Comparing four Nursery Production Methods on Chestnut Hybrid Seedling Quality

Summary

Seedling quality is important for successful restoration projects and nursery propagation techniques can affect seedling morphology to increase the likelihood of survival. Four production methods, two standard and two novel, will be compared across several measures of seedling health - height, root collar diameter, number of first-order lateral roots, and root volume, in order to determine which technique produces seedlings most likely to survive out-planting. Total cost per seedling will be determined to provide additional information for nurseries and practitioners who would like to produce their own chestnut hybrid seedlings for restoration plantings.

Principal Investigator(s) and Institutional Affiliation(s)

Taylor Evans: Master's Student - James Madison University Department of Biology

Heather Griscom: Professor - James Madison University Department of Biology

Duration of project: November 2019 - April 2021

Total amount requested: $1500 and 580 BC3F4 seeds.

Short and long-term goals of the project

Short term - Compare the effect of propagation techniques on several measures of hybrid seedling health while accounting for overhead and labor costs so that nursery manager and restoration practitioners can perform cost-benefit analysis for planting.

Long term - Plant seedlings from each treatment to assess the effect of propagation technique on seedling survival, height, basal diameter, and age at first mast. Test air-prune bed methodology for propagating chestnuts at planting site to reduce transport cost and transplant shock.

Narrative

Introduction

Successful restoration of chestnut hybrids is reliant on both genetic resistance to chestnut blight (*Cryphonectria parasitica*) and the long-term survival and reproduction of resistant individuals. The extensive breeding program to confer genetic resistance to chestnut blight has produced individuals capable of surviving to breeding age - survival of seedlings to reproductive maturity is especially important in re-establishing the hybrid chestnuts within temperate forests in the original range of American chestnut. Producing field-ready seedlings able to survive abiotic stresses and compete effectively with existing vegetation will require a renewed focus on seedling quality assessment (Oliet 2012).

There are a variety of methodologies suitable for the propagation of hardwood tree species, each with their advantages and disadvantages for both seedling quality and overall cost. We intend to compare four propagation methods, the Root Production Method®, bare-root, bare-root air-prune, and small containers, to determine which propagation method produces the highest quality seedlings at the lowest cost to the grower and, ultimately, to restoration practitioners. While various hardwood species, such as *Quercus* spp, have been studied regarding the effect of propagation method on species survival and overall health (Dey 2004; Walter 2013; Van Sambeek 2016) there is a gap in research on the effect of propagation methodology on the quality of

American chestnut seedlings. There are several morphological characteristics of both root and shoot architecture that are predictive of field planting success for hardwood seedlings including root volume, number of first order lateral roots, shoot height, root collar diameter, and biomass (Jacobs 2005). Additional research has suggested that root system development and preservation upon transplanting may be of particular importance in determining survival as seedlings mitigate water stress by establishing root-soil contact through the proliferation of new roots (Davis, 2005). Bare-root trees are often the most cost effective means of procuring large quantities of plant material for large planting projects. Without containers or soil to transport, moving bare root trees is often preferable to containerized seedlings, though when planted out in the field, bare-root seedlings can experience greater transplant shock and reduced survival from a reduction in the overall root system and a resulting increase in shoot:root ratio (Watson and Syndor 1987; Struve 2009) when compared to seedlings grown in containers (Wilson 2007). Transplant success is also greatly decreased if bare-root seedlings are planted outside of tree-dormancy (Struve, 2009; Richardson-Calfee and Harris, 2005). Container grown systems have shown reduced shock due to the transfer of the entire root system of the tree upon transplanting (Davis 2005) but may be subject to circling roots which may decrease drought stress tolerance, nutrient uptake capacity, and anchorage, resulting in a reduced lifespan (Warren and Blazich 1999).

The Root Production Method® (RPM®) developed by Wayne Lovelace of Forrest-Keeling nursery is a multistep propagation procedure that involves weighing and selecting the heaviest seeds which are then stratified in a mesh flat under moist conditions at 34°F for several months using a medium of four parts composted rice hulls, four parts pine bark, and two parts sand by volume (35% to 40% air pore volume). These flats are then moved to bottomless tables in a heated greenhouse in early February to initiate germination. In early March 50% of the largest and most vigorous seedlings are selected and transplanted into low (2.75”x2.75”x5.5”) plastic band containers using the same medium. After sixty days, these seedlings are moved outside under mist to harden off for 48 hours before being transplanted into larger, 2.5 gallon container containing the same growing medium for the remainder of their approximately 210 day growing cycle (Lovelace, 1998, 2002). RPM® has been shown to produce seedlings with greater average height and basal diameter, more fibrous root systems, earlier age at first nut production, and higher survival in sub-optimal site conditions compared to bare-root seedlings (Walter 2013; Van Sambeek 2016; Dey 2004) (Figure 1). RPM® seedlings are labor and infrastructure intensive to produce, requiring several transplants and culling of 50% of the seedlings produced, in addition to the transport of containerized seedlings. There have been several studies (Dey 2004; Walter 2013; Van Sambeek 2016) comparing the quality and survival of RPM® and bare-root seedlings that have primarily focused on *Quercus spp.* suggesting that other members of the *Fagacea* family, such as American chestnut, should respond similarly to RPM® propagation treatment.

Figure 1: Comparison of root structure of bare-root (left) and RPM seedling (right) at 210 days.

Bottomless bare-root beds, henceforth labeled as air-prune beds, have the potential to offer the best of both worlds by producing seedlings with the fibrous root systems of RPM® without the need for large quantities of individual containers and the potential for root circling. Air-pruning of seedling roots by growing trees in bottomless containers has been shown to produce seedlings with larger, more fibrous root systems with increased survival when planted in harsh, i.e. compacted or poorly drained, sites (Dey 2004) while preserving the ease of transport of standard bare-root seedlings. For example, bare-root air-pruned tree seedlings in the *Quercus* genus were found to establish more robust fibrous root systems compared to standard bare-root seedlings growing in soil (Dey 2004; Walter 2013). These beds can be built with lightweight growing media for ease of transportation and can be fitted with covers to protect seedlings from predation. The modular nature of these beds allow them to be scaled up and down with project needs and transported to the site itself for propagation, potentially reducing the time between removal from the bed and transplanting. While bottomless containers are common in tree propagation, there is a gap in research on the effects of growing seedlings in bottomless raised beds and its effects on both cost and quality of seedlings.

Seedling quality, planting density, and planting costs are all considered when determining the least-cost approach to achieving a desired stocking density (Dey 2008; Van Sambeek 2016) with seedling quality often compromised. Cost-benefit analysis should also be taken into consideration when choosing seedling stock for large plantings. Labor and material intensive cultivation techniques, such as RPM® and containerized seedlings, are more expensive than their bare-root counterparts to purchase and transport. For example, RPM® Hybrid chestnut stock sold as containerized seedlings are sold at $14.25-$14.75 per RPM® seedling compared to $1.51 for a 24-inch bare-root seedling from Forrest-Keeling nursery. The higher up-front cost may make them inaccessible for large plantings. However, research is still needed to determine if increased survival and decreased cost of replanting may even costs over time (Van Sambeek 2016). This proposed research will provide additional insight into the relationship between nursery production techniques and seedling quality. The goal is to disseminate results to restoration practitioners so that they can propagate their own planting stock at a reasonable cost to produce the highest quality seedlings for out-plantings.

Methodology

Site Location

Seedlings grown outdoors will be planted in open-field conditions in Rockingham County, Virginia. Seedlings undergoing greenhouse treatments will be located at James Madison University in Harrisonburg, Virginia.

Seed Collection and Storage

Of the 580 hybrid chestnut seeds received, 280 will be graded for use in the RPM® treatment by selecting the heaviest 50% (n=140). The remaining 50% will be mixed back into the remaining seeds for use in the other treatments. This seed will be separated into four equal lots of approximately 145 seeds each. Three lots will be stored under refrigeration in plastic bags with moist peat moss at 34°F until March before planting. The seed selected for use in the RPM® treatment will be sown in bottomless mesh flats (Treepots TR6 Flat, 15.75”x15.75”x7.5”) filled halfway with a potting medium consisting of composted rice hulls, pine bark, and sand (4:4:2 by volume) amended with slow release fertilizer with micronutrients (Scotts® Osmocote 19-6-2), mycorrhizal spores (MycoApply® Ultrafine), and a wetting agent (Miracle-Gro® Water Storing Crystals) creating a growth medium with a 35% to 40% air pore volume. Germination flats will be wrapped in plastic and held at 34°F until March before planting.

Growing Medium

Each lot of seeds will be split into three sub-treatments with three different growing mediums – field soil collected from the experiment site, potting mix used recommended by The American Chestnut Foundation (1/3 peat, perlite, and vermiculite by volume), and potting mix used in the RPM® process (4 parts pine bark, 4 parts rice hulls, 2 parts sand by volume).

RPM®

Germination flats will be removed from cold-storage and moved into a greenhouse in early February to germinate. In early March, the largest and most vigorous seedlings graded by height will be selected (50% cull rate, n=70) and potted in plastic bottomless band containers (2.75” x 2.75” x 5.5”). Band containers will be split into three groups of 23 filled with field soil, TACF potting mix, and RPM® potting mix. All treatments will include the same fertilizer concentration. These containers will be placed on a wire bench in a greenhouse and grown for approximately 60 days. Seedlings will be monitored bi-weekly and watered as needed. In early May, seedlings will be moved outside for hardening off for two days. After two days seedlings will be transplanted into seventy, 2.5 gallon pots in three treatments using the same three potting mixes used in the band pots. Pots will remain outside underneath a 5’x10’x3’ cage covered in ¼” hardware cloth to prevent rodent predation. Pots will be visited bi-weekly, checked with a soil moisture probe, and irrigated as needed until early December - approximately 210 days.

Figure 2: An example of a raised, bottomless air-prune bed. Source: Twisted Tree Farm.

Air-Prune

Three, 3’x4’x8” beds lined with 1⁄4” hardware cloth will be constructed in November, placed on cinder blocks, and filled with fertilized field soil, fertilized TACF potting mix, or RPM® potting mix (Figure 2). One lot of seeds will be removed from cold storage in March and planted 1” deep on 5” centers in the beds. A 3’x5’x3’ cage will be installed over each bed, covered in ¼” hardware cloth to prevent predation from rodents. Beds will be visited bi-weekly, checked with a soil moisture probe, irrigated as needed, and have competing plants removed.

Bare-root

One 3’x12’ bed will be tilled and prepared in November – one 3’x4’ section will be left with fertilized field soil and the remaining 3’x8’ section will be excavated to a depth of 14” and the field soil removed for use in other treatments. The bed will be split into three, 3’x4’ sections divided by a rhizome barrier. One section will contain amended field soil, one section will contain fertilized TACF potting mix, and one section will contain RPM® mix. One seed lot will be removed from cold storage in March and planted 1” deep on 5” centers in the bed. For protection, a 12” rhizome barrier will be installed 12” deep around the perimeter of the bed and a 3’x12’x3’ cage will be installed over the bed, covered in ¼” hardware cloth to prevent predation from rodents. Beds will be visited bi-weekly, checked with a soil moisture probe, irrigated as needed, and have competing plants removed.

Containers

Treepots (n = 145, 4”x4”x14”) will be split into three groups and filled with fertilized field soil, fertilized TACF mix, or RPM® mix. Seeds will be removed from cold storage in March and planted 1” deep in the pots. Treepots will be stored in a greenhouse for the growing season and will be visited bi-weekly, checked with a soil moisture probe, and irrigated as needed.

Measurement and Analysis

After one year, measured by the date that seedlings were placed in cold storage (approximately November 2021), seedlings from each treatment will be removed from the treatment site and measured for height, root collar diameter, number of first-order lateral roots greater than 1mm, and root volume. An ANOVA analysis and appropriate post-hoc tests will be performed for each measurement (data may need to be transformed) across the four treatments to test for significant differences.

Costs for each production method will also be calculated by totaling overhead costs and recording time spent on planting and management of each treatment group including time spent preparing/assembling beds, planting, cultivating, and harvesting in order to determine labor costs. These totals will be used to determine a cost per tree for each treatment.

Post-measurement

After seedling measurement is complete; a minimum of twenty trees per propagation treatment will be out-planted at a site in West Virginia for continued measurement and comparison of survival, height, basal diameter, and age at first mast.

Treatment Timeline

Month	***RPM®***	***Air-Prune***	***Container***	***Bare-root***
Nov 2019	Make potting mix, Grade and stratify Chestnut Seeds	Make potting mix, Stratify chestnut seeds	Make potting mix, Stratify chestnut seeds	Till/prepare bed, Stratify chestnut seeds
Dec 2019	Build rodent cages	Build beds/rodent Cages		Build rodent cages
Jan 2020				
Feb 2020	Move seeds in trays to greenhouse			
Mar 2020	Measure and cull seedlings (50%), transplant to band pots	Plant seeds in beds	Plant seeds in containers	Plant seeds in beds
Apr 2020	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep
May 2020	Transplant into 2.5 gallons pots, move outside	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep
June 2020	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep
July 2020	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep
Aug 2020	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep
Sept 2020	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep
Oct 2020	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep	Monitor/upkeep
Nov 2020	Data collection; Out-planting	Data collection; Out-planting	Data collection; Out-planting	Data collection; Out-planting

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Timeline, showing start and completion dates for each goal.

Timeline	Nov 2019	Dec 2019	Jan 2020	Feb 2020	Mar 2020	Apr 2020	May 2020	Jun 2020	Jul 2020	Aug 2020	Sep 2020	Oct 2020	Nov 2020	Dec 2020	Jan 2021	Feb 2021	Mar 2021	Apr 2021
Stratify Seeds	🟥	🟥	🟥	🟥	🟥													
Build/Prepare Beds	🟪	🟪																
Plant					🟥													
Monitor/Upkeep						🟨	🟨	🟨	🟨	🟨	🟨	🟨						
Data Collection													🟩					
Data Analysis													🟦	🟦	🟦			
Prepare Report																🟪	🟪	
Submit Report																		🟥

How results will be measured and reported.

This project will be prepared as a Master’s thesis with the goal of submitting the report results as a peer-reviewed publication. Results will also be reported to the public via TACF and presentations at regional conferences.

Breakdown of how and when funds will be spent

Item	**Amount**	**Purchased**
Three, 2"x8"x16'	$30.00	November 2019
Eight 1"x2"x8'	$24.00	November 2019
Thirty-four, 3/4" x 5' PVC Pipe	$85.00	November 2019
Fourteen, 3/4" x 10' PVC Pipe	$38.50	November 2019
Forty, PVC Side outlet connectors	$80.00	November 2019
Eight, PVC T-connectors	$6.00	November 2019
Four, PVC coupling	$2.00	November 2019
Zip-ties	$10.00	November 2019
One, 1/4" Hardware Cloth, 3'x100'	$124.00	November 2019
One, 1/4" Hardware Cloth, 3'x50'	$64.00	November 2019
One, 1/4" Hardware Cloth, 2'x50'	$28.00	November 2019
50’ Rhizome Barrier	$62.00	November 2019
70 3”x3”x5.5” Bottomless pots	$43.50	November 2019
70 10”x7” 2.5 Gal pots	$49.00	November 2019
153 Tree Pots/Containers 4”x14”	$136.00	November 2019
TR6 TreePotTrays	$79.00	November 2019
14 cu ft Rice Hulls	$20.00	November 2019
14 cu ft Pine Bark	$24.00	November 2019
7 cu ft Sand	$49.00	November 2019
14 lb Osmocote 19-6-12 Fertilizer	$52.00	November 2019
48 oz Wetting Agent	$24.00	November 2019
Mycorrhizal spores	$30.00	November 2019
12 cu ft Peat Moss	$40.00	November 2019
12 cu ft Perlite	$111.00	November 2019
12 cu ft Vermiculite	$126.00	November 2019
Shipping and Tax	$163.00	November 2019
Seeds	Provided by TACF	November 2019
TOTAL	**$1,500.00**	

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PROFESSIONAL PREPARATION

- **Professor 2017 - present**, **James Madison University**; 2011-2017, Associate Professor, 2006-2011, Assistant Professor
- 2004-2006, Visiting Professor, Sweet Briar College, Sweet Briar, Virginia
- 2000-2004, Ph.D., Forest Ecology, 2004, School of Forestry and Environmental Studies, Yale University and The New York Botanical Garden, Ashton Lab.
- 1993-1997, B.A., Biology, Smith College, Northampton, MA

SELECTED REFERRED JOURNAL ARTICLES *(N=21) (* denotes undergraduate/graduate collaborator)*

Thyroff, E*, **Griscom. H.** 2019. Experimental Study of Soil and Aspect on American Ginseng in an Appalachian Cove Ecosystem. ***Natural Areas Journal.*** In press (July).

Horstman, E., Ayon, J., **Griscom, H.** 2018. Growth, Survival, Carbon Rates For Some Dry Tropical Forest Trees Used In Enrichment Planting in the Cerro Blanco Protected Forest on the Ecuadorian Coast. ***Journal of Sustainable Forestry.*** 37 (2): 82-96. doi: 10.1080/10549811.2017.1387153

Hartman, CJ, DeMars, C., **Griscom, H**, Butner, H. 2017. Assessment of Undergraduate Students' Environmental Stewardship Reasoning and Knowledge. ***International Journal of Sustainability in Higher Education****.* 18 (4): 492-502, doi: 10.1108/IJSHE-07-2015-0128

Edgar, B.*, **H. Griscom**. 2017. The Effect of Controlled Burns on Abundance of Woody Species in Appalachian Pine-Oak Forests at Buck Mountain, West Virginia. ***Natural Areas Journal.*** 37 (1): 30-38.

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Maskiewicz, A., **Griscom, H**. & Welch, N. 2012. Using targeted active-learning exercises and diagnostic question clusters to improve students' understanding of carbon cycling in ecosystems. ***Life Sciences Education. CBE.*** 11. 58-67.

Meister, K. M.S. Ashton, D. Craven, **H. Griscom***.* 2012. Carbon dynamics of tropical forests. *In* M.S. Ashton, M.L. Tyrrell, D. Spalding & B. Gentry. (eds.), Managing Forest Carbon in a Changing Climate. Springer-Verlag, New York, NY.

TEACHING

Assistant/Associate/Full Professor (2006-Present) James Madison University, Harrisonburg Virginia

- Majors courses include: *Ecology and Evolution* lecture and lab (93 students), *Forest Ecology* lecture and lab (20 students), *Tropical Ecology and Restoration* (10 students),
- Graduate courses include: Seminar in *Tropical Ecology,* Seminar in *"The Art of Reviewing Manuscripts in Ecology and Evolution"; Restoration Ecology* (12 graduate students)

Visiting Professor (2004-2006), Department of Biology, Sweet Briar College, Virginia

- Majors courses include: *General Ecology, Field Natural History, Plant Biology, Physiological Plant Ecology, Introduction to Cells,* and *Introduction to Biology* Lab.
- Non-Majors Courses include: *Plant and Human Affairs*

FUNDED GRANTS – post-dissertation (Total = $90,000.00)

- ***The American Chestnut Foundation.*** Reintroduction of chestnuts into an Appalachian cove ecosystem. 2014-2016. $1500 (in addition to 400 hybrid chestnuts seeds)
- ***Jeffress Memorial Trust.*** Shifting Competitive Hierarchy? Implications for Restoration of the American chestnut, *Castanea dentata*. Supplemental Grant 2011-2013. $10,000.
- ***USDA Forest Service***, Southern Research Station. Predicting the future forest underneath a dying eastern hemlock (*Tsuga canadensis*) stand in a headwater ecosystem. Implications for native brook trout (Salvelinus fontinalis) populations. Supplemental Grant, 2010-2011. $10,000
- ***Jeffress Memorial Trust.*** Shifting Competitive Hierarchy? Implications for Restoration of the American chestnut, *Castanea dentata*. Supplemental Grant 2008-2009. $10,000.
- ***Jeffress Memorial Trust.*** Shifting Competitive Hierarchy? Implications for Restoration of the American chestnut, *Castanea dentata*. 2007-2008. $25,000.
- ***USDA Forest Service***, Southern Research Station. Predicting the future forest underneath a dying eastern hemlock (*Tsuga canadensis*) stand in a headwater ecosystem. Implications for native brook trout (Salvelinus fontinalis) populations. A proposal to the Southern Research Station, USDA Forest Service 2007-2008. $20,000.
- Summer Support For Undergraduate Research. James Madison University. 2008, 2013, 2016 $8000
- ***Faculty Assistance Summer Research Grant***. James Madison University. 2007, 2014. $6000.
- ***Seed Research Grant***. Sweet Briar College. 2006. $1000

PROFESSIONAL MEMBERSHIPS

ESA (Ecological Society of America), **ASB** (Association of Southeastern Biologists), **SEB** (Society of Economic Botany), **NAJ** (Natural Areas Journal), **TACF** (The American Chestnut Foundation)

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PROFESSIONAL PREPARATION

- 2018-present M.S., Biology. James Madison University. Expected Graduation May 2021
- 2009-2013 B.S. with Honors, Biology. James Madison University.

RESEARCH

Evans, T. Griscom, H. 2013. *The Effect of Evergreen Understory on Forest Regeneration after Tsuga Canadensis Removal.* James Madison University Honor's Thesis.

AWARDS

2009-2013 STEM Second Century Scholar

PROFESSIONAL MEMBERSHIPS

TACF (The American Chestnut Foundation)

SERVICE

- 2018-Present, Black's Run Forest Farm. Nursery maintenance and riparian restoration
- 2019, Cumberland Island National Seashore. Trail restoration.

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.

There is no known COI or COC