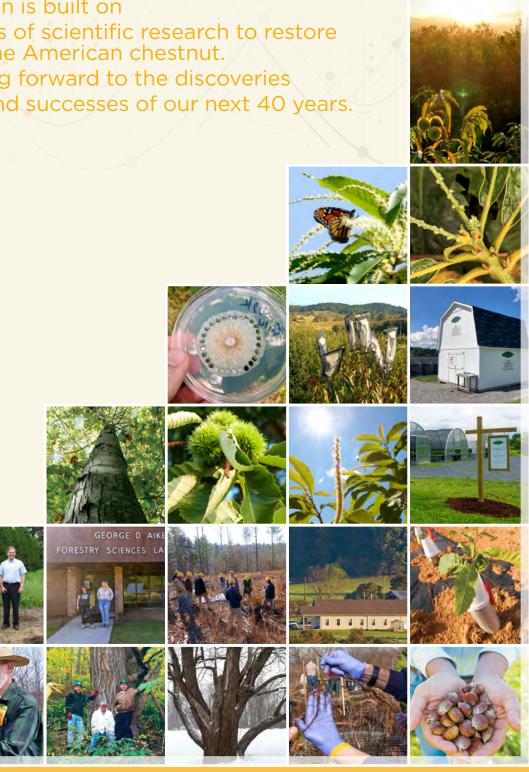


Our foundation is built on four decades of scientific research to restore the American chestnut. We are looking forward to the discoveries and successes of our next 40 years.









CHAIRMAN'S LETTER

Dear colleagues:

It seems like every year is an eventful one at The American Chestnut Foundation (TACF), and this past year is no exception. In 2023 we celebrated the 40th anniversary of our organization, and though we're looking to the future and our next 40 years, it's important to take a moment to look at the past fiscal year's successes.



Among the many accomplishments are:

- First and foremost, we hired Dr. William Pitt as our new President & CEO. Will has excellent credentials to lead both the expansion of our scientific research efforts and our restoration planning and implementation. Welcome, Will!
- 2 Reflecting a strong vote of confidence in our mission, TACF finished one of its best fundraising years ever during fiscal 2022-23.
- 3 After more than a year of development, our science staff and Science & Technology Committee released an updated, multi-year science strategy. You can view it on our website, https://tacf.org/science-strategies/
- TACF continued to receive notable media coverage including articles from *The New York Times*, *The Washington Post*, and the *Wall Street Journal*. In addition, we completed and released our documentary film *CLEAR DAY THUNDER*: *Rescuing the American Chestnut*, and we launched a new, updated website in December 2022.
- **5** TACF expanded important research alliances with major universities including Virginia Tech,

- University of Georgia, Villanova, and Penn State. The Foundation continues to cultivate relationships with more than 500 public and private organizations, and over 100 are covered by formal partnership agreements.
- **(6)** TACF began development of a tree improvement cooperative concept aimed at bringing together diverse stakeholders to focus on the restoration of the American chestnut and other threatened native tree species.
- Our new Government Affairs Committee met for the first time and is planning a strategy aimed at improving our exposure to both elected officials and important government agencies.
- We have gained extensive knowledge toward gene discovery that can be used in the creation of cisgenic products, created a pipeline for new transgenic lines, and continue our capacity to navigate federal and state regulatory processes. TACF is poised to release ongoing iterations of improved, disease-resistant trees for research and evaluation to members and supporters in the coming years.

Finally, organization-wide successes are not just measured by the "big wins," but also by the myriad of small successes that our staff and volunteers achieve every day.

Thanks to all for a great year.

William J. (Jay) Cude III Chairman. Board of Directors

PLEASE NOTE: The information published in this report was gathered and written during the 22-23 fiscal year, and reflects our partnerships and research during that time.





RESTORATION OF THE AMERICAN CHESTNUT IS A LONG-TERM ITERATIVE PROCESS



Our work to restore the American chestnut is a long-term, multigenerational process. For our existing transgenic and traditional breeding approaches, it will take perhaps a decade to optimize, adequately evaluate disease resistance and growth in field trials, and scale up seed production. It will take several decades more to develop and deploy new biotechnology pathways for further improvement. Many decades will be required to plant disease-resistant American chestnuts over millions of acres of suitable habitat. We are making progress each year and need your support for continuous improvement.

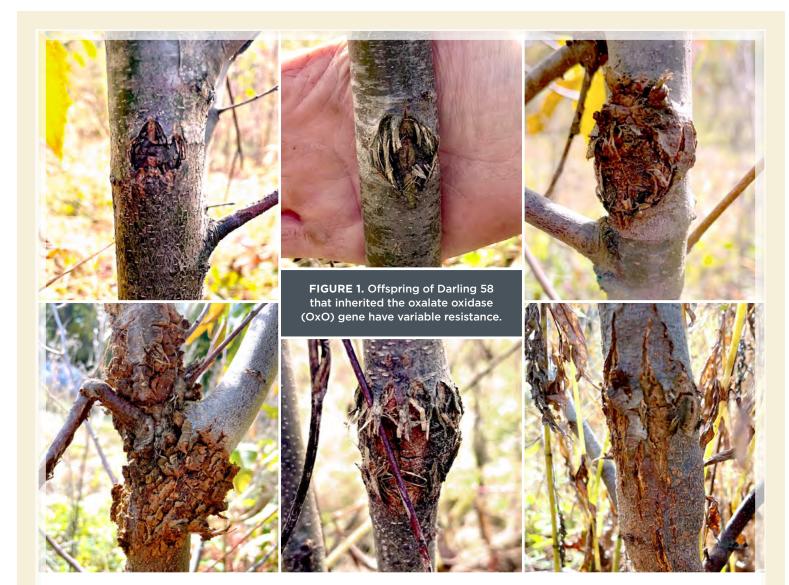
D58 WILL NOT BE THE RESTORED AMERICAN CHESTNUT TREE

Our collaborators from the State University of New York College of Environmental Science and Forestry (SUNY-ESF) developed the Darling 58 (D58) variety of American chestnut. The D58 tree contains a wheat oxalate oxidase (OxO) gene which detoxifies oxalic acid produced by the chestnut blight fungus. Assessments from early D58 field trials at ESF and Purdue indicate that offspring of D58 that inherited the OxO gene have significantly smaller chestnut blight cankers on average than siblings that did not inherit the gene; however, the blight resistance of D58 progeny that inherited OxO varies from highly resistant to fully susceptible (Figure 1). Variable resistance of D58 progeny may be related to variation in expression of the OxO gene. Research from ESF shows that the levels of expression of the OxO gene in second generation progeny of D58 varies from zero to levels higher than the D58 founder tree (Matthews, 2020). Beyond variable resistance, D58 progeny that inherited OxO tend

to grow slower and have lower survival rates on average than full siblings that did not inherit the gene (**Figure 2**, page 4). The growth and survival penalties are of concern because they may affect the competitiveness of trees in the forest during establishment and over the long term.

WE ARE TAKING A MEASURED APPROACH WITH D58 WHILE COLLECTING MORE DATA ON PERFORMANCE

Before learning about the growth penalties and variable resistance, we were planning to breed D58 progeny with a range-wide collection of American chestnuts and planting the diversified progeny in seed orchards to increase seed production. Now, we are still interested in breeding D58 with a wide variety of American chestnut backgrounds, but our main goal is research to assess growth and resistance of D58 progeny over longer time frames and a wider variety of environmental conditions. In future years, if we obtain stronger evidence that growth and survival penalties are not severe



enough to be of great concern for restoration, we will further increase our efforts in diversification and seed production for D58.

WE ARE CREATING NEW OXO FOUNDER LINES

We hypothesize that variable resistance and the growth penalty in D58 progeny is related to constitutive expression of the OxO gene, meaning the gene is always expressed in all tissues. Constitutive expression may lead to a phenomenon called gene silencing, which is a process plants use to reduce the expression of foreign DNA (Kumar et al. 2015). Furthermore, previous research indicates that constitutive OxO expression activates the expression of other genes involved in disease resistance (Hu et al. 2003). This is akin to an overactive immune system and may explain the growth penalties in D58. We are working with multiple partners to develop new OxO founder lines that only express the OxO gene in tissues that are wounded or infected with chestnut blight. Our goal is to get high enough OxO expression

to confer sufficient blight resistance in infected tissues, but to keep expression low in non-infected tissues so that the trees otherwise grow normally.

WE ARE EXPLORING STACKING OTHER RESISTANCE GENES WITH OXO

Evidence suggests OxO enhances blight resistance more than other genes that have been inserted into American chestnut to date (Nelson et al. 2014). However, the durability and efficacy of resistance conferred by OxO over the time period that trees are expected to live with blight is not yet known. With the recent sequencing of reference genomes for two Chinese chestnuts and an American chestnut, we have engaged in collaborations with researchers from Virginia Tech and Hudson Alpha Institutive of Biotechnology to discover candidate genes from Chinese chestnut that potentially contribute to blight resistance. To date, we have discovered 73 candidate blight resistance genes that are supported by three or more lines of evidence. Namely, these candidate

FIGURE 2. There is a potential growth penalty from constitutive expression of OxO.

The tree on the left inherited the constitutively expressed OxO gene. The tree to its right is a full sibling, meaning it shares the same two parents, just not the OxO gene.



genes are expressed specifically in Chinese chestnut but not American chestnut in response to blight infection. These genes map to genomic regions associated with elevated resistance in TACF's

Chinese-American hybrids. Inserting multiple genes together may potentially lead to more robust blight resistance. The genes would also be inherited together, simplifying the downstream breeding for genetic diversification.

BREEDING IS POTENTIALLY AN EFFICIENT METHOD TO STACK RESISTANCE GENES

The multigene insertion approach may require decades to develop, evaluate performance of the trees in field trials, and obtain federal regulatory approval for widescale deployment. An alternative gene stacking approach is to breed promising OxO founder lines with partially resistant large surviving American chestnuts (LSA) and backcross hybrids. The LSAs

and hybrids potentially contribute additional genes and mechanisms of resistance beyond the oxalic acid detoxification conferred by OxO. Selection of progeny with increased blight resistance and forest competitiveness will likely be required to realize gains beyond what is conferred by OxO alone. To increase the efficiency of selection, we are developing a cost-effective genotyping platform to

predict blight resistance and forest competitiveness of the progeny, enabling selection at the seedling stage prior to planting in seed orchards. TACF's goal is to generate trees with a high level of blight

> resistance, competitive ability for population growth, and genetic diversity for adaptive ability in forested ecosystems.

A multitude of approaches will be required to restore American chestnuts to Eastern forests. Thank you to all who have supported TACF over the last four decades. The coming decades will require continued creativity and perseverance to effectively pursue the above, and other emerging scientific directions. In the meantime, the crucial work that you, our members and volunteers, have been doing in gathering as much remaining wild-type diversity as possible and growing trees across diverse climates and landscapes becomes more pressing. With accelerating

environmental changes such as habitat loss, climate change, and novel pests, we move forward with steadfast resolve to rescue this iconic species and bring it into the ecosystems of the future. We are honored to continue on this journey together, bringing ever newer scientific possibilities and engaging ever broader groups of people across the former and future range of the American chestnut.

TACF's goal is to generate trees with a high level of blight resistance, competitive ability for population growth, and genetic diversity for adaptive ability in forested ecosystems.

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RESTORATION SUCCESS IS ONLY POSSIBLE WITH OUR DEDICATED VOLUNTEERS

The American Chestnut Foundation consists of sixteen state chapters and thousands of volunteers, all of whom play an important role in the restoration of this iconic tree species. Each year, regional volunteers 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 U.S. forests.



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

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



NUMBER OF Chestnuts Planted

965
Harvested
212



NUMBER OF Volunteers 560



Attendees/ Beneficiaries 16,195



NUMBER OF Chestnuts

Planted

3,529

Harvested

13,700



NUMBER OF Volunteers 336



NUMBER OF Attendees/ Beneficiaries

88,313

MID-ATLANTIC: KY, MD, VA, WV





NUMBER OF Chestnuts

Planted

3,556

Harvested

8,657



NUMBER OF Volunteers

630



NUMBER OF Attendees/ Beneficiaries

7,402



NUMBER OF Chestnuts

Planted

2,188

Harvested

51,830



NUMBER OF Volunteers

586



Attendees/ Beneficiaries

1,567

REGIONAL REPORTS





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

CT CHAPTER

- Over the past year, the CT Chapter developed one new Germplasm Conservation Orchard (GCO) for a total of ten GCOs. Volunteers were hard at work planting, maintaining, and weeding the young trees.
- The Chapter is preparing to help perform controlled pollinations with transgenic pollen by holding a pollination workshop at an American chestnut tree in Old Lyme.
- In order to expand outreach, the Chapter had exhibitions at an Earth Day Event, the CT Agricultural Experiment Station's Plant Science Day, the Durham fair, and the CT Flower and Garden Show; Zoom presentations were held with several land trusts; the Chapter continued to hold chestnut hikes at various locations in CT where chestnut trees are growing tall enough to flower.
- An ongoing goal of the Chapter is to continue finding and harvesting as many wild trees as possible in an effort toward successful restoration.

MA/RI CHAPTER

 A two-acre chestnut orchard located at the Massachusetts Division of Fisheries and Wildlife (MassWildlife) headquarters in Westborough, MA was named in honor of long-time Chapter member Rufin Van Bossuyt.

- An agreement with Historic New England's Eustis Estate Museum in Milton, MA was signed to establish an educational planting of chestnut trees. The varying species and varieties will be ordered chronologically along a pathway complete with interpretive signage.
- A new partnership with the Birches School, a nature-based pre-kindergarten through eighthgrade campus in Lincoln, MA, has provided an opportunity for students and faculty to learn about American chestnut restoration.
- Dr. Laura Meyerson, a professor at the University of Rhode Island (URI), along with the students from her Restoration Ecology class, attended a MA/RI Chapter meeting at Smith College MacLeish Field Station. They were given an introduction to Chapter work and the opportunity to ask questions, and a proposal has been submitted for research at the URI Campus.
- Volunteers from the South Kingstown Land Trust and University of Rhode Island Master Gardeners attended pollination training. Master Gardener Intern Rachel McCrory developed a detailed spreadsheet that is easy to use and tracks the performance of individual trees.
- The Burrillville Land Trust announced a grant of \$7,100 from the Champlin Foundation for use in the American chestnut tree GCO in the Edward D. Vock Conservation Area in Pascoag, RI.





ME CHAPTER

- The Board explored and addressed important governance issues, such as liability and accident insurance, established digital archives, and began to review and revise the Chapter's bylaws.
- Eva Butler, outreach coordinator, continued to engage membership and expand volunteer participation across many aspects of the Chapter's work.
- The Chapter embraced a major pivot in the backcross breeding program by contracting with Fox Forestry to conduct a significant roguing of five of its six seed orchards. They prepared for best x best crosses in 2023 in two breeding orchards. Tom Klak at the University of New England advanced the capacity to produce and freeze D58 and DarWin pollen and established several D58 orchards in southern Maine under a USDA permit.
- The search for surviving American chestnuts doubled and the Chapter harvested about 7,000 nuts in the fall of 2022. Hundreds of seeds were distributed to members and the public, thousands were given to Howard Nursery in PA, and 1,500 seedlings were grown and sold.
- Members participated in many outreach events with diverse partner organizations like the Maine Woodland Owners, Viles Arboretum, Penobscot County Conservation Association, Upper St. John River Organization, Boothbay Regional Land Trust, Orono Land Trust, Soil and Water Conservation Districts, and more.

PA/NJ CHAPTER

• The PA/NJ Chapter held over two dozen community events and other activities across Pennsylvania and New Jersey. These events were attended by thousands of participants, including volunteers, Chapter members, and community members.

NY CHAPTER

• The NY Chapter planted over 3,500 trees and harvested over 13,000 nuts. The Chapter bagged and hand-pollinated 954 female flowers with D58 pollen and volunteers helped with the fall harvest.

REGIONAL REPORTS







MID-ATLANTIC: KY, MD, VA, WV

KY CHAPTER

- The KY Chapter increased its Facebook followers to 650 and has been actively recruiting new contacts via social media, personal email, and visits.
- Over the last four years, the Chapter has focused on adding skills and resources to its board. Dr. Jennifer Koslow, associate professor at Eastern Kentucky University, Dr. Ellen Crocker, assistant professor at the University of Kentucky, Charlie Saunders, environmental control manager at the Division of Forestry, Clint Patterson, head forester at the Berea College Outreach Center, and Scott Ray, forest supervisor of the Daniel Boone National Forest have all joined.
- The Chapter has increased engagement with the U.S. Forest Service Southern Research Station office located at the University of Kentucky Forestry Building.
- Two pilots and a skilled University of Kentucky forestry student have tested airplane and drone searches for blooming chestnuts for the Chapter.

MD CHAPTER

 The Chapter contributed to the best x best research program and continued research on small stem assays.

VA CHAPTER

• The VA Chapter made significant progress this past year. The Chapter made controlled pollinations of Darling 58 and best x best trees, planted over 2,800 trees in seed orchards, and hosted a chestnut science conference. They also measured restoration plantings and facilitated a LidAR drone study at Lesesne State Forest. The Chapter is currently growing 1,400 seedlings in a greenhouse, and harvested over 8,000 chestnuts.

WV CHAPTER

 The WV Chapter held pollination workshops, educational talks, and planted nearly 600 trees.
 The Chapter sent nine newsletters keeping members, volunteers, and other interested community members up to date on events and news.





SOUTHERN: AL, CAROLINAS, GA, TN

AL CHAPTER

 The AL Chapter held several orchard activities and planted 400 chestnuts.

TN CHAPTER

 The TN Chapter conducted numerous greenhouse activities, orchard activities, and community events.

CAROLINAS CHAPTER

• The Carolinas Chapter participated in dozens of community events and orchard activities across North and South Carolina. More than 500 volunteer hours were donated in tabling events, documentary screenings, and greenhouse activities. 550 trees were planted and more than 600 nuts were harvested.

GA CHAPTER

- The GA Chapter collected 1,234 seeds from 42 genetic lines made for germplasm conservation, blight, and *Phytophthora* root rot (PRR) resistance screening, phylogenetic study, and somatic embryogenesis.
- Several *C. dentata* and backcross lines from Georgia and other states were successfully transformed using OxO at the University of Georgia (UGA). Over 80 PCR-positive OxO transgenic events in nine Founder Line backgrounds, representing all regions of the range, have been generated so far at UGA.

- Alternative small stem assays were completed on approximately 350 seedlings (from 11 backcross lines) over the course of summer and fall 2022.
- Scion material from about 20 different GA
 C. dentata trees were sent to U.S. Forest Service for epicotyl grafting and several newly discovered C. dentata trees were added to dentataBase, including at least two large surviving American chestnuts.
- Pollen was collected and stored from 40 trees in June 2023.
- Blight evaluations, orchard inoculations, and culling were performed at Roy Richards, Flint, and Milner backcross orchards.
- Trees were added for PRR screening at the Flint site in October 2022 and for blight screening at Richards seed orchard in February 2023.
 Test/demonstration plantings were made at 30 locations, most notably Hurricane Creek in collaboration with the University of North Georgia.
- Financial support was obtained from Berry College (Laura Maddox-Smith Summer Research Institute for the Environmental Sciences; \$5,000 to Martin Cipollini and students), Lumpkin Coalition (\$6,000), Tom and Eleanor Ratchford (\$5,000), Rome Rotary (\$2,000), the Cipollini/Patrick Fund (\$1,800), and Brad Stanback (\$10,000 to Scott Merkle at UGA).

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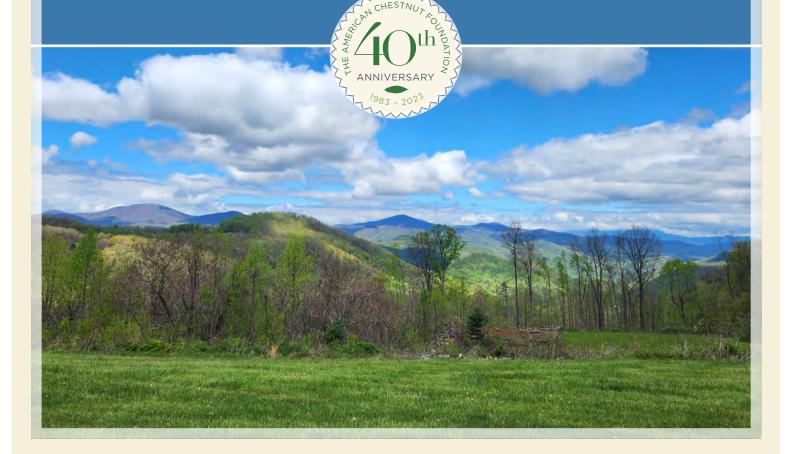
OUR CONSERVATION SUCCESS STORY CONTINUES WITH YOUR SUPPORT

The American Chestnut Foundation deeply appreciates the hard work of its chapter volunteers and committed supporters. Our mission is long-range, and your charitable contributions allow this conservation success story to continue. We are truly grateful for your help in restoring this iconic species.

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| PUBLIC SUPPORT AND OTHER REVENUES | | | |
|-----------------------------------|-------------|-------------|--|
| | | % OF INCOME | |
| Contributions | \$3,064,251 | 80.0% | |
| Membership | 521,670 | 13.6% | |
| Federal Grants | 64,235 | 1.7% | |
| Investment Income | 92,844 | 2.4% | |
| Merchandise Sales | 40,460 | 1.1% | |
| All Other Income | 46,015 | 1.2% | |
| TOTAL REVENUE | \$3,829,475 | | |

| | EXPENSES | |
|----------------------------|-------------|--------------|
| PROGRAM SERVICES | | % OF EXPENSE |
| External Research | \$296,635 | 8.7% |
| National Office Operations | 517,718 | 15.1% |
| Marketing & Communications | 278,587 | 8.1% |
| Science & Research | 798,939 | 23.4% |
| Chapter & Membership | 346,179 | 10.1% |
| Research Farm Operations | 614,029 | 18.0% |
| TOTAL PROGRAM SERVICES | \$2,852,087 | 83.4% |
| SUPPORTING SERVICES | | |
| Management & General | \$522,426 | 15.3% |
| Fundraising | \$43,807 | 1.3% |
| TOTAL SUPPORTING SERVICES | \$566,233 | 16.6% |
| TOTAL EXPENSES | \$3,418,320 | |

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