Managing effects of chestnut blight (*Cryphonectria parasitica*) on American Chestnut

Anya Collins

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Abstract

Cryphonectria parasitica is a parasitic fungus of chestnut trees that causes the disease known as chestnut blight. Chestnut blight causes diffuse cankers that rapidly grow, often girdling and killing the stem and colonizing the tree. Introduced in 1904 via nursery stock from East Asia, *C. parasitica* devastated the nearly four-billion-strong population of American chestnut (*Castanea dentata*) in the span of 40 years. The parasite has also been spread throughout Asia and Europe, with new cases being found as recently as 2003. While there are few adult American chestnut trees left, control of *Cryphonectria parasitica* may be possible through the breeding of canker-resistant hybrids or spread of the mycovirus *Cryphonectria hypovirus* (CHV-1).

Introduction

Chestnut blight is thought to have originated in China or Japan, where it coevolved with their native chestnut trees (*Castanea mollissima* and *Castanea crenata*). In the early 1900s, nursery stock from east Asia was commonly shipped to the US, which lead to the introduction of *C. parasitica* in 1904. The disease was first reported in the Zoological Park of New York City and named chestnut blight, though *C. parasitica* would not receive its current name until 1978,

decades after the American chestnut population had been decimated (Rigling and Prospero 2018).

C. parasitica is a necrotrophic bark pathogen that uses cracks in the bark, usually from fresh wounds or growth, to penetrate the host tissue. Once there, the fungus creates necrotic lesions (cankers) on the bark that girdle the tree part, leading to the death of that limb or sprout (Forest Pathology 2020). The life cycle of *C. parasitica* is not complex, having only a single host, but can still spread quickly throughout a population. The fungus can reproduce both sexually, forcibly ejecting ascospores through a perithecial neck that protrudes through the bark, or asexually via conidia that ooze out in tendrils after rain (Marra and Milgroom 2001). Conidia are primarily dispersed by rain, but birds, insects, and wind may also act as transport for these propagules over long distances. Those that are deposited on the ground can remain viable in the soil for many months (Heald *et al.* 1914).

The American chestnut was a culturally, ecologically, and economically important tree in eastern North America, where it comprised up to 45% of the forest canopy in some areas (Rigling and Prospero 2018). It was a large, straight growing tree with rot-resistant timber and edible nuts that provided food and shelter to both wildlife and people. Today, most remaining American chestnut trees exist as shrubs sprouting from old stumps and root systems, unable to grow to maturity before being killed back by chestnut blight (Barakat et al. 2009). Successful reintroduction will rely on the use of multiple control strategies, as hybridization or hypovirulence alone may not be enough to control *C. parasitica*.

Hybridization as a control method

While the European (*Castanea sativa*), Chinese (*Castanea mollissima*), and Japanese (*Castanea crenata*) chestnut are all hosts to *C. parasitica*, only the American chestnut has been completely devastated by its pathology. Because the fungus originated in East Asia, the Chinese and Japanese chestnut trees have evolved to be resistant and do not express serious disease symptoms. However, these resistant trees don't have the qualities that made American chestnut trees a crucial part of the Eastern US ecology and economy; they are more shrub-like than the American chestnut and the nuts that they produce are far less sweet. The American Chestnut Foundation (ACF) has been working to breed hybrid trees that retain the blight resistance of the Chinese chestnut and the physical qualities of the American chestnut using traditional breeding and backcrossing strategies (American Chestnut Foundation 2020). This involves breeding Chinese and American chestnut trees, and then breeding the offspring with American chestnut trees. There have been promising results from third backcross generations produced after hybridizing Chinese and American chestnuts for blight resistance and selected hybrids containing 60-90% American chestnut genome have been found to exhibit blight resistant intermediate to that of the American and Chinese chestnut. The success of these hybrids relies on their ability to produce progeny homozygous for blight-resistant alleles, which is currently being studied (Diskin *et al.* 2006). Reintroduction trials are being done in the eastern US to determine whether the hybrids are sufficiently competitive and blight resistant for restoration.

Even if a suitable hybrid is produced, there are still factors that might limit the success of the reintroduction of American Chestnut to the Eastern US. The range of the American chestnut once covered large portions of the eastern US, so reintroduction would need to take place over a wide range of environments. In some areas, fire may need to be utilized to ensure the saplings establish in areas with potential competitors. Degraded sites suitable for afforestation may need

to be plowed or disked (Jacobs *et al.* 2013). Seed predation may also limit recruitment, as it has been found that the American chestnut is not tolerant of damage done by weevils, a seed-predating beetle that affects the oak (*Quercus*) trees of eastern US (Dalgleish *et al.* 2012).

The ability of hybrid chestnuts to thrive in the same natural range as the American chestnut should also be considered. While the hybrid is planned to be more than 90% *Castanea dentata*, studies have suggested the presence of subtle differences between American and current hybrid chestnuts (Douglass 2007). Notably, limitations in cold tolerance may restrict the range of hybrid chestnuts. When compared to northern red oak (*Quercus rubra*) and sugar maple (*Acer saccharum*) both American and hybrid chestnuts were less tolerant to cold, with the hybrid chestnuts tending to fare worse than the American chestnuts (Gurney *et al.* 2011). This could limit the ability of hybrid chestnuts to establish in the northeast when faced with more cold-tolerant competitors. The hybrid chestnuts may also have lower litter flammability than American chestnut, reducing its ability to effectively replace the American chestnut in fire-prone ecosystems (Kane *et al.* 2019). This is particularly notable as American chestnut seedlings outcompete other species after burnings, but not clearcut events (Douglass 2007).

Hypovirulence as a control method

When *C. parasitica* was first introduced to Italy in 1938, European chestnut trees showed symptoms like those shown by the American chestnut tree. Cankers would form on the limbs of the trees, eventually girdling and killing the limbs and trunk. For two decades, the disease spread rapidly throughout chestnut-growing areas, causing significant dieback (Krstin *et al.* 2017). However, unlike the American chestnut, the European chestnut trees started to spontaneously

recover two decades after the introduction of *C. parasitica*. The cankers that formed would not grow to girdle the limbs and trunk of the trees, allowing the tree's survival.

The *Cryphonectria hypovirus* CHV1 was found to be the reason for the sudden recovery of European chestnut trees. This virus reduces the virulence of the fungus, which stops *C. parasitica* from creating girdling (lethal) cankers (Zamora *et al.* 2017). CHV1 also reduces sporulation and reduces pigmentation of *C. parasitica*. It is hypothesized that down-regulation of host genes by the virus is what causes the visual symptoms, but it doesn't appear to affect other host proteins and the mechanism by which the virus causes non-visual symptoms is unknown (Kazmierczak *et al.* 1996).

The biological control of *C. parasitica* through CHV1 infection has been found useful in Greece, where hypovirulence was introduced to 29 counties where *C. parasitica* was present and was able to spread profoundly and aid in the recovery of forests and orchards (Diamandis 2018). Research has also been done in Croatia, where it was established that different genotypes of European chestnut trees have varying responses to infection with a range of virulent and hypovirulent *C. parasitica* isolates (Krstin *et al.* 2017). However, treatment of American chestnut trees with CHV1 is limited by the restriction vegetative incompatibility puts on the transmission of CHV1 (Zhang *et al.* 2014). Horizontal transmission of the virus relies on fusion of vegetative hyphae, hyphal anastomosis, which is only possible between *C. parasitica* with vegetative compatibility. Vegetative compatibility is governed by at least 6 *vic* genes and dissemination of CHV1 is expected to be high when there is high vegetative compatibility throughout the population. While European *C. parasitica* populations have low diversity in vegetative compatibility types, North American populations have high diversity, leading to a lower rate of successful CHV1 transmission (Robin *et al.* 2009). Vertical transmission through

conidia is also possible, but the reduced sporulation of hypovirulent *C. parasitica* strains negatively impacts the spread of CHV1. Cankers may also contain fungi other than *C. parasitica* that may be weakly pathogenic, causing harm to the tree even when only hypovirulent *C. parasitica* are present (Kolp *et al.* 2020).

Discussion

Efforts to reintroduce the American chestnut tree currently focus on the production of blight-resistant hybrids that have the ecologically important traits of the American chestnut tree. However, full resistance relies on the ability to obtain hybrids that are homozygous for resistant alleles. I believe that focusing on increasing hypovirulent strains of *C. parasitica* in the US and engineering CHV-1 to spread through ascospores would increase the likelihood of hybrid chestnut trees fully establishing in the natural range of the American chestnut. Introduction of a transgenic strain of hypovirulent *C. parasitica* to a Connecticut forest site suggested that the spread of hypovirulence through ascospores may be possible (Anagnostakis *et al.* 2007). This suggests that hypovirulence could be spread by ascospores and conidia, increasing the application of the virus. Spores could artificially spread throughout the areas where hybrid saplings are planted to facilitate the spread of hypovirulence. In areas where *C. parasitica* has not yet been found, real-time PCR can be used to detect new introductions of the pathogen, allowing the co-introduction of CHV-1 (Chandelier *et al*. 2019).

I suggest that reintroduction be carried out as a series of smaller, experimental introductions of saplings using afforestation. This reduces the initial competition that the saplings must face, increases the abundance of planting sites, and eliminates the need for

controlled burns to eliminate competitors. Afforestation measures such as mine reclamation plantings are responsible for a significant number of plantings in the eastern US, and in 2004, around 40% of US coal mining operations occurred in the natural range of American chestnut trees (Douglass 2007). This may also help to lessen any potential public unease about introducing a new species to the area, as it does not require the removal of any trees.

To start this study, small stands of hybrid chestnut would be planted in mine reclamation sites throughout eastern US forests. These stands would be closely monitored for signs of infection so that treatment with hypovirulent strains of *C. parasitica* could be applied as soon as cankers develop. Given the success of these stands, focus would then be switched to planting larger aggregates of hybrid chestnuts and facilitating their spread to established forests along the historical range of the American chestnut. Hopefully, by allowing the hybrids to reach maturity in afforested areas, the need for controlled burns is mitigated and the hybrids will have sufficient competitive ability for successful reintroduction without further aid.

Concurrently, transgenic strains of CHV-1 could be experimentally tested on isolated orchards of both hybrid and American chestnuts. Though the hybrid chestnut trees will be at least partially resistant to chestnut blight, the development of faster spreading hypovirulent *C. parasitica* strains may allow American chestnut trees to be planted alongside hybrid chestnut trees in the future. It may also allow the resurrection of American chestnut trees that currently exist as shrubs sprouting from large root systems. However, the introduction of a transgenic CHV-1 virus is likely to garner significant pushback, so this aspect of the study should be a long-term goal. Education programs may help gain public approval and should be started as soon as a promising strain is developed. This would also allow a faster response in the case that the reintroduction of hybrid chestnut trees fail due to insufficient blight resistance.

Conclusion

The American chestnut was a culturally and ecologically important tree in the eastern US. Though it has been functionally extinct for over half a century, we are now closer than ever to being able to reintroduce it to its historical range. In doing so, we must be careful to not rely too heavily on any one method of control for *C. parasitica*, as this may jeopardize the success of the new hybrid chestnut trees. Successful reintroduction would set an example for the restoration of other decimated plants and expand the scope of forest manipulation, so we must be critical of every step and evaluate the possible interactions and impacts. There are still many challenges associated with the restoration of the American chestnut tree, but the success of this effort may prove to be one of the most significant restoration efforts yet.

Resources

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