CONSERVING MISSISSIPPI-ORIGIN AMERICAN CHESTNUT GENOTYPES—INITIAL RESULTS WITH A MODIFIED NUT-GRAFTING METHOD [1]

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Often considered the greatest ecological disaster in North America, the loss of the American chestnut (*Castanea dentata*) to chestnut blight (caused by *Cryphonectria parasitica*) greatly impacted the Appalachian forests along with local and regional economies (Clark et al. 2019, 2023). Thanks to tree breeding efforts made by various entities; blight-resistant seedlings are anticipated to become widely available in the next few years. However, before reintroduction to the forest, it is necessary to develop and produce both resistant and locally adapted seedlings. Fortunately, coppice regenerating stumps in the wild provide an opportunity to preserve local genotypes by establishing germplasm conservation orchards through clonal propagation. In addition, it is expected that American chestnut genotypes that evolved in the most southwestern area of the species' range (i.e., northeast Mississippi) will prove to be valuable sources of germplasm for breeding trees that are adapted to warmer environments. In the last couple of years, the Southern Research Station (SRS) has made substantial progress in developing a modified nut-grafting technique that is resulting in the conservation of rare American chestnut genotypes located in natural forests in Mississippi and adjacent Alabama.

Methods: In November 2022, we obtained seeds from Chinese chestnuts (C. *mollissima*) and American chestnuts from two cooperators, respectively-- Ron Revord, University of Missouri and Steve Hartman, Scottsville, Kentucky. During 2022/2023, additional cooperators provided scions for grafting in addition to scions we collected from the Mississippi and Alabama trees. In total, scions from 76 genotypes were collected from four areas within the southern US (northwest Alabama, northern Georgia, western Kentucky, and northeast Mississippi) (Table 1). The seeds for rootstock were germinated in December 2022 using peat pots and potting media (Fig. 1A). Once the seedlings were around 5 cm tall, they were cut for grafting using a razor blade (Fig. 1B-C) and grafted, with the graft unions being secured with fly-fishing line (Fig. 1D). The grafts were maintained in a growth-room with high relative humidity and artificial light (Fig. 1E). After 2-3 weeks in the growth-room, the surviving grafts were transplanted into half-gallon pots and transferred to a shadehouse (Fig. 1F). At various times during the season, the number and percentage of successful grafts for the two rootstock species and the four scion-areas were scored.

Results: We found that the peat pots and media allowed for good seedling growth and development and our environmental settings (temperatures, relative humidities, and light) in the growth-room (Fig. 1E) and shadehouse (Fig. 1F) provided adequate conditions for graft survival. Also, we noted that transplanting to one-gallon pots (Fig. 1F) at the time of transition to the shadehouse tended to be beneficial for survival (data not shown). Overall, for the 2022/2023

[1] Paper published in the Proceedings of the 37th Southern Forest Tree Improvement Conference, June 20-23, 2023, Knoxville, TN, http://www.sftic.org. pp. 28-30.

grafting season, 67.4% of the grafts survived through the transition to shadehouse stage, with modest differences observed among scion origins, ranging from 76.7% for north Georgia to 61.1% for northeast Mississippi (Table 1). In terms of rootstocks, Chinese chestnut resulted in a higher percentage of successful grafts overall, at 78% compared to 42% for American chestnut.

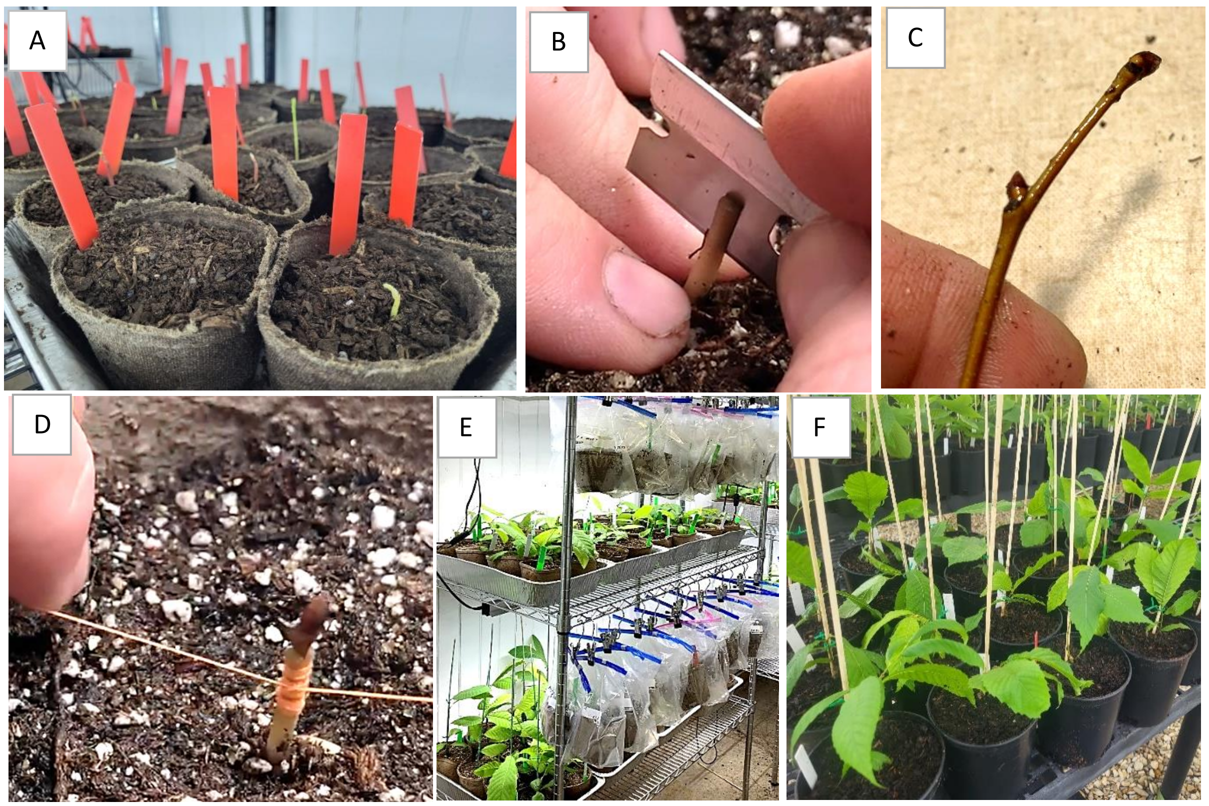

Fig. 1. Preparation of the American and Chinese chestnut rootstock and the modified root-grafting method. **A.** Rootstock germination using peat pots and potting media. **B.** Preparing the rootstock using a razor blade to cut the tissue. **C**. Scion ready to be grafted. **D**. Securing graft union using fly-fishing line. **E**. Acclimating the grafts from higher to lower humidity and appropriate light quality in the growth-room. **F**. Transferring the surviving grafts into half-gallon pots and placing them in the shadehouse.

Table 1. Results achieved in 2023— attempted and successful grafts and number of scion genotypes by state of scion-origin.

State of scion-origin	Surviving grafts	Attempted grafts	% Surviving grafts	No. genotypes grafted
Alabama	157	224	70.1	20
Georgia	165	215	76.7	19
Kentucky	126	187	67.4	14
Mississippi	248	406	61.1	23
Total	**696**	**1,032**	**67.4**	**76**

Discussion: In comparison to our results, researchers in New Zealand observed a significant range in success rates (0 to 100%) in grafting 18 Castanea spp. selections (Oraguzie et al. 1998). The choice of rootstock is essential for successful chestnut grafting and graft failure can be attributed to scion genotype and environmental conditions (Ackerman and Jayne 1980; Oraguzie et al. 1998). In our case, it took several attempts to modify the standard nut-grafting method for chestnut. Key adjustments included timing of scion collection, 1- or 2-bud scion preparation, rootstock preparation, type of cutting blade, and acclimation of grafted plants from one stage to the next. Previous studies have supported the importance of the choice of blade and scion preparation for grafting success (Ackerman and Jayne 1980; Xie et al. 2022). Through experience, we found that maintaining adequate post-grafting microclimates was critical for graft survival. Most importantly, was high relative humidity in the growth-room during the post-grafting acclimation phase (Fig. 1E). In our hands, the modified nut-grafting method has proven quite robust over the initial two seasons of scaling-up the number of grafts (the overall survival rate in 2021/2022 on over 1000 grafts was 63%, Nance and Parker, unpublished data). In addition, grafting on Chinese chestnut rootstock appears to be a good option (Ackerman and Jayne 1980), as survival rates were higher than grafting on American chestnut and the grafts will likely be resistant to Phytophthora root rot (Crandall et al. 1945).

Acknowledgments: We thank Gay Flurry (Southern Research Station) for assisting with rootstock preparation and tending the grafted plants and Ron Revord (University of Missouri), Jamie Van Clief (TACF Southern Regional Science Coordinator), and Steve Hartman (Kentucky chestnut grower) for their collaboration and supply of rootstocks and scions.

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