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Grant Title: Herbivory and Stress Responses of Outplanted Wild-Type American and Backcross Hybrid Chestnuts in Western North Carolina

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Ecological Discoveries: Advancing American Chestnut Hybrid Restoration Through Research and Education

Introduction

In 2022, a grant from the American Chestnut Foundation (TACF) provided critical funding to investigate the stress responses and herbivory patterns of wild-type and backcross hybrid American chestnuts in Western North Carolina. This pilot study, led by researchers at the University of North Carolina Asheville, aimed to address two of TACF's priority objectives: comparing the ecological functionality of different chestnut cultivars and evaluating their adaptation to abiotic and biotic stressors. With the overarching goal of aiding the reintroduction of this iconic species, the project explored how drought stress and herbivory interact to impact tree health, resilience, and the broader ecosystem surrounding chestnut trees. This research was motivated by a pressing need to develop robust planting strategies and to ensure the long-term survival of chestnuts in their native habitats amid changing climatic conditions and ecological pressures.

The American chestnut (*Castanea dentata*) once reigned as a keystone species in the eastern forests of North America, forming a vital component of the ecosystem by providing food, shelter, and economic resources. Tragically, the introduction of chestnut blight in the early 20th century decimated its populations, reducing this majestic tree to a memory in its native range. Efforts to restore the species began in the 1980s, and recent efforts by the American Chestnut Foundation have shown promise not only in producing hybrids that can survive the fungal threat but also in reestablishing ecological roles critical to forest health, including providing sustenance for wildlife and stabilizing food web dynamics.

However, the reintroduction of hybrid chestnuts into their native habitats is not without challenges. As these trees begin to reclaim their ecological niche, they bring with them the potential for unintended consequences, particularly with regard to insect interactions. The hybrid chestnuts may encounter a resurgence of pests, such as the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) and the lesser chestnut weevil (*Curculio sayi*), which threaten their growth and reproduction. Additionally, the influence of hybridization on insect herbivore diversity and abundance remains poorly understood. Insects, as primary consumers, play a significant role in shaping the health and structure of ecosystems, making it critical to study how these hybrids interact with native and invasive insect species.

Beyond pest management, reintroducing chestnut hybrids raises important questions about plant physiology, soil biodiversity and ecosystem functionality. Chestnuts contribute significantly to nutrient cycling and soil health, creating habitats for subterranean organisms essential for decomposition and nutrient retention. Soil biodiversity, in turn, supports aboveground plant health and forest productivity. Studying the interactions between chestnut hybrids and soil communities provides an opportunity to assess how these trees influence and are influenced by the intricate web of life belowground. As hybrid chestnuts adapt to environmental stressors like drought and herbivory, understanding their ecological role and physiological resilience will be pivotal to ensuring the long-term success of reintroduction efforts in restoring the American chestnut to its historic prominence.

This project aimed to evaluate how American chestnut hybrids interact with their environment by examining insect diversity and herbivory, soil biodiversity, and plant physiology. These objectives provide a comprehensive understanding of hybridization's ecological impacts, guiding efforts to restore this iconic species. The following methods outline the field and laboratory approaches used to achieve these goals.

Methods

To understand how American chestnut hybrids interact with their environment, researchers conducted a series of carefully designed field experiments in Western North Carolina. These studies focused on three types of chestnut stands: pure American chestnuts, hybrids with 94% American and 6% Chinese genetics, and hybrids with 75% American and 25% Chinese genetics. Scientists used a combination of survey and trapping methods and techniques to gather data on insect activity, herbivory, and soil health, as these are critical indicators of ecological balance. For example, pyramid-shaped traps were set up to collect insects from the trees, helping to identify which species were present and how they might be impacting the trees through herbivory or other interactions.

Additionally, researchers collected samples of soil and leaf litter to study the communities of tiny organisms living in and around the roots of the chestnuts. These

samples were carefully processed in the lab to identify different types of invertebrates, such as insects and arachnids, and to measure their diversity. This approach allowed scientists to explore how introducing chestnut hybrids might affect not only the plants themselves but also the broader ecosystem, including the important relationships between soil organisms and plant roots. By combining these methods, the team gained a comprehensive view of how chestnut hybrids function in their environment and what challenges they might face during reintroduction efforts.

In addition to these ecological assessments, researchers analyzed leaf physiology to measure how the trees adapt to their environment. They evaluated steady-state photosynthesis and diurnal patterns of water use by sampling leaves from the trees in controlled settings. By exposing leaves to different light levels, the team constructed light response curves to determine key physiological traits such as the maximum rate of photosynthesis, the efficiency of light use, and the balance point between photosynthesis and respiration. These measurements were complemented by daily tracking of water stress, using specialized instruments to measure water potential in the tree's xylem at various times of the day. This provided insights into how well the trees manage water under different environmental conditions. Together, these methods painted a detailed picture of how chestnut hybrids function physiologically and how they might perform in expanded conservation efforts.

Results

Aboveground Insect Diversity

The reintroduction of American chestnut hybrids has significant implications for aboveground insect communities. The study revealed eight major orders of insects, including Hymenoptera, Coleoptera, and Lepidoptera, across the different chestnut hybrid stands. Interestingly, while the total abundance of insects did not differ significantly among the hybrid types, there were marked differences in community composition. The 94% hybrid stands exhibited unique insect community structures compared to the 75% and pure American chestnut stands, driven in part by the presence of certain insect orders like Diptera and Coleoptera. Additionally, the study highlighted the impact of the Asian chestnut gall wasp (*Dryocosmus kuriphilus*), a parasitic wasp that forms galls on chestnut trees. Gall infestation was most severe in the 75% hybrids, with these trees being 3.7 times more likely to be infested compared to the 94% hybrids. Pure American chestnut trees showed no galling, perhaps due to their smaller size and resprouting growth habits. These findings emphasize how subtle genetic differences in hybrids influence both pest dynamics and insect community structure, underscoring the ecological complexity of chestnut hybrid reintroduction.

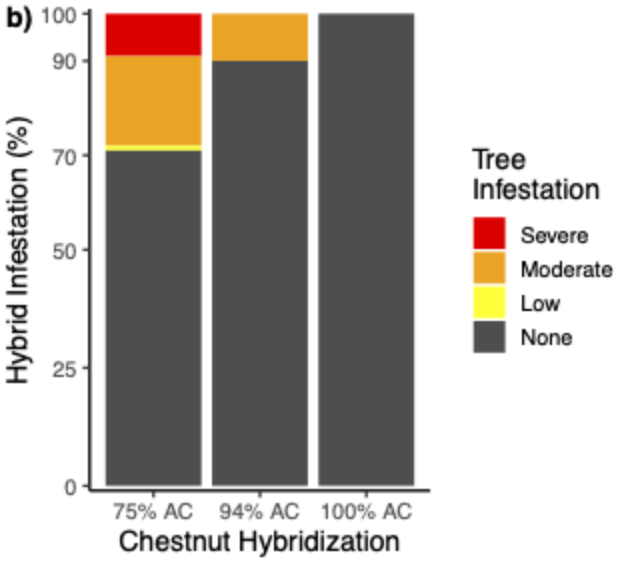

Figure 1: Asian Chestnut Gall Wasp infestation. Specifically, percent infestation across stands by Asian Chestnut Gall Wasp on American Chestnut Hybrids. Severity of infestation was assessed on a per tree basis. From Reed et al. 2024.

Soil Biodiversity

Belowground, the diversity of soil arthropods also varied across chestnut hybrid stands. Five primary arthropod classes, including Collembola, Arachnida, and Protura, were identified, with distinct differences between the 94% and 100% stands. Notably, the 94% hybrids showed greater overall soil biodiversity than the pure American chestnuts, which had a lower Shannon diversity index. These results suggest that the genetic makeup of the hybrids might contribute to more complex soil ecosystems, potentially enhancing nutrient cycling and soil health. However, seasonal variations also influenced the diversity, emphasizing the need for long-term monitoring to fully understand the implications of these findings.

Leaf Physiology

Physiological analyses of chestnut leaves provided crucial insights into the environmental adaptability of the hybrid stands. Steady-state photosynthetic parameters such as maximum net photosynthesis and yield varied among the 75%, 94%, and pure American chestnuts. The 94% hybrids demonstrated a higher yield and greater photosynthetic efficiency under optimal light conditions compared to the 75% hybrids. Meanwhile, diurnal measurements revealed that the 75% hybrids maintained higher gas exchange rates and showed better tolerance to midday water stress. These findings suggest that the 75% hybrids may be better suited for areas with fluctuating water availability, while the 94% hybrids align more closely with the physiological characteristics of pure American chestnuts.

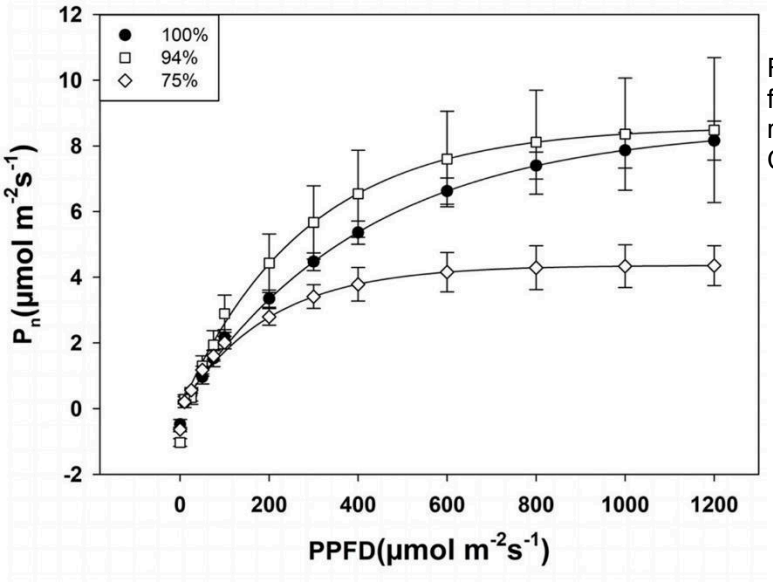

Figure 2. Steady-state light response curves for 100, 94%, and 75% stands. Points represent the mean (± 1 SE) of six trees. From Cleary et al 2024.

Impacts

This project has provided valuable insights into the ecological and physiological impacts of reintroducing American chestnut hybrids, advancing our understanding of their role in forest ecosystems. Aboveground, the study revealed distinct insect community compositions across hybrid types, shedding light on how genetic variations influence ecological dynamics. Belowground, the greater soil biodiversity observed in hybrid stands underscores the potential for hybrids to enhance ecosystem health and function. Physiological analyses highlighted the unique adaptations of different hybrid types, with the 75% hybrids demonstrating resilience under water stress and the 94% hybrids aligning more closely with the pre-blight characteristics of pure American chestnuts. These findings collectively inform strategies for the successful reintroduction of chestnuts into their native habitats.

Beyond its scientific contributions, this project had a profound impact on education and professional development. Six undergraduate students were actively involved in the research, with three directly funded through the American Chestnut Foundation grant. These students gained hands-on experience in fieldwork, data analysis, and scientific communication. Their work culminated in presentations at the annual conference of the Association for Southeastern Biologists, where they shared their findings with the broader scientific community. A particularly meaningful outcome for the students was developing a deep understanding of the legacy of the American chestnut and an appreciation for the cultural and ecological significance of its restoration. Furthermore, the students co-authored two publications resulting from this project, one of which has already been published, with the other currently in progress. These achievements demonstrate the power of collaborative research in fostering the next generation of scientists and advancing both ecological restoration and education.

This project has advanced our understanding of the ecological and physiological dynamics of American chestnut hybrids, revealing critical insights into their interactions with insect communities, soil biodiversity, and environmental stressors. By combining rigorous research with hands-on student involvement, we have not only informed strategies for the successful reintroduction of this iconic species but also fostered the next generation of scientists. The support from the American Chestnut Foundation was instrumental in achieving these outcomes, and we are deeply grateful for the opportunity to contribute to the restoration of a species so integral to both the cultural and ecological heritage of North America. Together, these efforts bring us closer to a future where American chestnuts once again thrive in our forests.

Figure 3: UNC Asheville undergraduate students working on the project. From top left to bottom right: Measuring chestnut leaf herbivory; collecting soil samples; group picture in the field; collecting pyramid trap specimens; evaluating photosynthesis; group work in the lab.

<u>Manuscripts resulting from funded work:</u>

- Reed*, J, E Hausler*, A Levinson*, JL. Horton, DS Willett, and CC Filgueiras. 2024. Ecological impact of American chestnut hybridization on invertebrate communities above and belowground. *Forests* 15:1159 https://doi.org/10.3390/f15071159 *<u>published in July 2024</u>*

- Cleary*, MS, JL Horton, and CC Filgueiras (*in review*). Investigating physiological differences among outplanted wild-type and hybrid chestnuts in western North Carolina. *Castanea. Submitted June 27, 2024*

Presentations from funded work

- Cleary*, MS, JL Horton and CC Filgueiras. Investigating physiological differences among outplanted wild-type and hybrid chestnuts in western North Carolina. Poster presentation. Annual Meeting of the Association of Southeastern Biologists March 20-23, 2024 Chattanooga TN

- Hausler*, E, JL Horton, and CC Filgueiras. Ecological impact of American chestnut hybridization on insect communities. Poster Presentation. Annual Meeting of the Association of Southeastern Biologists March 20-23, 2024 Chattanooga TN

- Middleton*, H and CC Filgueiras. Life underground: Exploring behavior of *Curculio sayi* larvae in soil. Poster Presentation. Annual Meeting of the Association of Southeastern Biologists March 20-23, 2024 Chattanooga TN

- Reed*, J, A Levinson*, JL Horton, and CC Filgueiras. Impact of American-Chinese chestnut hybridization on soil arthropod abundance and diversity and entomopathogenic nematode presence. Poster Presentation. Annual Meeting of the Association of Southeastern Biologists March 20-23, 2024 Chattanooga TN