THE AMERICAN CHESTNUT FOUNDATION
Research Proposal 2024

a. Project Title
Conservation of rare American chestnut genotypes through modified nut grafting and speed breeding pollen production

b. Summary (not more than 100 words)
Our research team will continue to develop and implement a modified nut-grafting technique as previously reported (Burdine et al. 2024). The current proposal will provide funding to assist in the establishment of up to four germplasm conservation orchards (GCO) as well as a speed breeding facility for accelerating pollen production. We have two GCO sites in the early stages of establishment (south and northeast MS) and are aiming to develop two additional sites (north MS and east-central MS). The speed breeding facility will be established at Mississippi State University (MSU) in cooperation with Drs. Joshua Granger (MSU) and Thomas Klak (University of New England, UNE).

c. Principal Investigator(s) and Institutional Affiliation(s)
Charles S. Burdine, USDA Forest Service, Mississippi State University
C. Dana Nelson, USDA Forest Service, Southern Research Station

d. Duration of project
Two years, November 2024 through October 2026.
*Additional funding will be requested for year two

e. Total amount requested. Please list sources and amount of matching funding for the same project.
Total requested: $10,000

We are funding a MS student's training on chestnut propagation and genetics and the effort to make and care for 1000-1200 grafts in the winter/spring/summer of 2024/25.

f. Short and long-term goals of the project
Short-term goals:
- Establish southwestern origin American chestnut grafts into GCOs to conserve this valuable germplasm
- Implement speed breeding at MSU to accelerate pollen production and breeding of American chestnut grafts

Long-term goals:
- Develop multiple GCOs within the southwestern portion of the American chestnut range using scion sources from Mississippi and northwest Alabama.

- Produce enough pollen from grafted American chestnut genotypes, that originated in the southwestern portion of the range, to share with other interested parties for breeding.
- Perform controlled crosses to produce locally adapted and resistant seedlings that can be returned to the location where the scion material used for grafting was collected.

g. Narrative (no more than five (5) pages)

Background

The Southern Institute of Forest Genetics (SIFG, SRS-4160) has grafted 1100-1200 American chestnut and chinquapin scions for each of the past three grafting seasons. The grafting technique implemented was originally developed by retired SIFG scientist, Warren Nance. During the first season (2021/2022) of large-scale grafting, all scions were sourced from American chestnut and chinquapin genotypes located in the wild throughout the northeastern quadrant of Mississippi. With assistance from TACF, additional cooperators supplied scion material during the 2022/23 season. This provided an opportunity to further asses the robustness of the grafting technique and the inclusion of the added material expands the geographic range the grafts represented. During the last two years, grafts include genotypes collected from Mississippi, Alabama, Georgia, Kentucky, and Arkansas. Success rate is variable depending on the source of the scions, cultivar used as root stock, and possibly the scion x root stock combination. However, 60-70% grafting success rates have been achieved each year. The partnership continued during the 2023/24 season and TACF cooperators provided additional scion material. The results from the last grafting season were similar, but are still be analyzed and will be presented at TACF's 2024 Annual Meeting in Cromwell, CT. For the upcoming grafting season (2024/25) we will attempt 1000-1200 grafts propagating both our rare MS and northwest AL genotypes as well as scions provided by TACF

Evidence has shown that American chestnut individuals in southwestern portion of the range or more genetically diverse (Sandercock et al. 2022). This genetic variation may provide increased adaptability to various environmental factors and this important for TACF breeding and ultimately species restoration. The number of ramets available from each of the rare southwestern genotypes grafted allows for preservation in the greenhouse, establishment in GCOs, and forced pollen production using speed breeding protocols developed by Dr. Tom Klak (personal communication). We will establish ramets of each genotype in two GCOs that have been initiated. In addition, we will evaluate two additional sites for GCOs to make sure we cover the range of environments in MS and provide ample back-ups for all genotypes. In addition, we will establish ramets of each genotype in an indoor environment meant to speed reproductive development using high light and nutrition under constant long-days (adapting Klak's speed breeding protocol). We will measure growth and flower production on these trees, collect and store pollen for TACF collaborators, and make crosses if female flowers develop.

Approach

Goal 1: Establish southwestern origin American chestnut grafts into GCOs to conserve this valuable germplasm

Objective— Establish new grafted genotypes at two existing (near Saucier and Columbus, MS) and evaluate two potentially new GCO sites (near Holly Springs and near Meridian) for future development

Methods— Plant, protect, and maintain grafted trees at two GCOs. Record survival and measure heights to monitor progress of the different genotypes and root stocks. The GCOs are located at the SIFG campus near Saucier, MS in far south Mississippi and on private land near Columbus, MS (northeast MS) controlled by Mississippi Wildlife, a non-profit partner in our American chestnut conservation work. Both GGOs are deer-fenced, but we will experiment with trees shelters (Plantra grow tubes) to enhance chestnut development by additional protection against predation and enhancement of the grafts' micro-environment. At least 6 ramets of each genotype will be planted in randomized complete blocks at each site. Planted grafts will be monitored annually for survival and growth.

Goal 2: Implement speed breeding at MSU to accelerate pollen production and breeding of American chestnut grafts

Objective— Adapt SRS's seed lab at MSU for speed breeding and evaluate grafted and seedling American chestnuts for vegetative and reproductive development

Methods— Develop speed breeding protocol for American chestnut grafts to accelerate both male and female flower production. We will establish at least three ramets per genotype (and American chestnut seedlings as controls) in an indoor environment meant to accelerate reproductive development using high light and nutrition under constant long-days (adapting Klak's speed breeding protocol). We will measure growth and flower production on these trees, collect and store pollen for TACF collaborators, and make crosses if female flowers develop.

h. Timeline, showing start and completion dates for each goal

- Goal 1— GCO establishment
    - Assess GCOs sites (two already initiated) in Mississippi to layout plantings of newly grafted American chestnuts
        - November and December 2024
    - Plant grafted American chestnuts into the two initiated GCOs
        - February-March 2025
    - Tend and measure grafted trees. Pring through Fall 2025.

- Goal 2— Speed breeding
    - Consult with Dr. Tom Klak to better understand speed breeding protocol and obtain recommendations for implementing at MSU October 2024
    - Retro-fit SRS seed lab at MSU for speed breeding—purchase and add lights and irrigation set-up
        - November/December 2024- January 2025
    - Plant grafted American chestnuts (and American chestnut seedlings as controls) in containers in speed breeding environment

        - March 2025
    - Monitor trees for reproductive and vegetative development
        - Anticipate first pollen collection by August/September 2025
        - Monitor reproductive development monthly August 2024-November 2026
        - Measure tree size quarterly from time of planting through November 2026

i. How results will be measured and reported

- Goal 1—GCO establishment
    - We will score the initial and final survival of the grafted American chestnuts at both GCO locations. The initial score will be taken in June 2025 and the final in October 2025. The trees will be measured for height the time of planting and at the end of the growing season (October 2025). The results will be reported to TACF upon request and by November 2025 and November 2026.

- Goal 2—Speed breeding
    - On a monthly basis through 2025 and 2026, we will monitor the American chestnut grafts and control American chestnut seedlings for reproductive and vegetative development. Male and female flowers will be counted and recoded by month of first appearance. Tree heights will be collected every three months, starting at the time of planting. The results will be reported to TACF upon request and by November 2025 and November 2026.

j. Breakdown of how and when funds will be spent

We will spend the funds between November 2024 and October 2025.

Approximately $3200 is requested to support (equipment and supplies) the establishment and maintenance of grafted American chestnuts in the two GCOs, and approximately $5300 is requested to support (equipment and supplies) the retrofit of the seed lab at MSU for speed breeding and establishment of grafted American chestnuts. The total of ~$8500 does not include overhead. SRS's usual 16% overhead would put the total closer to $9800.

k. Brief Curriculum Vitae (CV) for each Principal Investigator, including recent publications and grants received. Please restrict each CV to two (2) pages.

Attached

l. A Conflict of Interest or Commitment (COI or COC) statement. If a COI or COC is known, please document them here. If there is no known COI or COC, please certify as such with a statement in this section.

Co-PI Nelson is on TACF's science advisory board and will need to be recused from evaluating proposals this year. Also, co-PI Nelson is not a member of TACF due to Conflict-of-Interest concerns.

m. Bibliography

Burdine, C.S., C.K. Parker, W.L. Nance, E. Galeano, C.D. Nelson. 2023. Conserving Mississippi-origin American chestnut genotypes— Initial results with a modified nut-grafting method. In: Proc. 37th South. Forest Tree Improvement Conf., June 20-23, 2023, Knoxville, TN, pp. 28-30. https://sftic.org

Klak, T. Restoring the American chestnut tree by speed breeding. News Center Maine YouTube channel. https://www.youtube.com/watch?v=aKHCmxWu7LE#:~:text=UNE%20Profesor%20Thomas%20Klak%20is%20speed

Sandercock, A.M., J.W. Westbrook, Q. Zhang, H.A. Johnson, T.M. Saielli, J.A. Scrivani, S.F. Fitzsimmons, K. Collins, M.T. Perkins, J.H. Craddock, J. Schmutz, J. Grimwood, and J.A. Holliday. 2022. Frozen in time: Rangewide genomic diversity, structure, and demographic history of relict American chestnut populations. Molecular Ecology 31(18):16. https://doi.org/10.1111/mec.16629.