of seedlings around the transgenic trees (Newhouse et al., 2018). Furthermore, Goldspiel et al. (2019) raised wood frogs (*Lithobates sylvaticus*) on transgenic American chestnut leaf litter and leaf litter from other trees including non-transgenic American chestnuts, hybrid chestnuts, Chinese chestnuts,

American beeches, and sugar maples to determine whether transgenic American chestnut leaves had any negative effects on the growth and survival of larval wood frogs compared to non-transgenic leaves. Larval survival, growth, and development were not impacted by the transgenic leaf litter, although there was some delay in metamorphosis which could have negative effects in drought conditions (Goldspiel et al., 2019). Since drought conditions are expected to increase with climate change (Arias et al., 2021), this could become an issue in the future. Overall, these studies suggest that transgenic trees would likely not have significant negative impacts on organisms in the surrounding ecosystem.

The researchers at SUNY-ESF have submitted the transgenic American chestnut tree to the USDA, FDA, and EPA for approval before introducing it into the environment (Powell et al., 2019). The “Darling 58” transgenic American chestnut was submitted to the USDA under the Animal and Plant Health Inspection Service (APHIS) whose focus includes regulating biotechnology in order to maintain plant and animal health (APHIS, n.d.; Powell et al., 2019). The transgenic American chestnut must be regulated by the USDA because a strain of the bacterium *Agrobacterium tumefaciens*, which is derived from bacteria that are harmful to plants, was used in the process of creating the transgenic American chestnut (Powell et al., 2019). As of July 2022, the USDA has drafted an Environmental Impact Statement (EIS) regarding the possible impacts of introducing the transgenic American chestnut tree to the environment (APHIS, 2022). The EIS provides two alternatives: taking no action and denying the petition for nonregulated status or to approve the petition and no longer regulate the introduction and reproduction of the transgenic tree (APHIS, 2022). No longer regulating the transgenic chestnut is listed as the preferred alternative because the tree has been determined to be “unlikely to pose a plant pest risk” (APHIS, 2022). The transgenic tree was submitted to the FDA since chestnuts

can be eaten and used as feed and to the EPA because it is possible the EPA could consider the OxO gene as a pesticide, in which case it would fall under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Powell et al., 2019). To the best of my knowledge, no information from the FDA or EPA regarding their review of the transgenic American chestnut has been released.

While the “Darling 58” chestnut is under federal review, researchers are also working on producing more lines of transgenic American chestnut trees (Westbrook et al., 2019a). Introducing multiple lines of transgenic chestnuts would provide more genetic diversity and avoid negative effects that could be caused by low genetic diversity, such as the gene being silenced due to offspring inheriting more than one copy of the same OxO gene (Westbrook et al., 2019a). To try to decrease this possibility of gene silencing in the wild, different promoters controlling the expression of the OxO gene are also being considered (Westbrook et al., 2019a). For example, the “Darling 58” chestnut uses the CaMV 35s promoter (Westbrook et al., 2019a), but another promoter, win3.12 from poplars (*Populus deltoides*), has been shown in studies to also provide American chestnuts with blight resistance when used with the OxO gene (Carlson et al., 2021). Overall, transgenic American chestnut trees are a promising strategy to provide blight resistance that addresses some of the issues that backcross breeding has.

Iic. Biocontrol

A third strategy for American chestnut restoration is biocontrol, which consists of introducing a hypovirus, which is a strain of the blight fungus containing a mycovirus that weakens the blight, to infected trees (Powell et al., 2019; Rigling & Prospero, 2018). Strains of hypoviruses impacting the blight have been found occurring naturally in Europe and in some areas of the U.S. (Milgroom & Cortesi, 2004) and artificial introduction of hypoviruses is being

used as a method to control the chestnut blight on both continents (Rigling & Prospero, 2018; Powell et al., 2019). Introducing the hypovirus to infected trees involves putting a liquid containing the hypovirus into a hole made in a canker caused by the blight, allowing the mycovirus within the hypovirus to infect the canker and possibly spread to the pathogenic fungus in other areas of the tree (Prospero & Rigling, 2016). In Europe, the *Cryphonectria hypovirus* (CHV1) has been shown to make cankers stop growing and successfully spread throughout and between infected trees, impacting not only the cankers that received the hypovirus treatment, but other cankers on the tree as well (Prospero & Rigling, 2016).

Introduction of hypoviruses to the American chestnut population has not been as successful in the U.S. as it has in Europe (Powell et al., 2019; Rigling & Prospero, 2018). Chestnut blight was introduced to Europe in 1938 and began causing the same symptoms in European chestnuts (*Castanea sativa*) as it did in American chestnuts (Heiniger & Rigling, 1994). Natural strains of hypovirulent blight fungus were found in stands of European chestnuts in 1951, which spread between trees and decreased the rate of canker growth and mortality of infected trees (Heiniger & Rigling, 1994). Methods for inoculating cankers with hypoviruses were eventually developed and used to further treat blight infections in Europe (Heiniger & Rigling, 1994). The spread of the hypovirulent strains of the blight fungus was aided by the relatively low number of vegetative compatibility (vc) types (Anagnostakis et al., 1986; Rigling & Prospero, 2018; Liu et al., 2000), or groups of the blight fungus that are able to merge with each other and pass on the hypovirus (Milgroom & Cortesi, 2004). One reason hypovirulence has not been as successful in the U.S. is because American chestnuts succumb to the blight more easily than European chestnuts, so it is more likely for the blight to kill an infected American chestnut before the hypovirus can infect the blight fungus (Rigling & Prospero, 2018).

Additionally, there are more vegetative compatibility (vc) types of the blight fungus in the U.S. than in Europe, preventing the spread of the hypovirus within and between the blight fungus infecting trees (Anagnostakis et al., 1986; Powell et al., 2019; Jacobs et al., 2013; Rigling & Prospero, 2018; Liu et al., 2000). Filamentous ascomycetes fungi such as the chestnut blight can integrate with genetically similar individuals of the same species to form heterokaryons, a fungal body that contains genetic information from both of the integrating individuals (Jacobson et al., 1998; Saupe, 2000). The ability to form heterokaryons is controlled by regions within the fungal genome known as vegetative incompatibility (vic) loci, which can recognize self vs non-self (Jacobson et al., 1998; Saupe, 2000). Individuals that have different versions of the genes (or alleles) in these loci will recognize each other as “non-self,” triggering cell death, which effectively blocks the formation of a heterokaryon (Jacobson et al., 1998; Saupe, 2000). Therefore, individuals that are unable to form heterokaryons are vegetatively incompatible and individuals that can are vegetatively compatible (Milgroom & Cortesi, 2004). The spread of viruses between fungal individuals requires horizontal transmission (Milgroom & Cortesi, 2004), which in the blight fungus involves the exchange of cytoplasm (anastomosis) during the integration of two fungal individuals forming a heterokaryon (Zhang & Nuss, 2016). Consequently, contact between the cytoplasm from each individual will trigger the vic loci to initiate cell death for individuals that are vegetatively incompatible, which can hinder hypovirus transmission, whereas those that are vegetatively compatible will form a heterokaryon and transmit the hypovirus (Biella et al., 2002; Caten, 1972).

As a result, having more vegetative compatibility (vc) types of the chestnut blight in an area means infected strains of the blight fungus are more likely to come into contact with individuals they are incompatible with and are therefore more likely to be unable to spread the

virus to them. In models created by Liu et al. (2000), it was found that the spread of hypoviruses decreased as vc type diversity of the blight fungus increased. This relationship between vc type diversity and the spread of the hypoviruses held true even with genetically modified hypoviruses, although the modified hypoviruses did have greater spread than the unmodified hypoviruses (Liu et al., 2000).

Despite the difficulties faced so far, researchers are continuing to look for ways to increase the effectiveness of hypoviruses in the U.S. (Rigling & Prospero, 2018). One possible solution is developing “super donor” strains of the chestnut blight that will allow hypoviruses to spread across strains of the fungus that are not vegetatively compatible (Zhang & Nuss, 2016; Stauder et al., 2019). Since the mechanism preventing transmission of hypoviruses between blight fungus individuals and by extension chestnut trees is controlled by certain genetic loci (Jacobson et al., 1998; Saupe, 2000), disrupting these genes could allow transmission to occur between vegetatively incompatible individuals (Choi et al., 2012; Zhang et al., 2014). Zhang & Nuss (2016) developed super donor strains of the blight fungus that increased hypovirus transmission between different vegetative compatibility types in lab conditions by disrupting the vic loci in the donor strains. Using the information on gene silencing from Zhang & Nuss (2016), Stauder et al. (2019) found that these super donor strains increased viral transmission in the field as well, indicating that they could be used as biocontrol in wild chestnut populations. While hypovirulence has not been successful in the U.S. yet, there is promising research that may allow it to be more successful in the future.

III. Challenges for American Chestnut Restoration

IIIa. Climate Change

Modern climate change is a global phenomenon in which long-term climate conditions, such as temperature and weather patterns, are changing, largely due to human emissions of greenhouse gases (Arias et al., 2021). Climate change has wide-reaching effects including increasing temperatures, droughts, and severity of storms as well as contributing to sea level rise (Arias et al., 2021). Climate conditions are an important determiner of species distribution (Mackey & Lindenmayer, 2001), so changes in climatic conditions due to climate change are causing shifts in species distributions and increased species extinctions (Root et al., 2003; Pimm et al., 2014; Arias et al., 2021).

Based on climate models, the American chestnut is projected to experience a northward shift in suitable habitat range due to climate change (Figure 5; Barnes & Delborne, 2019; Noah et al., 2021). Studies conducted by Barnes & Delborne (2019) and Noah et al. (2021) found that the amount of suitable American chestnut habitat in models decreased in the southern part of its range and increased in the northern part of its range in New England and the Adirondacks under moderate and severe climate change projections. American chestnut range was found to push farther into Canada as well (Barnes & Delborne, 2019). Shifts in suitable habitat were more drastic under severe climate change projections than moderate projections (Figure 5; Barnes & Delborne, 2019; Noah et al., 2021). For example, the projected shift in suitable habitat by 2100 under moderate climate change was similar to the projected shift by 2070 under severe climate conditions (Noah et al., 2021). This suggests that trees planted in the southern portion of the chestnut's range may not survive as the effects of climate change increase (Barnes & Delborne, 2019).

While these models are helpful tools to try to predict how climate change might impact the range of American chestnuts, it is important to keep in mind that they are only predictions

based on current data and possible actions society could take (Noah et al., 2021). This means that climate change could impact American chestnut range differently than expected based on these models (Noah et al., 2021). These models also only look at the impacts of climate change and do not take the effects of diseases, pests, or changes in land use into account (Barnes & Delborne, 2019; Noah et al., 2021). However, they do suggest that northern parts of the current American chestnut range will be more suitable for chestnuts in the future, so reintroduction efforts should focus on these areas to decrease the chance of climate change killing reintroduced trees (Noah et al., 2021). American chestnuts could also be introduced into Canada, but the possible impacts of introducing them to Canada as a non-native species should be explored before this avenue of reintroduction is implemented (Barnes & Delborne, 2019). Additionally, using trees from the southern part of the American chestnut range that are more tolerant to warmer temperatures in restoration programs could provide trees that are more resistant to warming temperatures due to climate change (Barnes & Delborne, 2019).

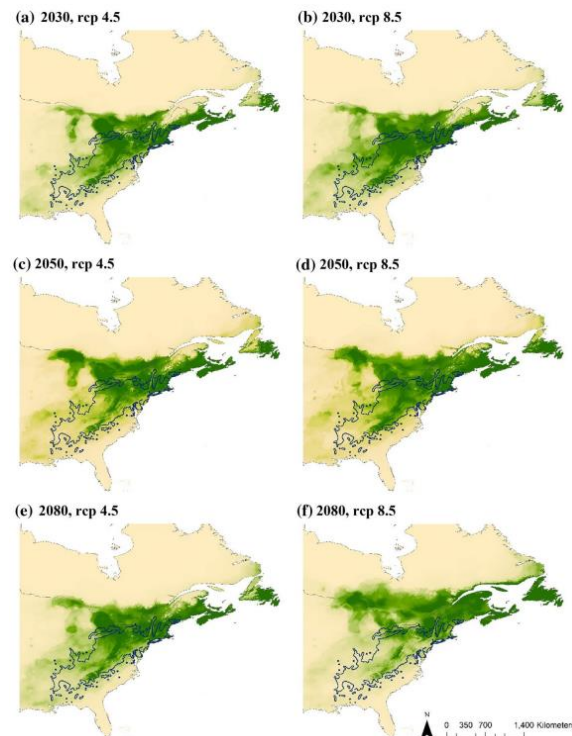


Figure 5: Suitable habitat for American chestnuts under moderate (rcp 4.5) and severe (rcp 8.5) climate projections (Barnes & Delborne, 2019).

IIIb. Phytophthora Root Rot

In addition to the blight, another introduced pathogen is threatening the American chestnut: *Phytophthora cinnamomi*, also known as *Phytophthora* root rot (Gustafson et al., 2021; Jeffers et al., 2007) or ink disease (Anagnostakis, 2012; Jeffers et al., 2007). Root rot was likely introduced to the southern United States prior to 1824 by trees brought from Portugal and contributed to American chestnut mortality before the chestnut blight was introduced (Anagnostakis, 2012). *P. cinnamomi* is a pseudofungus in the class Oomycetes, which contains other plant pathogens including the one that caused the Irish potato famine (Cavalier-Smith & Chao, 2004). *P. cinnamomi* enters the roots of plants through wounds and then kills the root tissue, which turns the rotting roots black (hence the names ink disease and root rot) (Anagnostakis, 2012; Hardham & Blackman, 2018). By causing the roots to rot, the pathogen prevents the plant's roots from taking water up from the soil and transporting it to the rest of the plant (Hardham & Blackman, 2018). Some infected plants die quickly while others do not show symptoms of the infection for years (Hardham & Blackman, 2018). This is likely due to *P. cinnamomi* being a hemibiotroph, meaning it has both biophytic (does not kill the plant after infection) and necrophytic (kills the plant it is infecting) phases (Hardham & Blackman, 2018; Crone et al., 2013). In certain species of plants and environmental conditions, the biophytic phase can last longer, causing the plants to be asymptomatic (Hardham & Blackman, 2018; Crone et al., 2013). Unlike with the chestnut blight, root rot kills the entire plant, not just the stems, so American chestnuts killed by root rot will not resprout (Westbrook et al., 2019b; Anagnostakis, 2012).

Due to the American chestnut's susceptibility to Phytophthora root rot, it is important to consider the impacts it may have on reintroduced chestnut populations, including under different climate conditions (Gustafson et al., 2021). Root rot is currently only found below latitude 40°N in the United States because temperatures are too cold above that latitude, but it is expected to move further north as the climate warms (McConnell & Balci, 2014; Burgess et al., 2017). Gustafson et al. (2021) created a model to determine how root rot may affect chestnut restoration efforts and what strategies could be used to decrease these effects. The model showed that increases in temperature due to climate change will increase the impacts of *P. cinnamomi* on American chestnut populations and even American chestnut populations with higher root rot resistance would be unable to fully recover their populations to what they were pre-blight (Gustafson et al., 2021).

Research is being done to develop American chestnuts that are resistant to root rot through backcross breeding (Jeffers et al., 2007). As with the chestnut blight, Chinese chestnuts show greater resistance to root rot than American chestnuts (Jeffers et al., 2007). While resistance to root rot is variable between backcross-bred chestnuts (Jeffers et al., 2007; Westbrook et al., 2019b), some of the hybrids were found to have greater resistance to root rot and could be used in the future to create hybrid chestnuts that are resistant to both the blight and root rot (Westbrook et al., 2019b). There is also interest in using bioengineering to produce chestnuts that are resistant to both pathogens (Powell et al., 2019).

IV. Summary & Conclusions

The chestnut blight has caused a huge disruption of American chestnut populations and forest ecosystems (Paillet, 2002; Dagleish et al., 2016; Dagleish & Swihart, 2012; Diamond et al., 2000). Restoration efforts are ongoing, and it is important to consider whether the restoration

of American chestnuts is feasible and necessary. Based on the current body of research, I have concluded that American chestnut restoration is feasible with a combination of restoration techniques and careful attention to shifts in climate and pathogens. Additionally, American chestnut restoration is necessary due to the tree's niche remaining partially open and its ability to provide a precedent for the restoration of other tree species in the future.

American chestnut restoration is feasible with a combination of the three main restoration methods: backcross breeding, bioengineering, and biocontrol. Backcross breeding is the oldest method and some American chestnuts that have been backcrossed for multiple generations and have been implemented in places such as Longwood Gardens (Figure 6; Longwood Gardens, n.d.; *personal observations*) and at test sites on USDA Forest Service National Forest System lands (Clark et al., 2014). There are some concerns, though, about resistance being bred out of the chestnuts as they reproduce in the wild (Steiner et al., 2017) and the hybrids having different morphological characteristics from fully American chestnuts due to having a mixed genome from two different species (Diskin et al., 2006). These concerns have been addressed through bioengineering. Transgenic American chestnuts with the OxO gene are resistant to the blight, the gene is passed on like a dominant trait so it will not be lost (Powell et al., 2019), and only the desired gene will be added to the trees, so there will be no morphological impact (Powell et al., 2019; Merkle et al., 2006; Diskin et al., 2006). There are some concerns about introducing GMOs into the environment surrounding the transgenic trees that are not raised with the backcrossed trees, but studies suggest that these trees will not have significant negative impacts on ecosystems (Newhouse et al., 2018, 2021; Goldspiel et al., 2019). While these two methods focus on introducing resistant trees to ecosystems, biocontrol allows for the reduction of the effects of the blight on trees that are already infected in the wild by injecting infected trees with

hypoviruses (Powell et al., 2019; Rigling & Prospero, 2018). Despite biocontrol having limited success in the U.S. so far, a more effective process still remains under investigation for researchers (Rigling & Prospero, 2018; Zhang & Nuss, 2016; Stauder et al., 2019). Each of these methods has limitations, but implementing all three of them simultaneously will help to decrease the impact of these limitations on restoration. Organizations such as the American Chestnut Foundation (TACF) are already using this three-pronged approach to American chestnut restoration (TACF, n.d.). Additionally, having a greater diversity of trees in the wild will allow for greater genetic diversity in American chestnut populations which will allow these populations to be more resilient against disturbances (Schaberg et al., 2008).



Figure 6: Backcrossed chestnut tree at Longwood Gardens. This is one of several backcrossed chestnuts planted across Longwood Garden's property; working with the team in charge of this tree planting inspired my SCE (Photograph by author).

While methods for reintroduction exist, factors such as climate change and other diseases including *Phytophthora* root rot should be considered when determining where to focus restoration efforts (Barnes & Delborne, 2019; Noah et al., 2021; Gustafson et al., 2021). Climate

change is likely to shift the range of suitable habitat for American chestnuts and the *Phytophthora* root rot pathogen northward, so reintroduction should be focused on the northern parts of the American chestnut range that are predicted to be less impacted by climate change and root rot (Noah et al., 2021; Gustafson et al., 2021). I think that focusing restoration on these areas of the range in consideration of climate change would be prudent and increase the effectiveness of restoration efforts along with using chestnut trees that are more tolerant to warmer temperatures in restoration programs, as suggested by Barnes & Delborne (2019). Producing chestnuts that are resistant to *Phytophthora* root rot would help increase the effectiveness of reintroduction as well.

In addition to the feasibility of American chestnut restoration, the necessity of this restoration should be considered. As a person who cares about the environment, it can be easy to say that any restoration project is necessary in order to try to reverse the impacts that humans have had on the environment and to keep ecosystems healthy. However, it is important to ask whether the ecosystems in question would actually benefit from having a species reintroduced or if we are simply trying to restore a species that we feel guilty for letting disappear. Based on my research, I have found that Eastern forest ecosystems have not fully filled the niche the American chestnut left open and are still being impacted by its loss (Dalglish & Swihart, 2012; Smock & MacGregor, 1988). This suggests that American chestnut restoration is necessary in order to return forest ecosystems to their previous health. Furthermore, 30% of tree species are threatened worldwide (BGCI, 2021), so this will not be the last time that we are faced with the decision of whether and how to save a tree species. The efforts for the restoration of the American chestnut have been going on for a long time, and if they are implemented successfully, they could set a precedent for the restoration of other threatened tree species in the future.

Acknowledgements:

I would like to thank Dr. Fox for being my advisor, supporting me through the process of writing my SCE, and giving me such helpful feedback on all of my drafts. Thank you to all of the professors who have taught me at Washington College and who have supported me throughout my time at WAC. Thank you to the Starr Center for providing me with the opportunity to spend a summer working at Longwood Gardens, and to Longwood Gardens for providing me with the opportunity to work with backcrossed American chestnuts, which was the initial inspiration for me to write about American chestnut restoration. Thank you to my friends and family who have been supportive of me throughout my college career. And especially, thank you to my mom, who sent me news articles about trees and led me to discover more about the topic of American chestnut restoration, which then allowed me to narrow into my specific SCE topic. I couldn't have done it without you all.

References:

- Anagnostakis, S. L., Hau, B., Kranz, J. (1986). Diversity of Vegetative Compatibility Groups of *Cryphonectria parasitica* in Connecticut and Europe. *Plant Disease*. 70(6):536-538.
- Anagnostakis, S. L. (2012). Chestnut Breeding in the United States for Disease and Insect Resistance. *Plant Disease*. 96(10):1392-1403.
- Anderson, P. J. & Rankin, W. H. (1914). *Endothia canker of chestnut*. Cornell University.
- Animal and Plant Health Inspection Service. (n.d.) *Biotechnology Regulatory Services (BRS)*. United States Department of Agriculture.
<https://www.aphis.usda.gov/aphis/ourfocus/biotechnology>
- Animal and Plant Health Inspection Service. (2022). *The State University of New York College of Environmental Science and Forestry Petition (19-309-01p) for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut (Castanea dentata): Draft Environmental Impact Statement* (OECD Unique Identifier: ESF-DAR58-3). United States Department of Agriculture.
<https://www.regulations.gov/document/APHIS-2020-0030-8289>
- Arias, P.A., N. Bellouin, E. Coppola, R.G. Jones, G. Krinner, J. Marotzke, V. Naik, M.D. Palmer, G.-K. Plattner, J. Rogelj, M. Rojas, J. Sillmann, T. Storelvmo, P.W. Thorne, B. Trewin, K. Achuta Rao, B. Adhikary, R.P. Allan, K. Armour, G. Bala, R. Barimalala, S. Berger, J.G. Canadell, C. Cassou, A. Cherchi, W. Collins, W.D. Collins, S.L. Connors, S. Corti, F. Cruz, F.J. Dentener, C. Dereczynski, A. Di Luca, A. Diongue Niang, F.J. Doblas-Reyes, A. Dosio, H. Douville, F. Engelbrecht, V. Eyring, E. Fischer, P. Forster, B. Fox-Kemper, J.S. Fuglestedt, J.C. Fyfe, N.P. Gillett, L. Goldfarb, I. Gorodetskaya, J.M. Gutierrez, R. Hamdi, E. Hawkins, H.T. Hewitt, P. Hope, A.S. Islam, C. Jones, D.S. Kaufman, R.E. Kopp, Y. Kosaka, J. Kossin, S. Krakovska, J.-Y. Lee, J. Li, T. Mauritsen, T.K. Maycock, M. Meinshausen, S.-K. Min, P.M.S. Monteiro, T. Ngo-Duc, F. Otto, I. Pinto, A. Pirani, K. Raghavan, R. Ranasinghe, A.C. Ruane, L. Ruiz, J.-B. Sallée, B.H. Samset, S. Sathyendranath, S.I. Seneviratne, A.A. Sörensson, S. Szopa, I. Takayabu, A.-M. Tréguier, B. van den Hurk, R. Vautard, K. von Schuckmann, S. Zaehle, X. Zhang, and K. Zickfeld. (2021). 2021: Technical Summary. In *Climate Change 2021: The Physical Science Basis*. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 33–144. doi:10.1017/9781009157896.002.
- Barnes, J. C. & Delborne, J. A. (2019). Rethinking restoration targets for American chestnut using species distribution modeling. *Biodiversity and Conservation*. 28:3199-3220.
- BGCI. (2021). *State of the World's Trees*. BGCI.

- Biella, S., Smith, M. L., Aist, J. R., Cortesi, P., Milgroom, M. G. (2002). Programmed cell death correlates with virus transmission in a filamentous fungus. *Proceedings of the Royal Society London B*. 269:2269-2276.
- Bowen, L., Megalos, M., Moorman, C. (2019, April 5). *Wildlife Terms*. NC State Extension. <https://content.ces.ncsu.edu/wildlife-terms>
- Burgess, T. I., Scott, J. K., McDougall, K. L., Stukely, M. J., Crane, C., Dunstan, W. A., Brigg, F., Andjic, V., White, D., Rudman, T., Arentz, F., Ota, N., Hardy, G. E. St. J. (2017). Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. *Global Change Biology*. 23:1661-1674.
- Burnham, C. R., Rutter, P. A., French, D. W. (1986). Breeding blight-resistant chestnuts. *Plant Breeding Reviews*. 4:347-397.
- Burnham, C. R. (1987a). Historical Overview of Chestnut Breeding in the United States. *The Journal of the American Chestnut Foundation*. 2(1):6-8.
- Burnham, C. R. (1987b). Backcross Breeding: I. Blight-Resistant American Chestnut Trees II. Hardier Chinese Chestnuts. *The Journal of the American Chestnut Foundation*. 2(1):9-13.
- Carlson, E., Stewart, K., Baier, K., McGuigan, L., Culpepper II, T., Powell, W. (2021). Pathogen-induced expression of a blight tolerance transgene in American chestnut. *Molecular Plant Pathology*. 23:370-382.
- Carrillo, M. G. C., Goodwin, P. H., Leach, J. E., Leung, H., Vera Cruz, C. M. (2009). Phylogenomic Relationships of Rice Oxalate Oxidases to the Cupin Superfamily and Their Association with Disease Resistance QTL. *Rice*. 2:67-79.
- Caten, C. E. (1972). Vegetative Incompatibility and Cytoplasmic Infection in Fungi. *Journal of General Microbiology*. 72:221-229.
- Cavalier-Smith, T. & Chao, E. E-Y. (2006). Phylogeny and Megasytematics of Phagotrophic Heterokonts (Kingdom Chromista). *Journal of Molecular Evolution*. 62:388-420.
- Choi, G. H., Dawe, A. L., Churbanov, A., Smith, M. L., Milgroom, M. G., Nuss, D. L. (2012). Molecular Characterization of Vegetative Incompatibility Genes That Restrict Hypovirus Transmission in the Chestnut Blight Fungus *Cryphonectria Parasitica*. *Genetics*. 190(1):113-127.
- Clark, S. L., Schlarbaum, S. E., Pinchot, C. C., Anagnostakis, S. L., Saunders, M. R., Gundy, M. T-V., Schaberg, P., McKenna, J., Bard, J. F., Berrang, P. C., Casey, D. M., Casey, C. E., Crane, B., Jackson, B. D., Kochenderfer, J. D., Lewis, R. F., MacFarlane, R., Makowski, R., Miller, M. D., Rodrigue, J. A., Stelick, J., Thornton, C. D., Williamson, T. S. (2014). Reintroduction of American Chestnut in the National Forest System. *Journal of Forestry*. 112(5):502-512.

- Crone, M., McComb, J. A., O'Brien, P. A., Hardy, G. E. St J. (2013). Annual and herbaceous perennial native Australian plant species are symptomless hosts of *Phytophthora cinnamomi* in the *Eucalyptus marginata* (jarrah) forest of Western Australia. *Plant Pathology*. 62:1057-1062.
- Dahiya, T., Yadav, S., Chauhan, N., Handa, P., Pundir, C. S. (2010). Strawberry Fruit Oxalate Oxidase – Detection, Purification, Characterization and Physiological Role. *Journal of Plant Biochemistry and Biotechnology*. 19:247-250.
- Dalgleish, H. J., Nelson, C. D., Scrivani, J. A., Jacobs, D. F. (2016). Consequences of Shifts in Abundance and Distribution of American Chestnut for Restoration of a Foundation Forest Tree. *Forest*. 7(4).
- Dalgleish, H. J. & Swihart, R. K. (2012). American Chestnut Past and Future: Implications of Restoration for Resource Pulses and Consumer Populations of Eastern U.S. Forests. *Restoration Ecology*. 20(4):490-497.
- Diamond, S. J., Giles Jr., R. H., Kirkpatrick, R. L., Griffin, G. J. (2000). Hard Mast Production Before and After the Chestnut Blight. *Southern Journal of Applied Forestry*. 24(4):196-201.
- Diskin, M., Steiner, K. C., Hebard, F. V. (2006). Recovery of American chestnut characteristics following hybridization and backcross breeding to restore blight-ravaged *Castanea dentata*. *Forest Ecology and Management*. 223:439-447.
- Elliott, K. J., Swank, W. T. (2007). Long-term changes in forest composition and diversity following early logging (1919-1923) and the decline of American chestnut (*Castanea dentata*). *Plant Ecology*. 197:155-172.
- Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., Foster, D. R., Kloepfel, B. D., Knoepp, J. D., Lovett, G. M., Mohan, J., Orwig, D. A., Rodenhouse, M. L., Sobczak, W. V., Stinson, K. A., Stone, J. K., Swan, C. M., Thompson, J., Von Holle, B., Webster, J. R. (2005). Loss of Foundation Species: Consequences for the Structure and Dynamics of Forested Ecosystems. *Frontiers in Ecology and the Environment*. 3(9):479-486.
- Goldspiel, H. B., Newhouse, A. E., Powell, W. A., Gibbs, J. P. (2019). Effects of transgenic American chestnut leaf litter on growth and survival of wood frog larvae. *Restoration Ecology*. 27(2):371-378.
- Gravatt, F. (1949). Chestnut Blight in Asia and North America. *Unasylva*. 3(1):2-7.
- Gustafson, E. J., Miranda, B. R., Dreaden, T. J., Pinchot, C. C., Jacobs, D. F. (2022). Beyond blight: *Phytophthora* root rot under climate change limits populations of reintroduced American chestnut. *Ecosphere*. doi: 10.1002/ecs2.3917
- Hardham, A. R. & Blackman, L. M. (2018). Pathogen profile update: *Phytophthora cinnamomi*. *Molecular Plant Pathology*. 19(2):260-285.

- Heiniger, U. & Rigling, D. (1994). Biological Control of Chestnut Blight in Europe. *Annual Review of Phytopathology*. 32:581-599.
- Jacobs, D. F., Dalglish, H. J., Nelson, C. D. (2013). A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. *New Phytologist*. 197:378-393.
- Jacobson, D. J., Beurkens, K., Klomparens, K. L. (1998). Microscopic and Ultrastructural Examination of Vegetative Incompatibility in Partial Diploids Heterozygous at *het* Loci in *Neruospora crassa*. *Fungal Genetics and Biology*. 23:45-56.
- Jeffers, S. N., James, J. B., Sisco, P. H. (2009). Screening for Resistance to *Phytophthora cinnamomic* in Hybrid Seedlings of American Chestnut. *Phytophthoras in Forests and Natural Ecosystems*. 188-194.
- Kubisiak, T. L., Hebard, F. V., Nelson, C. D., Zhang, J., Bernatzky, R., Huang, H., Anagnostakis, S. L., Doudrick, R. L. (1997). Molecular Mapping of Resistance to Blight in an Interspecific Cross in the Genus *Castanea*. *Phytopathology*. 85:751-759.
- Liu, Y-C., Durrett, R., Milgroom, M. G. (2000). A spatially-structured stochastic model to simulate heterogenous transmission of viruses in fungal populations. *Ecological Modelling*. 127:291-308.
- Longwood Gardens. (n.d.). *Castanea dentata x mollissima*. Longwood Gardens Plant Explorer. https://plantexplorer.longwoodgardens.org/weboi/oecgi2.exe/INET_ECM_DisPI?NAME=3824&startpage=0&refreshlist=1&DETAIL=1#map.
- Mackey, B. G., & Lindenmayer, D. B. (2001). Towards a hierarchical framework for modelling the spatial distribution of animals. *Journal of Biogeography*. 28:1147-1166.
- McConnell, M. E., & Balci, Y. (2014). *Phytophthora cinnamomi* as a Contributor to White Oak Decline in Mid-Atlantic United States Forests. *Plant Disease*. 98(3):319-327.
- Merkle, S. A., Andrade, G. M., Nairn, C. J., Powell, W. A., Maynard, C. A. (2006). Restoration off threatened species: a noble cause for transgenic trees. *Tree Genetics & Genomes*. 3(2):111-118.
- Milgroom, M. G., Cortesi, P. (2004). Biological Control of Chestnut Blight with Hypovirulence: A Critical Analysis. *Annual Review of Phytopathology*. 42:311-338.
- Murrill, W. A. (1906). A New Chestnut Disease. *Torreya*. 6(9):186-189.
- Newhouse, A. E., Polin-McGuigan, L. D., Baier, K. A., Valletta, K. E. R., Rottmann, W. H., Tschaplinski, T. J., Maynard, C. A., Powell, W. A. (2014). Transgenic American chestnuts show enhanced blight resistance and transmit the trait to T1 progeny. *Plant Science*. 228:88-97.
- Newhouse, A. E., Oakes, A. D., Pilkey, H. C., Roden, H. E., Horton, T. R., Powell, W. A. (2018). Transgenic American Chestnuts Do Not Inhibit Germination of Native Seeds of

- Colonization of Mycorrhizal Fungi. *Frontiers in Plant Science*. doi: 10.3389/fpls.2018.01046.
- Newhouse, A. E., Allwine, A. E., Oakes, A. D., Matthews, D. F., McArt, S. H., Powell, W. A. (2021). Bumble bee (*Bombus impatiens*) survival, pollen usage, and reproduction are not affected by oxalate oxidase at realistic concentrations in American chestnut (*Castanea dentata*) pollen. *Transgenic Research*. 30:751-764.
- Noah, P. H., Cagle, N. L., Westbrook, J. W., Fitzsimmons, S. F. (2021). Identifying resilient restoration targets: Mapping and forecasting habitat suitability for *Castanea dentata* in Eastern USA under different climate-change scenarios. *Climate Change Ecology*. doi: 10.1016/j.ecochg.2021.100037.
- Paillet, F. L. (2002). Chestnut: history and ecology of a transformed species. *Journal of Biogeography*, 29(10/11), 1517-1530.
- Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T. M., Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M., Sexton, J. O. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science*. 344(6187). doi: 10.1126/science.1246752.
- Powell, W. A. et al. (2019). Developing Blight-Tolerant American Chestnut Trees. *Cold Spring Harbor Perspective in Biology*. doi: 10.1101/cshperspect.a034587
- Prospero, S., Rigling, D. (2016). Using molecular markers to assess the establishment and spread of a mycovirus applied as a biological control agent against chestnut blight. *BioControl*. doi: 10.1007/s10526-015-9713-0
- Rigling, D., Prospero, S. (2018). *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. *Molecular Plant Pathology*. 19(1):7-20.
- Robbins, M. (2019, November 14). *Backcrossing, Backcross (BC) Populations, and Backcross Breeding*. Plant Breeding Genomics. <https://plant-breeding-genomics.extension.org/backcrossing-backcross-bc-populations-and-backcross-breeding/>.
- Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*. 421:57-60.
- Saupe, S. J. (2000). Molecular Genetics of Heterokaryon Incompatibility in Filamentous Ascomycetes. *Microbiology and Molecular Biology Reviews*. 64(3):489-502.
- Schaberg, P. G., DeHayes, D. H., Hawly, G. J., Nijensohn, S. E. (2008). Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. *Forest Ecology and Management*. 256(5):855-862.
- Smith, D. M. (2000). American Chestnut: Ill-fated Monarch of the Eastern Hardwood Forest. *Journal of Forestry*. 98(2):12-15.

- Smock, L. A. & MacGregor, C. M. (1988). Impact of the American chestnut blight on aquatic shredding macroinvertebrates. *Journal of the North American Benthological Society*. 7(3):212-221.
- Stauder, C. M., Nuss, D. L., Zhang, D-X., Double, M. L., MacDonald, W. L., Metheny, A. M., Kasson, M. T. (2019). Enhanced hypovirus transmission by engineered super donor strains of the chestnut blight fungus, *Cryphonectria parasitica*, into a natural population of strains exhibiting diverse vegetative compatibility genotypes. *Virology*. 528:1-6.
- Steiner, K. C., Westbrook, J. W., Hebard, F. V., Georgi, L. L., Powell, W. A., Fitzsimmons, S. F. (2017). Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. *New Forests*. 48:317-336.
- Stephenson, S. L. (1986). Changes in a Former Chestnut-Dominated Forest after a Half Century of Succession. *The American Midland Naturalist*. 116(1):173-179.
- TACF. (n.d.). 3BUR. The American Chestnut Foundation. <https://acf.org/science-strategies/3bur/>.
- TACF. (2016). *TACF Fact Sheet: Identifying Cankers on American Chestnut: Canker IDing*. The American Chestnut Foundation. <https://acf.org/wp-content/uploads/2016/09/Identifying-Blight-Cankers.pdf>.
- Van Fleet, W. (1914). Chestnut Breeding Experience: How the American Chestnut, Threatened by Extinction, May Be Saved to Horticulture, Even if not to Forestry – Promising Crosses Between Chinquapin and Asiatic Species – Trees Bear Early, Produce Good Nuts and Show Resistance to Blight. *The Journal of Heredity*. 5:19-25.
- Wang, T., Zhang, E., Chen, X., Li, L., Liang, X. (2010). Identification of seed proteins associated with resistance to pre-harvested aflatoxin contamination in peanut (*Arachis hypogaea* L). *BMC Plant Biology*. doi: 10.1186/1471-2229-10-267.
- Westbrook, J. W., Holliday, J. A., Newhouse, A. E., Powell, W. A. (2019a). A plan to diversify a transgenic blight-tolerant American chestnut population using citizen science. *Plants People Planet*. 2:84-95.
- Westbrook, J. W., James, J. B., Sisco, P. H., Frampton, J., Lucas, S., Jeffers, S. N. (2019b). Resistance to *Phytophthora cinnamomic* in American chestnut (*Castanea dentata*) Backcross Populations that Descended from Two Chinese Chestnut (*Catanea mollissima*) Sources of Resistance. *Plant Disease*. 103:1631-1641.
- Youngs, R. L. (2000). “A Right Smart Little Jolt”: Loss of the Chestnut and a Way of Life. *Journal of Forestry*. 98(2):17-21.
- Zhang, B., Oakes, A. D., Newhouse, A. E., Baier, K. M., Maynard, C. A., Powell, W. A. (2013). A threshold level of oxalate oxidase transgene expression reduces *Cryphonectria parasitica*-induced necrosis in a transgenic American chestnut (*Castanea dentata*) leaf bioassay. *Transgenic Research*. 22:973-982.

- Zhang, D-X, Spiering, M. J., Dawe, A. L., Nuss, D. L. (2014). Vegetative Incompatibility Loci with Dedicated Roles in Allorecognition Restrict Mycovirus Transmission in Chestnut Blight Fungus. *Genetics*. 197(2):701-714.
- Zhang, D-X & Nuss, D. L. (2016). Engineering super mycovirus donor strains of chestnut blight fungus by systematic disruption of multilocus *vic* genes. *Proceedings of the National Academy of Sciences*. 113(8):2062-2067.