

Investigating the development and mycorrhizal communities of American, Chinese, and hybrid chestnut seedlings.

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Introduction

Eastern North American forests were once dominated by enormous American chestnut trees, until the introduction of a fungal blight killed every adult tree within a few decades, forever altering the ecology of the region.¹ The American Chestnut Foundation (TACF) has a breeding program to create blight resistant backcross hybrids between American and Chinese chestnuts, that are 94% American, for restoration of chestnut to its former range. In order to introduce the hybrid, successful seedling establishment will be imperative. For this, we must understand the role of ectomycorrhizal (ECM) fungal relationships in seedling success. Such ECM relationships have been shown to aid in the uptake of resources and protect against environmental stresses like drought,² with inoculated seedlings having improved development and survival when compared to uninoculated seedlings.³

American chestnuts gain ECM relationships from their surrounding environments⁴, but because the American chestnut went functionally extinct before the advent of modern mycorrhizal research, there is little known about such relationships and how they contribute to their development. This leaves a large gap in the knowledge that is likely going to have a monumental role in the recovery of our forests, as common mycorrhizal networks between individuals allows for the redistribution of nutrients to others that are “tapped in” to the same network and may be struggling. For example, if a seedling is in a shaded area and gets little sunlight, the ECM community is able to send extra nutrients from a well established tree in the same community. We do not yet know whether ECM community composition is impacted by chestnut genotypes, nor do we know how chestnut growth and ECM communities reinforce one another under different tree hybridization regimes. This research will provide data to ascertain the relationship between chestnut genotype and ECM community composition in the American chestnut’s natural range and environment.

Methods

At TACF’s Meadowview Research Plantation in Virginia, we randomly planted seeds of three chestnut types (100% American, 100% Chinese, and F₁ American-Chinese hybrids; Fig.1) in April 2025, in pure stands of each chestnut type and a non-ectomycorrhizal control stand composed of sugar maple (*Acer saccharum*). There were 66 seeds of each chestnut type (3) planted in each experiment stand (4) totalling 792 seeds. These seeds were sourced from TACF and/or commercial vendors. The seedlings were protected from predation by plastic tubes (Fig. 2) and wire fences. In mid-August 2025, we harvested five seedlings per site x seed combination (60 total) (Fig.3), leaving the remainder to grow another year. To investigate ECM colonization, roots were rinsed and 20 cm of fine root tips were taken from the top, middle, and bottom sections of the root system.^{5,6} About 100 root tips per seedling were randomly assessed for mycorrhizal colonization (percentage of sampled root tips that were colonized), using methods by Fischer and Colinas (1996 revised in 2014)⁷, under a dissecting microscope (Fig.4-5). Then fifteen to 25 root tips per seedling were collected for DNA barcoding to characterize the ECM communities within each stand. Barcoding will be done with high-throughput nanopore sequencing with UNCA's Oxford Nanotech^(R) Minion Sequencer (Fig. 6) and barcodes will be compared with the NIH GenBank using B.L.A.S.T. algorithm to identify the ECM sample to species. Species richness data will then be used to determine the ECM richness of the three monotypic stands and will be compared by calculating the Coefficient of Community (CC)⁸:

$$CC = 2c / (a + b)$$

Where a is the total number of species in the first community, b is the total number of species in the second community, and c is the total number of species in both communities.



Figure 1. Random planting scheme. Each flag color represents a different seed type.



Figure 2. Seeds were protected from predation with plastic tubes.



Figure 3. Harvesting seedlings.



Figure 4. Inoculated root tips of a Hybrid planted in a Chinese plot (587).



Figure 5. Inoculated root tips of a Chinese planted in the control plot (666).



Figure 6. Oxford Nanotech^(R) Minion Sequencer. Taken from the Oxford Nanopore Technologies website

Expected Outcomes/Discussion

Germination of Chinese seeds was delayed relative to the American and hybrid seeds, likely due to incomplete cold stratification. They were replanted with seeds from a different source six weeks after the initial planting

Root systems of harvested seedlings were not as established as expected, potentially because of competition with adjacent seedlings in the 0.33 m spacing. It is also possible that some root tips were lost in harvest from the compacted clay soil. Some seedlings were unharvested to allow for another season of growth and colonization.

This preliminary study will improve our understanding of the ECM communities that are developed in American, Chinese, and F₁ hybrid monotypic stands, as well as our understanding of the ECM colonization of chestnut seedlings, which is crucial to successful seedling establishment. ECM colonization and connection to common mycorrhizal networks can increase seedling survival and growth, especially in stressful environments.^{2,3} This study will provide information on the ECM communities maintained in mono-specific stands of different chestnut types and if there are any benefits to conspecific seedlings establishing in these stands through connection to common mycorrhizal networks with adult trees. We hope to have sequences by the end of the semester.

Literature Cited

- Jacobs DF, Dalgleish HJ, Nelson CD. 2012. A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. *New Phytologist*. 197(2):378–393.
- Van der Heijden MGA, Horton TR. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*. 97(6):1139–1150.
- Aryal P, Meiners SJ, Carlswald BS. 2020. Ectomycorrhizae determine chestnut seedling growth and drought response. *Agroforestry Systems*. 95(7):1251–1260.
- Reazin C, Baird R, Clark S, Jumpponen A. 2019. Chestnuts bred for blight resistance depart nursery with distinct fungal rhizobiomes. *Mycorrhiza*. 29(4):313–324.
- Caruso KE, Horton JL, Hove AA. 2021. Assessing the Effect of Eastern Hemlock (*Tsuga canadensis*) Decline from Hemlock Woolly Adelgid (*Adelges tsugae*) Infestation on Ectomycorrhizal Colonization and Growth of Red Oak (*Quercus rubra*) Seedlings. *The American Midland Naturalist*. 186(1).
- Walker JF, Miller OK, Horton JL. 2008. Seasonal dynamics of ectomycorrhizal fungus assemblages on oak seedlings in the southeastern Appalachian Mountains. *Mycorrhiza*. 18(3):123–132.
- Fischer C, Colinas C. 1996, revised 2014. Method for the evaluation of plant quality and mycorrhizal status of *Quercus ilex* seedlings inoculated with *Tuber melanosporum*.
- Baird, R, CE Stokes, A Wood-Jones, M Alexander, C Watson, G Taylor, K Johnson, T Remaley and S Diehl. 2014. Fleshy saprobic and ectomycorrhizal fungal communities

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