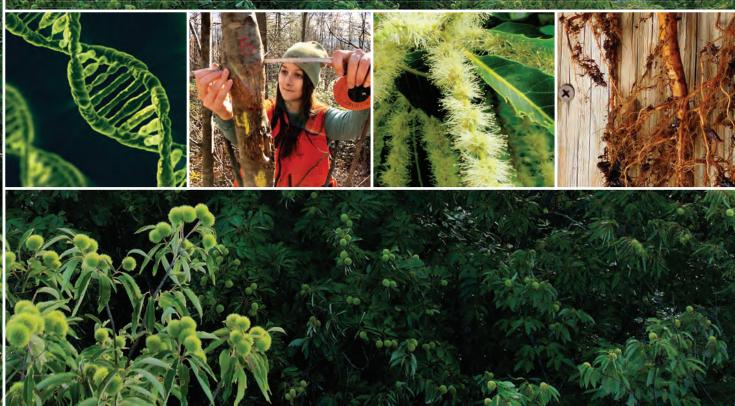


ANNUAL REPORT July 1, 2023 – June 30, 2024

# ADAPTING to a changing world





ANNUAL REPORT July 1, 2023 - June 30, 2024

Regional Reports 7



Dear colleagues and friends:

As an ecologist by training, I am often drawn to the ideas of adaptation and resilience, which can be used to tell the story of the American chestnut. In thinking about post-glacial retreat, I wonder how it was that chestnut was able to adapt and survive so successfully to become a dominant tree in eastern forests? Was it better able to flourish across the blank canvas of a landscape scraped bare by post-glacial retreat? Were the seeds so palatable and nutritious to animals that they were carried far and wide across the landscape? Was it able to take advantage of microclimatic conditions created through landscape disturbances over time? These and other questions speak to the resilience and adaptation of this majestic tree, which has a more complex genetic resistance than we previously thought.

In December 2023, TACF made the difficult decision to discontinue its support of the Darling 58 American chestnut due to significant performance limitations. We realize that our research efforts must transition towards solutions that provide better opportunities for meeting these complex challenges. Mother nature has truly shown us that ecology is hard.

A Research Advisory Council (RAC), made up of external researchers, was also assembled. This group assisted TACF science leadership by providing new directions in developing blight resistance, helping to advance our mission of restoring the American chestnut to its native range. The RAC provided an initial set of recommendations in July 2024, which are being incorporated into the 2025-2027 Strategic Plan. We look forward to sharing more about this in future communications.

The RAC is also contributing to a donor-supported workshop that will explore biotechnology and molecular genetic techniques, and will expand the network of scientific partnerships.

I am excited about the new direction that we are taking with science at TACF. We do this in partnership with our staff, board, membership, and volunteers. Sometimes taking a few steps backwards allows us to take large leaps forward. We are continuing with our efforts to develop both a more resilient tree as well as a more resilient organization. I look forward to writing to you next year about our new directions and successes.

With best regards,

Elist af

Liz Kramer, Board of Directors Chair The American Chestnut Foundation

The American Chestnut Foundation 2023 - 2024 Annual Report

#### SCIENCE MILESTONES

By Jared Westbrook, TACF Director of Science

### **RECURRENT GENOMIC SELECTION**

### to improve pathogen resistance in American chestnut populations

Breeding disease resistance into American chestnut is more complex than initially envisioned. Many genetic variants, each with small effect, influence blight resistance. We are also introducing resistance to Phytophthora root rot (PRR), which has limited American chestnut reintroduction in the South. Finally, we aim to represent sufficient genetic diversity from the wild population so that the species has capacity to adapt to a changing world. Recurrent genomic selection (RGS) is the main strategy we are pursuing to accomplish these objectives. Genomic selection involves associating trees' DNA profile with population-wide variation in disease resistance. The correlation between genotype and disease resistance enables us to make accurate predictions of trees' resistance from DNA alone, given the trees for which we are predicting resistance are closely related to the trees we have evaluated for resistance. Predicting trees' disease resistance enables us to select the most resistant individuals for planting in seed orchards, where they will begin to produce large quantities of seed with improved resistance in the next 7 to 10 years. "Recurrent" refers to performing multiple generations of genomic selection to incrementally improve resistance. RGS is currently the global standard method to improve complex traits, such as yields in crops or milk production in cows, which are controlled by many unknown genes.

#### OUR RGS STRATEGY IN CHESTNUT INVOLVES TWO MAIN STEPS:

- O Controlled crosses between American/ Chinese hybrid parents that maximize average blight and/or PRR resistance while also maintaining majority American chestnut ancestry (e.g., > 70%).
- Genotyping to select roughly 10% progeny that have disease resistance greater than their parents while retaining majority American chestnut ancestry.

We will repeat the cycle of controlled crosses and selection of improved progeny until average population resistance exceeds that required for long-term survival. We started building the foundation for RGS over the last six years by assessing more than 5,500 trees across 100 orchard locations for eight stem traits related to trees' long-term blight resistance. We also partnered with Dr. Jason Holliday's Forest Population Genomics Lab at Virginia Tech to genotype approximately 5,000 trees to assess their American chestnut ancestry and predict resistance of trees that have not yet been evaluated. Over the last two years, we have begun making controlled crosses between selected parents.

This year, we partnered with Dr. Hill Craddock's and Dr. Martin Cipollini's labs at the University of Tennessee at Chattanooga and Berry College, respectively, to evaluate the average blight resistance of 1,015 seedling progeny from 30 families where the parents varied in average estimated blight resistance and *C. dentata* ancestry. As we have observed in the past, there was a trade-off between family mean blight resistance ratings and average *C. dentata* ancestry of the

#### FIGURE 1. SMALL STEM ASSAY (SSA) RESULTS FOR BLIGHT RESISTANCE 2024 trials at The University of Tennessee at Chattanooga and Berry College.

Family mean blight resistance of progeny from American chestnut backcross hybrids

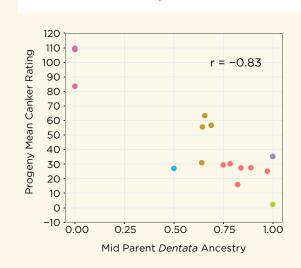
(● = backcross x F<sub>1</sub>, ● = backcross x backcross) COMPARED TO:

One large surviving American chestnut family ( $\bigcirc$ ), three resistant Chinese chestnut families ( $\bigcirc$ ), one intermediately resistant Chinese x American F<sub>1</sub> family ( $\bigcirc$ ), and one susceptible American chestnut control family ( $\bigcirc$ ).

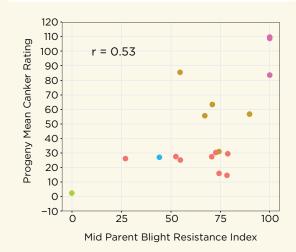
Family mean resistance of families in the SSAs CORRELATED WITH:

Average C. dentata ancestry

of the parents



Average blight resistance index values of the parents estimated from eight parental blight stem traits

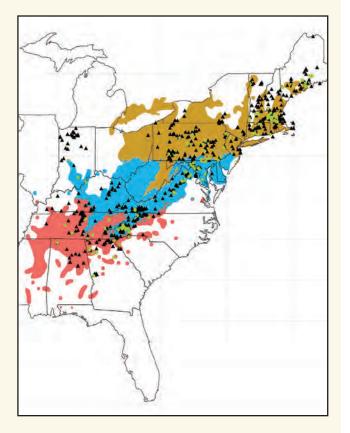


#### SCIENCE MILESTONES

parents (r = -0.83). However, four first *C. dentata* backcross families, where the parents had ~ 66% *C. dentata* ancestry on average, had mean progeny blight resistance ratings ranging from 55 to 85 on a 0 = average American chestnut, to 100 = average Chinese chestnut scale (Figure 1, previous page). This means backcross trees that inherited a majority of their genome from American chestnut have captured substantial heritable blight resistance. Further gains in resistance may be made through genomic selection of the most resistant individuals in these families. Another interesting result from this study was that we observed significant

#### FIGURE 2. MAP OF THREE BREEDING ZONES

where breeding activities will be regionalized, which includes location of our current orchards  $(\bigcirc)$  and wild type American chestnuts used in breeding  $(\blacktriangle)$ .



heritable blight resistance in American chestnut. We crossed two progeny of so-called large surviving American chestnuts (LSA) that survived the original chestnut blight epidemic. The mean blight resistance rating of this family exceeded the susceptible *C. dentata* control (LSA mean = 35 v. *C. dentata* mean = 2), suggesting that blight resistance in 100% *C. dentata* progeny may be further improved through selection of the most resistant parents and progeny.

#### OUR PLAN IS TO PURSUE THREE BREEDING TRACKS WITH BACKCROSS HYBRIDS TO

- maximize blight resistance,
- balance gains in blight and PRR resistance simultaneously, and
- maximize PRR resistance.

#### PLUS, A FOURTH NON-HYBRID BREEDING TRACK TO

 maximize blight resistance among progeny of large surviving American chestnuts

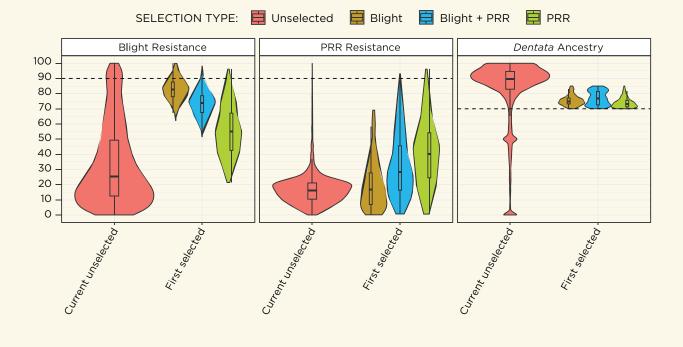
We are pursuing this fourth, non-hybrid breeding track to maximize blight resistance in the progeny of large surviving American chestnuts (LSAs). To represent genetic diversity from C. dentata, we also plan to regionalize breeding efforts into three breeding zones (northeast, central, and southern) corresponding to three genetically distinct American chestnut subpopulations (Figure 2; Sandercock et al. 2024). Our main priority for all three breeding regions will be to maximize blight resistance, but for the central and southern zones, we will also pursue breeding tracks 2 and 3 to incorporate PRR resistance as this disease is expected to limit chestnut reintroduction in these regions. For the hybrid breeding track to maximize blight resistance (track 1), genetic simulations predict that it is possible to increase population mean blight resistance to a mean of 82 via intercrossing selected backcross hybrid and LSA parents and genomic selection of the top 10% most blight-resistant progeny. For the track that balances gains in blight and PRR (track 2), predicted mean resistance is expected to be 69 and 40 for blight and PRR resistance, respectively. For the PRR resistance breeding track (track 3), we estimate that resistance to this disease can be improved to a mean of 49 after the first generation of selection. For breeding tracks 1-3, the *C. dentata* ancestry of the selected progeny is expected to vary between 70% and 85% with a mean of 75%, indicating that large gains in resistance may be balanced with selection for majority American chestnut ancestry. For the LSA breeding track (track 4), simulations predict that mean resistance of the first-generation selections can be improved to 61 (**Figure 3**). Over the next 20 years, we are optimistic that through a recurrent genomic selection program, we could develop populations where a subset of the progeny exceeds our thresholds for long-term blight or PRR resistance. To this end, approximately 10% of trees with the highest putative blight resistance (based on genomic selection), will be planted in seed orchards at Meadowview, a site in North Carolina, and Pennsylvania State University over the next three to five years. Ultimately, it will be necessary to inoculate the genomically selected trees with chestnut blight and compare their resistance to American chestnut and Chinese chestnut controls to confirm or update

#### FIGURE 3. NEXT STEPS FOR BREEDING TO IMPROVE BLIGHT AND PRR RESISTANCE in backcross hybrid and large surviving American chestnut populations.

Predicted genetic variation (%) in blight resistance, PRR resistance, and American chestnut ancestry among top 10% selected from three hybrid breeding tracks to

maximize blight resistance, *and* blight and PRR resistance, and *and* maximize PRR resistance.
 We will also pursue a fourth breeding track to maximize blight resistance in the progeny of large surviving American chestnuts.

Resistance estimates were scaled from 0 = susceptible American chestnut mean to 100 = resistant Chinese chestnut mean. Dotted lines indicate our targets for disease resistance to enable long-term survival (> 90) and > 70% American chestnut ancestry.



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#### SCIENCE MILESTONES

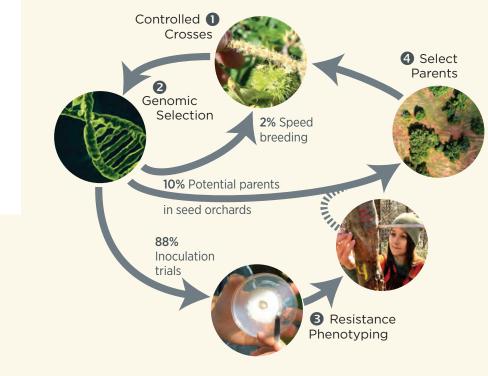
our predictions for gains in blight resistance. We plan to plant out the remaining 90% of the non-selected trees in progeny tests that will be planted across multiple sites so we can evaluate these trees' genetic potential in multiple contrasting environments. Phenotypes from these progeny tests will also be used to update genomic selection models to improve accuracy of selection of the next generation of parents (Figure 4). TACF's chapters, with their decades of experience growing chestnuts and established field sites, are uniquely positioned to establish a network of replicated progeny tests. Any tree that performs well in progeny tests could ultimately be used as a parent in the next generation of breeding and selection. Furthermore, we are exploring methods of shortening the breeding cycle by inducing trees to flower within one to two years using supplemental lighting and fertilizer treatments with the construction of

a new greenhouse at Meadowview Research Farms. Resistance of progeny could be predicted from DNA profiles of trees rather than having to inoculate all trees with the chestnut blight fungus. This 'speed breeding' approach opens exciting possibilities for rapid resistance gains, although it will still be necessary to ultimately grow selected trees in orchards to generate large numbers of seed for breeding and restoration (Figure 4).

We know from a millennia of agriculture that directed plant breeding works. The RGS method is a way to make this work more efficiently so we can get to levels of disease resistance required for restoration-worthy trees sooner. As always, we are on tree time and have decades of patient work before ecologically impactful, self-replicating populations are in place across the eastern US. I am deeply grateful to TACF's volunteers and donors for their support of our ambitious mission and the next chapter of our work.

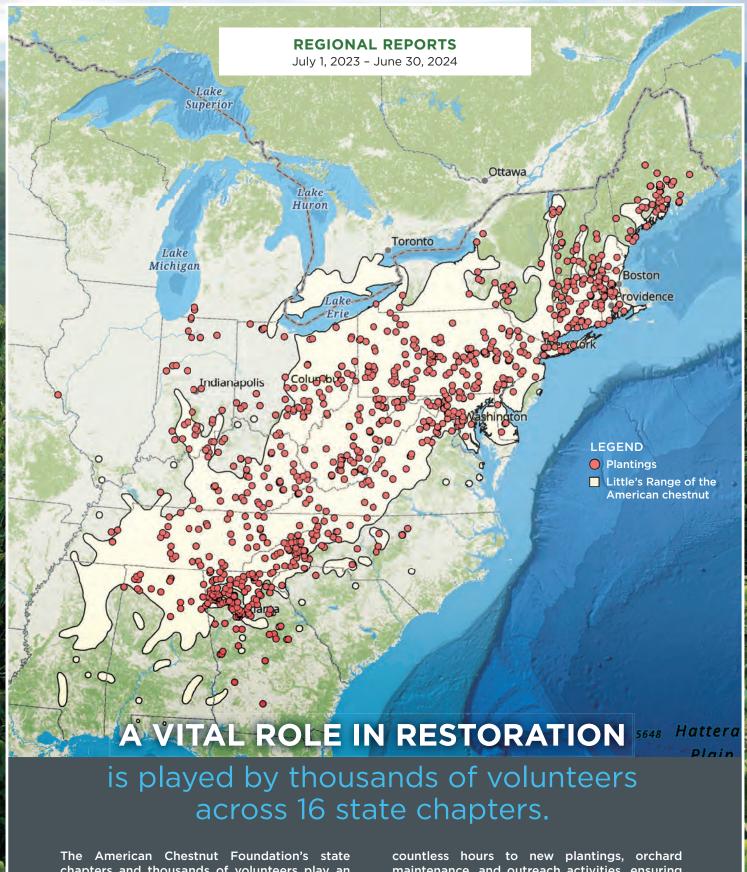
#### FIGURE 4. A DIAGRAM OF A SINGLE RECURRENT GENOMIC SELECTION CYCLE

Percentages indicate the number of individuals passed to each of the four breeding tracks. The dashed line indicates information from the resistance phenotyping used to determine which parents should be used in controlled crosses for the next generation.



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chapters and thousands of volunteers play an important role in the restoration of this iconic tree species. Each year, volunteers in the four regions of the tree's native range dedicate countless hours to new plantings, orchard maintenance, and outreach activities, ensuring that future generations will experience the splendor of healthy American chestnuts thriving in eastern US forests.

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#### **REGIONAL REPORTS**

#### NEW ENGLAND REGION: CT, MA/RI, ME, VT/NH

#### **CT CHAPTER**

- The CT Chapter is devoting much of its efforts to Germplasm Conservation Orchards (GCO), starting one new orchard this year, therefore adding to the five existing orchards, bringing their total number of GCOs to 10.
- The Chapter also conducted backcross orchard pollinations using pollen from a tree with *Phytophthora cinnamoni* resistance and adding the pollen into some of their existing seed orchards.
- Continued outreach by the Chapter includes giving presentations to many gardening and environmental groups.
- They have also embarked on a new partnership with Case Mountain Cabin, Inc., a volunteer group trying to restore an American chestnut log cabin built in 1917 in Manchester, CT.

#### **MA/RI CHAPTER**

- The MA/RI Chapter contributed more than 300 volunteer hours to maintain an existing orchard in South Kingston, RI.
- The Chapter also held or participated in more than 50 activities during the fiscal year, including orchard maintenance, screenings of *Clear Day Thunder*, and speaking events.
- A conservation symposium was hosted by the Chapter at Smith College in honor of TACF's 40<sup>th</sup> Anniversary.
- In collaboration with Historic New England, of which the Eustis Estate Museum is a part, the Chapter planted an orchard of American chestnut hybrids for the education of visitors to the museum which abuts the Blue Hills Reservation.

- Gould Farm, the first residential therapeutic community in the nation, established a test plot of hybrid chestnut seedlings with the hope of developing a full orchard in subsequent years.
- The Monterey Native Plants Working Group and the Monterey Community Center hosted a chestnut roast on Native American Heritage Day.
- The University of Rhode Island planted a common garden orchard in Kingston, Rhode Island. A common garden is composed of individual trees from different geographic areas that are then held and studied under the same environmental conditions. The Kingston orchard, one of three common gardens, is composed of 33 different hybrid crosses. These crosses have origins from states including Delaware, Maine, Maryland, Pennsylvania, Tennessee, Vermont, and Virginia.

#### **ME CHAPTER**

- The Maine Chapter launched a new program called Chestnuts Across Maine (CAM), designed to enlist land trusts, schools, conservation organizations, and their members to plant small orchards of chestnuts throughout Maine.
  - These plantings will help the Chapter preserve native chestnut diversity (a new model for GCOs) and serve as laboratories to test new restoration chestnuts. Most importantly, these orchards will enlist new partners, engage the Chapter's wide-spread membership, and serve to build communities around growing and caring for chestnut trees.
- The Chapter completed nine pilot projects in FY2023-24 with a goal of having 60 CAM plantings by 2030 and chestnut trees within a 10-minute walk of all Maine schools.



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#### **VT/NH CHAPTER**

- The VT/NH Chapter hosted an information booth at the New Hampshire Farm, Forest, and Garden EXPO. American chestnut seedlings were distributed. The Chapter found this to be a good method of generating interest in its activities and plans to greatly expand on this approach in 2025.
- They continued efforts to have American chestnut trees planted at all Vermont schools and their annual free nut giveaway is also ongoing; fortyseven packages of nine American chestnuts with planting pots were sent to Chapter members who requested them.
- The Chapter pollinated several American chestnuts with pollen collected from trees within Vermont and New Hampshire, and harvested more than 10,500 fertile nuts from trees used in the pollination effort.

- They tracked about twenty wild-type American chestnut trees that flowered in the past or the fiscal year. One new tree was discovered in 2024.
- The Chapter established a working relationship with the New London, NH Conservation Commission by planting a GCO and by expanding previously established plantings in New London. They also expanded the Windsor Grasslands GCO in Windsor, Vermont by 30 trees, yielding an orchard of 90 trees of nine selected sources.
- Chapter volunteers assisted in monitoring and maintenance of breeding orchards in Vermont, including Pikes Peak orchard in Perkinsville, Lake St. Catherine orchard in Poultney, and Green Mountain National Forest Orchard in Goshen.
- Leaders amended their Chapter bylaws to decrease the quorum requirement at annual meetings to 25 members or five percent of Chapter membership, whichever is less.

#### NORTH CENTRAL REGION: IN, NY, OH, PA/NJ



#### **PA/NJ CHAPTER**

• The PA/NJ Chapter participated in more than 25 events, including farm shows, native plant festivals, and earth day celebrations.

#### MID-ATLANTIC REGION: KY, MD, VA, WV

#### **KY CHAPTER**

- The KY Chapter utilizes Facebook (866 Followers), sends emails to 141 members, and connects with every "chestnutter" who contacts the Chapter.
- The Chapter continuously searches for future board members who are leaders that will bring their skills and resources through people or land to the organization.
- The Chapter has built an inventory of wild American trees via TreeSnap, with 1,628 "snaps" across the state, representing around 4,000 wild Americans observed.
- The TreeSnap Chestnut Map shows us good chestnut habitats, and that wild inventory provides any variety of scions that we decide to collect. The inventory also tells us where larger trees are growing that could be producing flowers for pollination.
- The Chapter follows the science as it develops.

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#### **REGIONAL REPORTS**



#### **MD CHAPTER**

- The MD Chapter held more than 20 events at locations across the state, including culling trees at the Central Maryland Research and Education Center Orchard, harvesting nuts at Monocacy Orchard, and planting trees at Beltsville.
- The efforts of the Chapter support Phytophthora root rot research, the Recurrent Genomic Selection program (formerly known as best x best), and small stem assay research.

#### **VA CHAPTER**

- The Chapter held more than 75 events at locations across the state during the fiscal year.
- Clear Day Thunder was screened several times, and maintenance, phenotyping and tours were offered at Lesesne State Forest; harvesting, shucking, and planting took place at several locations across the state.

#### WV CHAPTER

- The WV Chapter installed its first 100-tree Germplasm Conservation Orchard (GCO) in 2024. This orchard was established in conjunction with the US Army Corps of Engineers at the Burnsville Dam in central WV.
- The Chapter has nearly 40 sites where American chestnut orchards have been planted, but many of the sites have fewer than twenty trees.
  - Twenty-one sites have more than 30 American chestnuts. Other sites where 80 American chestnuts were planted include three WV state forests: Calvin Price, Greenbrier, and Seneca. Those orchards were established by the WV Division of Forestry.

#### SOUTHERN REGION: AL, NC/SC, GA, TN

#### **NC/SC CHAPTER**

 The NC/SC Chapter held more than 40 activities across the Carolinas, including educational hikes in partnership with Southern Appalachian Highlands Conservancy, leaf collection at Warren Wilson College, tabling at a Cradle of Forestry event, as well as planting, pollinating, and harvesting.

#### **GA CHAPTER**

- The GA Chapter collected 1,154 seeds from 37 genetic lines made for germplasm conservation in September and October 2024.
- Several *C. dentata* and backcross lines from Georgia and other states were successfully

transformed using OxO at UGA. Over 80 PCRpositive OxO transgenic events in nine Founder Line backgrounds, representing all regions of the range, have been generated so far at the University of Georgia.

- Several newly discovered *C. dentata* trees were added to *dentata*Base, including at least two large surviving American chestnuts.
- Trees were added for Phytophthora root rot screening at the Flint and Hurricane Creek sites in late 2023/early 2024.
- Members represented the Chapter at several outreach events as well as scientific conferences such as The Association of Southeastern Biologists and NE-1833 meetings in Chattanooga.

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a area a

July 1, 2023 - June 30, 2024



- The Seeds for Members program in January and February of 2024 resulted in about 35 new or renewed members and 350 American chestnut seeds dispersed, as well as \$1,500 in donations.
- Chapter board member Scott Laseter spent approximately 25 hours on the bylaws project including reviewing existing bylaws for the Chapter and national TACF.

#### **TN CHAPTER**

- A pollination workshop was held June 8, 2024.
- In 2023 and 2024, the Chapter focused on recurrent genomic selection (RGS), formerly known as best x best, crosses to introduce resistance to Phytophthora root rot into their breeding population. They selected pollen from Georgia, North Carolina, and South Carolina, which showed evidence of *Phytophthora* resistance. Additionally, the Chapter used some of their own F<sub>1</sub> hybrids with proven resistance. This strategy aims to strengthen the overall resistance in their breeding program. The workshop was held at the Tennessee Tech University Orchard.
- A small stem assay (SSA) inoculation workshop was held July 17, 2023.

- The Chapter is conducting another SSA this year, following the completion of one in the last fiscal year. As part of the RGS program, the Chapter is using molecular markers to assist in selections during the progeny tests. This year's project includes 900 inoculated trees from 38 randomized families.
- A shucking party was held on October 21, 2023.
- Chapter members hosted volunteers for a fall workshop and chestnut shucking party at the University of Tennessee at Chattanooga (UTC) Fortwood Street Greenhouse and Nursery. This crew, which included UTC faculty, students, and alumni, processed more than 14,000 chestnut seeds in one very long workday.
- The Chapter, in collaboration with volunteers from the NC/SC Chapter and several members of TACF staff, planted approximately 1,000 chestnut seeds at the Linville State Nursery in Newland, NC. The seeds sown during this planting are from RGS trees that were pollinated over the past year and represent some of the most advanced plant material the Chapter has.

#### NEW ENGLAND REGION:

Number of Chestnuts Planted: 1,313

Number of Volunteers: 643

Number of Attendees/ Beneficiaries: 18,287

#### NORTH CENTRAL REGION:

Number of Chestnuts Planted: 4,830

Number of Volunteers: 70

Number of Attendees/ Beneficiaries: 19,940

#### MID-ATLANTIC REGION:

Number of Chestnuts Planted: 4,200

Number of Volunteers: 1,025

Number of Attendees/ Beneficiaries: 7,736

#### **SOUTHERN REGION:**

Number of Chestnuts Planted: 4,509

Number of Volunteers: 746

Number of Attendees/ Beneficiaries: 4,393

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#### OUR DONORS July 1, 2023 - June 30, 2024

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who allow this long-range restoration mission to continue and thrive.

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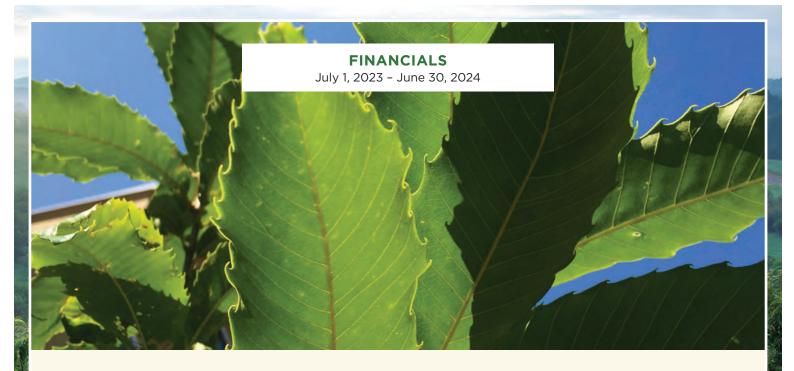
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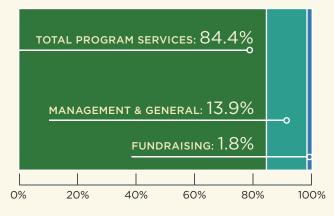


#### **PUBLIC SUPPORT AND OTHER REVENUES**

TOTAL REVENUE	\$3,300,992	
All Other Income	21,607	0.7%
Merchandise Sales	24,839	0.8%
Investment Income	141,757	4.3%
Federal Grants	10,765	0.3%
Membership	526,372	15.9%
Contributions	\$2,575,652	78.0%
		% Income

#### EXPENSES

		%
Program Services		Expense
External Research	\$44,478	1.2%
National Office Operations	614,534	16.7%
Marketing & Communication	s 227,551	6.2%
Science & Research	1,173,228	31.8%
Chapter & Membership	343,785	9.3%
Research Farm Operations	703,816	19.1%
Total Program Services	\$3,107,392	84.4%
Total Program Services	\$3,107,392	84.4%
Total Program Services Supporting Services	\$3,107,392	84.4%
	<b>\$3,107,392</b> \$511,573	<b>84.4%</b> 13.9%
Supporting Services		
Supporting Services Management & General	\$511,573	13.9%





The American Chestnut Foundation is a 501 (c)(3) non-profit organization. Donations are deductible to the maximum extent allowed by law. Tax ID: 41-1483019

The American Chestnut Foundation 2023 – 2024 Annual Report

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#### **ANNUAL REPORT**

July 1, 2023 - June 30, 2024

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Meadowview Research Farms 29010 Hawthorne Drive Meadowview, VA 24361 (276) 696-9142

Mid-Atlantic Regional Office Virginia Department of Forestry Central Office 900 Natural Resources Drive Charlottesville, VA 22903

New England Regional Office UVM Aiken Forestry Service Labs / USFS NRS 705 Spear Street South Burlington, VT 05403 (802) 999-8706

North Central Regional Office Pennsylvania State University 108 Business Services 206 Forest Resources Lab University Park, PA 16802 (814) 863-7192

Southern Regional Office 50 N. Merrimon Avenue Suite 115 Asheville, NC 28804 (828) 281-0047

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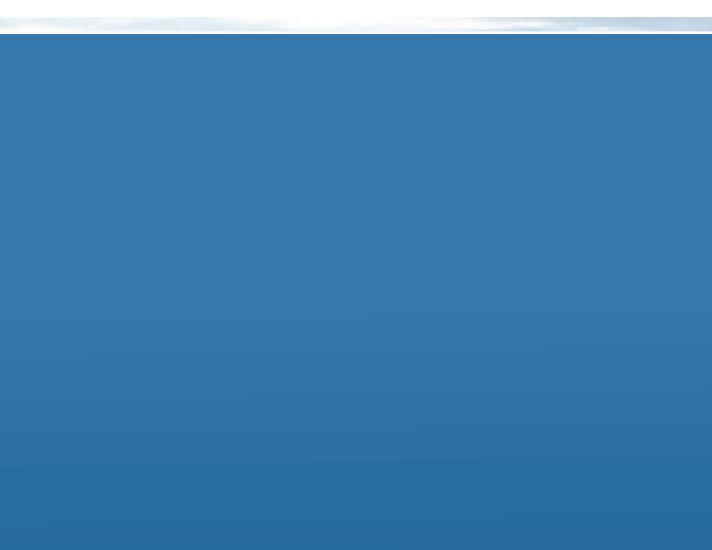
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