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

Submitted to:
The American Chestnut Foundation
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A. Project Title

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

B. Summary

Our pilot study investigates how wild-type and backcross hybrid American chestnuts respond to herbivory and addresses two of TACF's priority topics: 2. compare the ecological functionality of backcross and transgenic American chestnuts to wild type American chestnut and 4. local adaptation and abiotic stress tolerance in wildtype, transgenic, and backcross hybrid populations of American chestnut. We will study seasonal patterns of water stress and herbivory in outplanted stands of American chestnut of different origin. This information will provide a baseline to help guide reintroduction of American chestnut to the mountains of western North Carolina.

C. Principal Investigators and Institutional Affiliation

Dr. Camila C. Filgueiras, Entomologist
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Dr. Jonathan Horton, Plant Physiologist
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D. Duration of Project

01 April 2023 - 31 March 2024: 12 Months

E. Total Amount Requested: $10,000

Matching Funding: $16,910

PI Filgueiras will devote 0.5 month of summer effort to the project, the value of which is calculated at $3,945 for salary ($71,000 annual salary / 9), with $1,380 for fringe benefits (which are calculated at 35% of the salary), for a total of $5,325.

Co-PI Horton will devote 0.5 month of summer effort to the project, the value of which is calculated at $4749 for salary ($85,488 annual salary / 9), with $1,663 for fringe benefits (which are calculated at 35% of the salary), for a total of $6,412.

Unrecovered indirect costs, which cover items such as office and lab space, general office and lab supplies, administrative support, etc., are calculated at UNC Asheville's federally negotiated indirect cost rate of 59.5% of the PI and Co-PI's salary for summer time and effort. For this project that totals $5,173.

Separately, PI Filgueiras previously received several grants supporting chestnut research, which provided the foundation for the work in this proposed project. Those grants are:

- Northern Nut Growers Association, $5,000; supported research with population biology and phenology of the lesser chestnut weevil.
- Northeast Sustainable Agriculture Research and Education Partnership Grant, $29,684; supported work developing biological control of the lesser chestnut weevil.

F. Project Goals

Short-term Goals:

1. Characterize patterns of water stress during the growing season for different cultivars
2. Quantify insect communities feeding on chestnut cultivars of different backgrounds.
    a. Determine which insects are most responsible for this herbivory
    b. Determine which chestnut backgrounds (e.g. wild-type and backcross hybrids) are most susceptible to herbivory.
3. Determine if there are interactive effects between drought stress and herbivory in different cultivars.
4. Evaluate physiological and stress responses of different cultivars to abiotic conditions

5. Train undergraduate students in research methods and educate them about the American chestnut

Long-term Goals:

1. Develop chestnut varieties that are more locally adapted (less susceptible to water stress and herbivory) using knowledge of the attributes and cultivar backgrounds that drive stress responses and herbivore preferences.

   To ensure that the continued efforts of TACF to restore the American chestnut are successful, plantings must be able to withstand and thrive in the presence of abiotic (drought) and biotic (insect herbivore) stress. The knowledge gained from this project will directly inform development and planting of less susceptible, more resilient cultivars.
2. Develop planting protocols and pest management strategies to maintain abiotic stress and levels of herbivory below damage thresholds.

   Current and future plantings of chestnuts may need to be managed by TACF and related stakeholders to reduce herbivore populations in order to ensure continued health of these iconic trees.
3. Train the next generation of researchers investigating and improving the American chestnut and supporting efforts of reestablishment.

G. Narrative

Background

Understanding how plants of different genetic makeup respond to a range of abiotic and biotic stressors can help guide restoration efforts and management. Because of the near total extirpation of American chestnut from the landscape in the last century, there is a dearth of modern ecological knowledge of the species (Jacobs et al. 2013). These knowledge gaps need to be addressed to help with successful reintroduction of transgenic and backcross hybrids that are resistant to chestnut blight. Two major hurdles for tree regeneration are localized drought (abiotic) and herbivory (biotic) impacts on establishing seedlings and saplings., which often interact. Drought stress impacts the ability of plants to fix and utilize carbon. While herbivory, involves loss of biomass, and often elicits carbon and nitrogen expensive defensive responses. The combined impact of drought stress and herbivory, both of which are predicted to increase with climate change (Motha and Baier 2005; Mitchell et al. 2013), may overwhelm establishing seedlings and saplings. Understanding how the magnitude and interplay between these two stressors affects tree growth and physiology is needed to help guide chestnut restoration efforts.

Drought stress reduces stomatal conductance which can limit plants' ability to fix carbon, thus reducing growth and altering carbon allocation patterns. Studies have been conducted on shade tolerance of pure American chestnut seedlings outside the range of chestnut blight (Joesting et al. 2009), on water stress in pure American chestnut seedlings (Bauerle et al. 2006), and in BC_3F_2 seedlings (Brown et al. 2014), but we did not find studies that compared abiotic stress responses among outplanted American chestnuts of different genetic origin. We have the opportunity to conduct a pilot study on outplanted stands of American chestnuts of different genetic origin at Dupont State Recreational Forest (DSRF) in western North Carolina.

In natural settings, these trees interact with a number of native and introduced insect herbivores and pollinators. These insect herbivores range from generalist leaf-feeding insects such as Japanese beetles (*Popillia japonica*) to specialist problematic pests such as the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) and lesser chestnut weevil (*Curculio sayi*). In its former role as the dominant foundation species in Appalachian forests, American chestnut trees undoubtedly hosted and supported many insect herbivores integral to the ecology of eastern forests. With expanded plantings of blight resistant varieties, the role that insect herbivores will play in shaping the ecology of chestnut forests is unknown. On one hand, restoring chestnut trees in eastern forests could increase the diversity and abundance of herbivores that depend on chestnuts to complete their life cycle. On the other hand, insect herbivore populations may become unbalanced, threatening the continued expansion of this iconic tree.

The dearth of knowledge on this species and its wide range highlight the need for regionally-specific information on herbivory patterns on outplanted blight-resistant hybrids. Previous work found that of different cultivars can vary in the amount of carbohydrates in leaves; hybrid chestnuts can demonstrate differing amounts of growth; and some herbivores such as the gypsy moth (*Lymantria dispar dispar*; Rieske et al. 2003; Post and Parry 2011), Asian chestnut gall wasp (Anagnostakis et al. 2010; Anagnostakis 2012), and lesser chestnut weevil (Filgueiras *unpubl. data*) can perform differently on different cultivars. These findings highlight that effective management depends upon knowledge of cultivar effects on herbivory that can affect efficacy of entomopathogen controls (Brown et al. 2020). These observations, coupled with observations about levels of other herbivores, seem to suggest a large degree of variation in herbivory on chestnut trees, likely governed by cultivar background.

<u>Need</u>

To ensure continued expansion of the iconic American chestnut, we need to understand responses to abiotic (drought) and biotic (herbivory) stresses across chestnut backgrounds. Specifically, we need:

- An estimate of field-based drought stress and plant morphological and physiological responses to that stress.
- A baseline understanding of levels of herbivory - on leaves, nuts, and other parts of the trees - in forests of the eastern US for chestnuts of different genetic makeup.
- An understanding of the factors that drive the observed differences in herbivory.
- An understanding of how herbivory and drought stress might impact expanded chestnut plantings in a changing climate.

<u>Proposed Work</u>

Study Site

We propose to conduct a pilot study on outplanted chestnut trees of different genetic origin at DSRF in western North Carolina. We have a long-standing research relationship with Jordan Luff, Managing Forester at DSRF, and co-PI Horton is a member of the DRSF Advisory Committee. There are three stands of outplanted American chestnut at DSRF that differ in their genetic makeup. One stand is composed of BC_1F_1 (75% American/25% Chinese) trees planted in 2009. A second is composed of 100% American chestnut trees from seeds collected along the Blue Ridge Parkway in NC that were planted in 2012. The last is composed of BC_3F_3 (94% American and 6% Chinese) trees planted in 2014. These three stands are all in close proximity and share similar environments.

Assessing Levels of Drought Stress and Responses

Local environment in each stand will be monitored with soil temperature, air temperature and humidity, precipitation, and soil moisture sensors (HOBO Sensors and dataloggers, Onset Computer Corp, Bourne MA). These data will be monitored to identify periods when trees are well watered and times when they are experiencing soil water stress (soil water potential < 1.5 Mpa). During each period (well watered and droughted) plant water potential will be measured at predawn and mid-day on ten randomly chosen trees per stand using a Scholander-type Pressure Chamber (Scholander et al. 1964; PMS-1000, PMS Instruments, Albany, OR). Additionally, leaf gas exchange (photosynthesis and transpiration) will be measured in the morning (7:00 – 10:00) and afternoon (1:00 – 3:00) under ambient conditions of temperature, RH, and CO_2 concentrations using a Portable Photosynthesis System (Li-6400, LiCor Biosciences, Lincoln, NE). From these measurements, several parameters (e.g. diurnal change in plant water potential, instantaneous water-use-efficiency, etc.) can be compared among the different genetic stocks of American chestnut and related to abiotic conditions.

Assessing Herbivory Patterns

We will conduct insect and leaf surveys to understand baseline levels of herbivory across cultivar backgrounds. Leaf surveys will be conducted alongside assessments of plant drought and responses in order to potentially correlate observations. Leaf surveys will be conducted by randomly selecting a minimum of 10 leaves on 10 trees of each available genotype and backcross and photographing them with leaf-area-eaten processing software. These leaves will be standardized by leaf stage and tree age to facilitate comparisons. In addition to leaf-area-eaten, each leaf will be analyzed as to the type of herbivore (to functional taxonomic group with a focus on insect herbivores) causing the herbivory.

Visual observations will be made, and any insects actively present on examined trees will be collected. During the course of insect surveys, we will pay close attention to catkin production and assess observed pollinators including any insects present on the flowering parts of the plants. To do so, observed insects will be aspirated and compared with insects caught in co-located pyramid traps. These traps catch a broad range of insects on chestnut trees. The results of these surveys will provide us with indices of alpha and beta diversity, species occurrence metrics, and indicator species information on a cultivar basis which can be used to shed light on which cultivars foster the most diverse communities.

Trees will also be assessed for Asian gall wasp using counts of galls as a primary metric. The results of these surveys will be: metrics of leaf area consumed, number and type of insect feeding, and counts of galls on a per cultivar basis. Additionally, a nut survey will be conducted at the end of the season where 100 chestnuts from each cultivar background will be collected and monitored for lesser chestnut weevil emergence. The result of the nut survey will be: metrics of numbers of nuts infested, number of weevils per nut, and timing of emergence.

Assessing Characteristics Affecting Herbivore Preference

To understand the factors driving the herbivory, we will conduct physiological assessments of the trees while standardizing by leaf stage, tree age, and location. In tandem with the leaf surveys described above, leaves will be assessed for toughness using a penetrometer and trichome density through visual inspection under magnification. These results will give us an idea about physical plant defenses across cultivars. Presumably leaves that are tougher (i.e. require more force for the penetrometer to puncture) and with higher trichome densities are better defended and less palatable to herbivores.

Impact

Together, understanding drought susceptibility and response, baseline levels of herbivory on chestnuts of different backgrounds in eastern forests, and understanding the factors that drive herbivore preference, will provide a better understanding of managing the reintroduction of American chestnut to forested landscapes. We will be able to compare drought responses among wild-type and two different backcrosses in natural field settings. Additionally, we expect to find that chestnut cultivars can support a broad level of feeding by diverse insect herbivores that contribute to a healthy functioning ecosystem. We also expect that we will identify one or two insect species that could require management.

Additionally, understanding the impact of drought and herbivory on plant growth and fitness is paramount for successful reintroduction of American chestnut in Appalachian forests. In addition to herbivory assessments, on each tree we examine, we will capture metrics of plant growth such as diameter, height, annual branch elongation, and nut production (if any). This should allow us to associate observed levels of drought, herbivory, and insect communities with chestnut performance and health.

The pilot study proposed here is the first step in reaching the long-term goal of effective reintroduction of chestnuts. The initial data gained from this project will provide insights critical to establishing a long-term integrated strategy to support chestnut and chestnut ecosystem health. The data and insights generated in this project can be used to inform additional experiments, control strategies, breed and select resistant cultivars, and identify potential problem areas before they arise. The intent of our research team is to continue working with TACF to pursue these questions and use the results from this year's pilot study to inform continued research on herbivory and stress response of American chestnuts.

Educational Outcomes

This work will directly involve and support undergraduate researchers who will be participating in the project. This direct, hands-on research and exposure to the ecology of American chestnut and interaction with TACF will be both a meaningful opportunity for the students and the broader community and an opportunity for TACF to interact with the next generation of researchers in this area. Conducting this work at DSRF contributes to their goal of educating the public on different forest management goals and activities and will continue ongoing collaborations between UNC Asheville and DSRF.

References

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Brown AJ, Newhouse AE, Powell WA, Parry D. Comparative efficacy of gypsy moth (Lepidoptera: Erebidae) entomopathogens on transgenic blight-tolerant and wild-type American, Chinese, and hybrid chestnuts (Fagales: Fagaceae). Insect science. 2020 Oct;27(5):1067-78.

Brown CE, Mickelbart MV, Jacobs DF. Leaf physiology and biomass allocation of backcross hybrid American chestnut (*Castnea dentata*) seedlings in response to light and water availabilty. Tree Physiology. 2014. 34:1362-1375.

Dalgleish HJ, Lichti NI, Schmedding N, Swihart RK. Exposure to herbivores increases seedling growth and survival of American chestnut (Castanea dentata) through decreased interspecific competition in canopy gaps. Restoration Ecology. 2015 Sep;23(5):655-61.

Jacobs DF, Dalgleish HJ, Nelson CD. A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. New Phytologist. 2013. 197:378-392.

Joesting HM, McCarthy BC, Brown KJ. Determining the shade tolerance of American chestnut using morphological and physiological leaf parameters. Forest Ecology and Management. 2009. 257:280-286.

Mitchell PJ, Battaglia M, Pinkard EA. Counting the costs of multiple stressors: is the whole greater than the sum of the parts? Tree Physiology 2013. 33:447-450.

Motha RP, Baier W. Impacts of present and future climate change and variability on agriculture in the temperate regions: North America. Climate Change. 2005. 70:137-164.

Post KH, Parry D. Non-target effects of transgenic blight-resistant American chestnut (Fagales: Fagaceae) on insect herbivores. Environmental entomology. 2011 Aug 1;40(4):955-63.

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H. Timeline

	2023									2024		
Activity	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
Prepare Field Season												
Purchase Supplies	■	■	■									
Train Students	■	■	■	■								
Organize & Map Field Plots		■	■									
Install Environmental Sensors and Datalogger		■	■									
Field and Lab Work												
Physiological Measurements			■	■	■	■						
Leaf Samples			■	■	■	■	■	■				
Insect Samples			■	■	■	■	■	■				
Insect Identification							■	■	■	■		
Leaf Evaluation						■	■	■				
Bioassays					■	■	■	■				
Climate Assessment									■	■	■	
Analysis and Report												
Data Analysis									■	■	■	
Report Writing										■	■	■
Paper Writing										■	■	■

I. Results

Data and metadata will be collected throughout the project and stored in tabular form in the cloud under management by PI Filgueiras. Results from this project will be presented in an annual report based on the work completed for this proposal. Results from this project will also be presented at the NE 1833 meeting and the annual meeting of the entomology society. Undergraduate students will present their work at UNC Asheville's Undergraduate Research Symposium and regional meetings, such as the Association of Southeastern Biologists Annual Meeting. In addition to publication in peer-reviewed journals, the results will be shared with stakeholders through extension publications.

J. Funding Breakdown

Budget

Expense	Amount Requested
Undergraduate Student - Insect Survey	**$3,600**
Undergraduate Student - Plant Physiology Assessment	**$3,600**
Fringe benefits	**$551**
Travel	**$1,499**
Lab & Field Supplies	**$750**
Total	**$10,000**

Budget Justification

Student Wages

Funds are requested to support two undergraduate students during the summer months ($15/hr at 20hrs/wk for 12wks, or $3,600 each) to work on this project. One student will be under the direct supervision of PI Filgueiras and will be responsible for conducting insect surveys and herbivory assessments. The second student will be under the supervision of Co-PI Horton and will be responsible for plant physiology assessments.

Fringe benefits

Funds are requested for FICA for the undergraduate students, which is calculated at 7.65% of the salaries and wages.

Travel

Funds are also requested to support travel to and from field sites in order to collect data. These funds will be used to rent fleet cars and pay for gas. Much of the field work will take place at Dupont State Recreational Forest (DSRF). Round trip from UNCA to DSRF averages 100 miles at current state vehicle rates ($0.48/mile), round trip costs will be $48. We plan 2-3 trips/week for 12 weeks of the summer field season.

Lab & Field Supplies

Funds are requested to support purchase of supplies critical to this project. These include a leaf penetrometer ($500), insect field traps ($100), and disposables ($150). These disposables include petri dishes and generalist herbivores for bioassays, labeling equipment, plastic bags, zip ties, etc., necessary for conducting field and laboratory trials. Purchase of most of these supplies will be part of preparing for the season in April, May, and June.

K. CVs

Camila C. Filgueiras, PhD - Abbreviated CV

Education:

Ph.D.	Federal University of Lavras, Lavras, Brazil	2015
M.S.	Federal University of the Rural Amazon, Belem, Brazil	2010
B.S.	Federal University of Lavras, Lavras, Brazil	2008

Professional Experience:

2022-Present	Assistant Professor, Department of Biology, UNC Asheville
2021-2022	University Faculty Fellow, Department of Biology, UNC Asheville
2018-2021	Research Associate, Department of Entomology, Cornell University, Cornell AgriTech at the NYS Agricultural Experiment Station, Geneva, NY
2012-2015	Graduate Research Associate, Department of Entomology, Federal University of Lavras
2014-2015	Docent, McGuire Center for Lepidoptera and Biodiversity
2013-2014	Consultant, Network of Organic Producers, MG, Brazil
2010-2012	Technical Coordinator, AgroTeste Inc.
2011	Visiting Scholar, Department of Entomology, University of Illinois

Professional Societies/Service:

Entomological Society of America, Society of Nematology, Metabolomics Society, The American Chestnut Foundation

Extension & Selected Presentations

2021	Managing the emerging pest on Chestnuts. Northeast Organic Farming Association of New York 2021 Winter Conference. (Invited Presentation).
2020	Biological Control Options for Specialty Crops. Orange County Cornell Cooperative Extension Meeting.
2020	Phenology and Biological Control of the Chestnut Weevil. (Invited Presentation - postponed due to COVID).
2020	Strategies for Managing Chestnut Weevil. Conversations with members of the New York Tree Crop Alliance. January-November 2020.
2020	Beneficial Nematodes to Manage Maggots. 2020 Orange County Onion School.

Selected publications (2015-present):

Filgueiras & Willett. 2022. Phenology and monitoring of the Lesser Chestnut Weevil (*Curculio sayi*). Insects. Invited Submission to Special Issue. Under Review.

Filgueiras & Willett. 2021. Non-lethal effects of entomopathogenic nematode infection. Scientific Reports.

Pereira, **Filgueiras**, Doria, Penaflor, & Willett. 2021. The effects of biostimulants on induced plant defense. Frontiers in Agronomy.

Barrett, **Filgueiras**, & Willett. 2021. Using *Cucumis sativus*, *Acalymma vittatum*, *Celatoria setosa*, and generalist pollinators as a case study for plant–insect interactions. Arthropod-Plant Interactions.

Agnello, Combs, **Filgueiras**, Willett, & Mafra-Neto. 2021. Reduced infestation by *Xylosandrus germanus* in apple trees treated with host defense compounds. Journal of Economic Entomology.

Crandall, Gold, Jimenez-Gasco, **Filgueiras**, & Willett. 2020. A multi-omics approach to solving problems in plant disease ecology. PLOS One.

Willett, **Filgueiras**, Benda, Zhang & Kenworthy. 2020. Sting nematodes modify metabolomic profiles of host plants. Scientific Reports.

Pereira, **Filgueiras**, Willett, & Penaflor. 2020. Sight unseen: belowground feeding influences the distribution of an aboveground herbivore. Ecosphere.

Filgueiras, Martins, Pereira, & Willett. 2019. The ecology of salicylic acid signaling: primary, secondary, and tertiary effects with applications in agriculture. International Journal of Molecular Science.

Sabino, Negrisoli, Andalo, **Filgueiras**, Moino, & Sales. 2018. Combined application of entomopathogenic nematodes and insecticides in the control of leaf-miner (*Tuta absoluta*) on Tomato. Neotropical Entomology.

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Filgueiras, Willett, Junior, et al. 2016. Stimulation of the salicylic acid pathway aboveground recruits entomopathogenic nematodes below- ground. PLOS ONE

Filgueiras, Willett, Pereira, et al. 2016. Eliciting maize defense pathways aboveground attracts belowground biocontrol agents. Scientific Reports

Filgueiras, Willett, Junior, et al. 2016. Stimulation of the salicylic acid pathway aboveground recruits entomopathogenic nematodes below- ground. PLOS ONE

Jonathan L. Horton, Ph.D. – Abbreviated CV

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Education

2000 - 2003	Postdoctoral Fellowship, Plant Physiological Ecology, Virginia Tech
1996 - 2000	Ph.D. Forestry, Northern Arizona University
1993 - 1996	MS Biology, Appalachian State University
1986 - 1990	BS Biology, University of North Carolina

Professional Experience

2020 – present	Professor and Chair, Biology Department, UNC Asheville
2016 – present	Professor, Biology Department, UNC Asheville
2009 – 2016	Associate Professor, Biology Department, UNC Asheville
2003 – 2009	Assistant Professor, Biology Department, UNC Asheville
2000 - 2003	Postdoctoral Fellow, Virginia Tech
1997 - 2000	EPA-STAR Research Fellow, Northern Arizona University

Professional Societies/ Service:

Ecological Society of America, Southern Appalachian Botanical Society, The American Chestnut Foundation

Recent Publications (2015 – present * denotes undergraduate author)

Caruso* KE, **JL Horton**, and AA Hove. 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. *American Midland Naturalist* 186:16-34.

Sabo*, IA, JR Ward, HD Clarke, and **JL Horton**. 2019. Partial-root harvest of American ginseng (*Panax quinquefolius* L.): a non-destructive method for harvesting root tissue for ginsenoside analysis. *Castanea* 84:310-321.

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<u>Current and Pending Support</u>

Filgueiras, CC and JL Horton (this proposal). $10,000. Herbivory and Stress Responses of Outplanted Wild-Type and Backcross Hybrid American Chestnuts in Western North Carolina. The American Chestnut Foundation.

L. COI

There are no known conflicts of interest for either of the PIs conducting this project.