

MISSION SUCCESS

ANNUAL REPORT JULY 1, 2021 – JUNE 30, 2022



THE  
AMERICAN  
CHESTNUT  
FOUNDATION™

AMBITIOUS GROWTH

GROWTH IS NEVER  
BY MERE CHANCE;  
IT IS THE RESULT OF FORCES  
WORKING TOGETHER.

JAMES CASH PENNEY



THE  
AMERICAN  
CHESTNUT  
FOUNDATION™

Dear Chestnut Community,

As I reflect on the past year's accomplishments, one theme emerges about The American Chestnut Foundation (TACF): we are poised for ambitious growth in order to achieve mission success. Although being a nimble, relatively small organization with a loyal donor base and dedicated corps of staff and volunteers, we will need to ratchet up our efforts to ensure the American chestnut is reintroduced at a scale never before attempted in the modern conservation movement.



## HOW DO WE GROW?

"How do we grow?" is a reoccurring theme in conversations with staff, board, and chapter leadership. We are fortunate to involve highly engaged thought leaders who take their service at TACF seriously and put in countless volunteer hours. This raises a call for recruitment. We need to continually build bench strength on the national and chapter boards to prepare for what will be pivotal and momentous years ahead. If you, or your family and associates, want to participate in this amazing and hopeful mission, join a committee at the national level or volunteer at your state chapter to help drive the future of TACF. Many of our current volunteer leaders have been involved for decades. They deserve the support and energy of "fresh troops" to carry the baton of chestnut restoration forward.

Our national committees are a hard-working group of professionals driven by goals, strategies, and actions. Depending on your interest and expertise, these committees are a terrific way to give back to TACF and determine your potential desire to be considered for national or chapter board service. Our committees (Science & Technology, Restoration, Promotion & Outreach, Chapters, Governance, and Finance) are chaired by talented and devoted individuals who move our mission forward with strategic guidance and open dialogue.

The board and staff have dedicated themselves to grow the organization's diversity as well. To better serve communities that have been historically underrepresented within TACF, we are embarking on a professionally guided journey to integrate diversity, equity, inclusion, and justice into the overall fabric of how we do business. It's not just the right thing to do, it will help TACF achieve its Mission and Vision.

At this juncture, please accept my sincere appreciation for all you do for TACF. We are arguably one of the strongest grassroots conservation organizations dedicated to one species. The durability and endurance of our mission is nothing short of remarkable and I am proud to be part of it.

A handwritten signature in black ink, reading "William J. Cude III".

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

DARLING 58 TREE

Preparing to  
incorporate  
Darling 58 into  
our breeding  
program

BREEDING PROGRAM



In 2022, The American Chestnut Foundation (TACF) welcomed news from the U.S. Department of Agriculture (USDA) that the agency intends to grant non-regulated status to grow and publicly distribute progeny of the blight-tolerant Darling 58 (D58) variety of American chestnut. For those who are new to our mission, our collaborators at the State University of New York – College of Environmental Science and Forestry (ESF) inserted a gene from wheat called oxalate oxidase (OxO) into the genome of an American chestnut tree from New York State to create the D58 variety. This gene encodes an enzyme that detoxifies oxalic acid produced by the chestnut blight fungus and reduces the fungus' ability to damage the tree. Decisions on whether or not to allow public distribution of D58 progeny are expected in 2023 from all three federal agencies that regulate genetically modified organisms: USDA, Environmental Protection Agency (EPA), and Federal Drug Administration (FDA). TACF staff, academic collaborators, and volunteers spent 2022 preparing for the possibility of incorporating D58 into our breeding program in four ways:

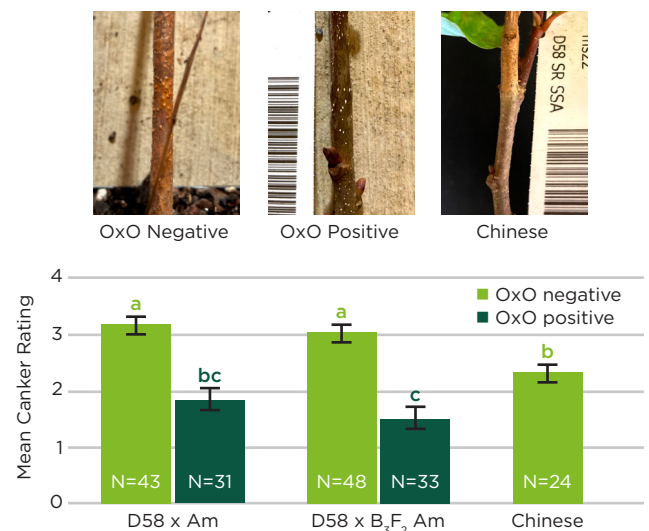
## 1 We confirmed that progeny of the Darling 58 tree are likely to have high levels of blight tolerance

When a Darling 58 tree is bred with a wild blight-susceptible or backcrossed American chestnut, approximately 50% of the progeny inherit the OxO gene. In 2022, staff at TACF's Meadowview Research Farms and ESF inoculated seedling progeny of wild and backcrossed American chestnuts that inherited OxO and compared the canker severity to full siblings that did not inherit the gene. We found that trees that inherited OxO had significantly less severe cankers than full siblings that did not inherit the gene. Furthermore, the average

canker severity of progeny that inherited OxO was similar to that of Chinese chestnut, which we know is resistant to chestnut blight (**Figure 1**). When D58 was bred with partially blight-resistant backcross trees, their progeny appeared to have slightly greater resistance than Chinese chestnut. While these results are preliminary, they give us confidence to scale up our efforts in breeding D58 with a diverse population of American chestnut trees. Long-term field trials are required to determine whether progeny of the D58 variety will have sufficient blight resistance and forest competitiveness over the long lifespan of a tree. Deregulation of D58 is a prerequisite to conduct these long-term trials in forest conditions.

**FIGURE 1**

Comparison of the blight resistance of Darling 58 progeny from the second outcross generation ( $T_2$ ) that were bred with wild-type American chestnuts (D58 x Am) and backcrossed American chestnuts (D58 x  $B_3F_2$  Am). Average canker ratings (larger ratings = more severe cankers) of progeny that inherited the oxalate oxidase gene (blue bars) compared to full siblings that did not inherit the gene (red bars) and to Chinese chestnut (Chinese). Numbers inside the bars are sample sizes for each cross type. Different letters above the bars indicate significant differences in average canker ratings ( $P < 0.05$ , Tukey Test). Examples of cankers on OxO positive (+), negative (-) and Chinese chestnut (Ch) are pictured to the right.



## 2 We learned how many American chestnut individuals we need to breed with D58 for sufficient climate resilience

Our plan is to breed the D58 variety to a genetically diverse sample of American chestnut trees so that the blight-tolerant restoration population has the capacity to adapt to a large range and to a changing climate. To characterize how genetic diversity in American chestnut is distributed across the landscape and how this diversity relates to climatic variation, our research collaborators at Virginia Tech sequenced the whole genomes of 384 American chestnut trees. They found that the American chestnut range can be divided into three seed zones. When we breed D58 with trees from each of these seed zones, the resulting progeny are likely to be locally adapted to the climates in these regions. Furthermore, they found that breeding D58 progeny with roughly 200 wild American chestnut trees from across the species range is sufficient to represent the climate-adaptive genetic variation. We have progeny from a wide-ranging collection of over 500 wild-type American chestnuts represented in TACF's breeding orchards (**Figure 2**). Thus, it is feasible to diversify the D58 population for enhanced climate resilience by breeding with the many accessible backcross and wild-type American chestnuts that are already flowering and producing nuts in TACF's orchards.

## 3 We identified American chestnut backcross hybrids that inherited resistance to *Phytophthora* root rot

*Phytophthora* root rot (PRR), caused by the imported pathogen *Phytophthora cinnamomi*, has been especially deadly for American chestnuts in the southern half of the species range. Breeding D58 progeny with American chestnut backcross trees that inherited resistance to PRR from Chinese chestnut is currently our primary strategy for generating progeny with resistance to both chestnut blight and PRR (**Figure 3**). To identify PRR-resistant backcross trees, we inoculate their seedling progeny with *P. cinnamomi* in contained greenhouse conditions and assess parental resistance based on the survival rates of the progeny. Since 2005, we have screened over 32,000 progeny of 920 backcross mother trees for resistance to *P. cinnamomi*, in collaboration with researchers from Clemson University and the U.S. Forest Service. We recently accelerated the identification PRR-resistant backcross parents through genomic prediction of PRR resistance in collaboration with researchers at Virginia Tech. We identified 70 backcross trees whose progeny survival rates after *P. cinnamomi* inoculation are expected to exceed 40%. In 2021 and 2022, we started breeding D58 with these PRR-resistant backcross trees and screening the progeny for the inheritance of OxO and

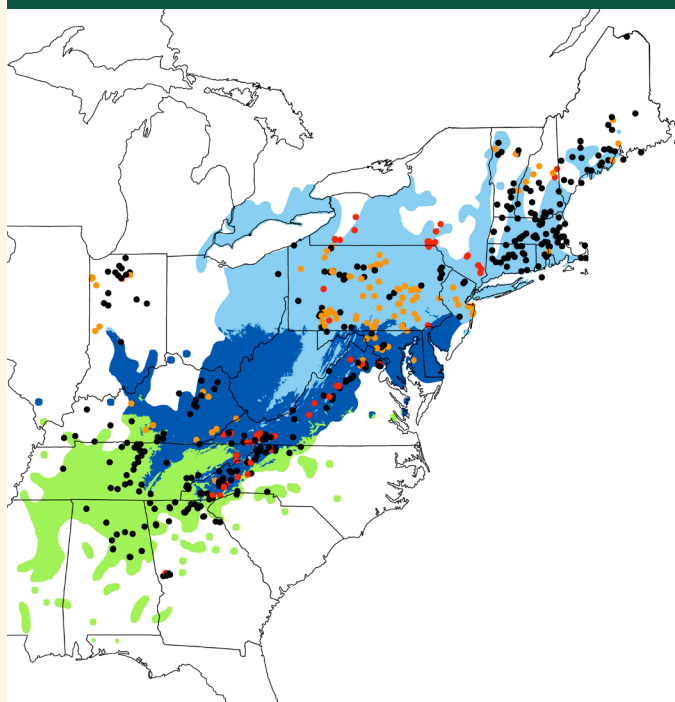
resistance to *P. cinnamomi*. Under permits from the USDA, we planted the first 'dual resistance' progeny at a site in Georgia that is infested with *P. cinnamomi*. The long-term exposure to both *P. cinnamomi* and chestnut blight will be the true test of these trees' dual disease resistance.

## 4 We developed a genotyping platform for D58 progeny

We will breed D58 progeny with American chestnut backcross trees to expedite population diversification. Currently, there are not enough unique lines of flowering wild-type American chestnuts conserved in orchards to meet our diversification objective, but we have more than enough unique backcross lines planted in orchards.

**FIGURE 2**

Three seed zones for American chestnut and geographic origins of the wild *Castanea dentata* parents in TACF's breeding program. Seed zone delineations by Alex Sandercock (Virginia Tech). Black dots are wild-type parents used in backcross breeding; red dots are wild trees whose progeny have been planted in germplasm conservation orchards; and orange dots are parents used in both breeding and germplasm conservation.



Backcross trees have been prioritized that, on average, inherited 97% of their genome from *C. dentata* for breeding with D58 progeny. We also developed a genotyping method that will enable us to select against residual inheritance of genes from Chinese chestnut and select progeny that inherited all of their genome

from *C. dentata*. Genotyping also enables us to select progeny that inherited less than 10% of their genome from the D58 founder tree. Selecting against the D58 genome minimizes the potential for deleterious inbreeding in the restoration population and ensures that the trees we deploy for restoration have locally adapted genetics.

**FIGURE 3** Steps to generate American chestnut trees with dual resistance (clockwise from top left).

**STEP 1:** Identify PRR-resistant backcross parents by inoculating progeny with *Phytophthora cinnamomi*. Photo by Steve Jeffers.

**STEP 2:** Apply pollen containing a blight resistance gene (OxO) to PRR-resistant parents. Pictured: TACF intern Israel Golden and John French from the GA Chapter.

**STEP 3:** Identify nuts that inherited OxO via a chemical detection method.

**STEP 4:** Select PRR-resistant progeny in the greenhouse. Pictured: Jamie Van Clief, Southern Regional Science Coordinator.

**STEP 5:** Plant progeny in an orchard site infested with *P. cinnamomi*. Pictured: Austin Flint (standing) and Johnny Paolozzi from the GA Chapter.

**STEP 6:** Allow the first-generation selections to intercross via open pollination.

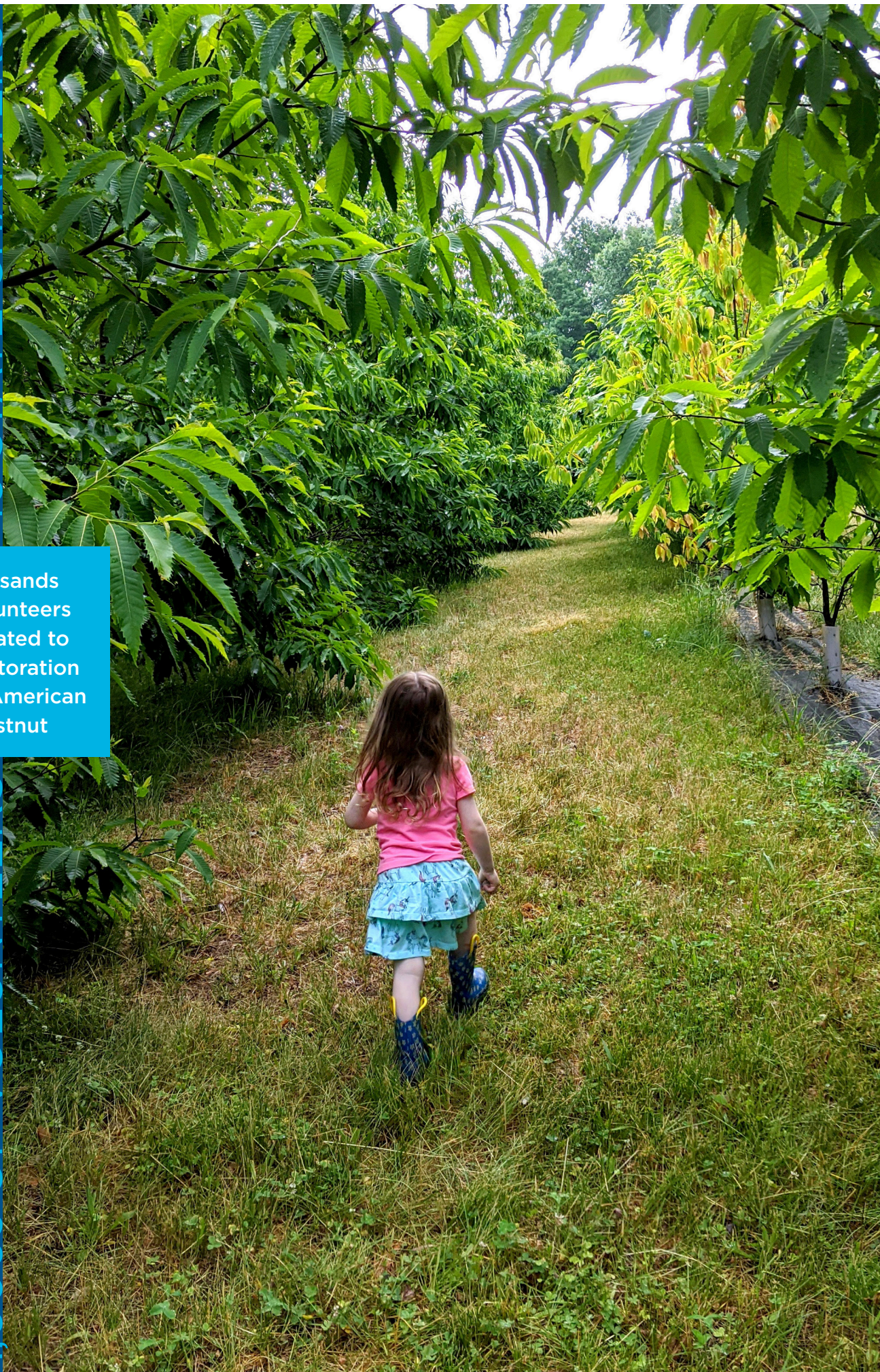


I am more hopeful than ever that we will be successful in generating a population of American chestnut that will have sufficient disease resistance and climate resilience for restoration. Deregulation of D58 will be a major milestone in the restoration effort, but not a solution in and of itself. Much work is still required to complete the breeding for diversification and dual disease resistance. We are building partnerships with tree nurseries to establish seed orchards that are capable of producing millions of seed within the next decade. Deregulation of D58 will enable us to begin planting blight-tolerant trees in forests to evaluate the durability of their disease resistance and forest competitiveness. The sustained dedication and enthusiasm of TACF members and volunteers will be crucial for all of these efforts. Thank you for your continued support and collaboration.

16 STATE CHAPTERS

Thousands  
of volunteers  
dedicated to  
the restoration  
of the American  
chestnut

1,000s OF 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

#### CT Chapter

- Over the past year, the CT Chapter developed two new Germplasm Conservation Orchards (GCOs) for a total of nine 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 pollination workshops in Roxbury and Middletown.
- In order to expand outreach, the Chapter had an exhibition at an Earth Day Event, the CT Agricultural Experiment Station's Plant Science Day, and the CT Flower and Garden Show; Zoom presentations were held with several Land Trusts; the Office of the CT Historian published a podcast about the tree's restoration; and hikes to discover wild American chestnuts were offered at various locations.

- 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

- The MA/RI Chapter, having planted seven seed orchards containing 35,000 trees within the last 10 years, is now planting best x best crosses. These crosses have been added to the existing orchard at Norfolk County Agricultural High School and a new orchard was established in Sandisfield, MA.
- Conserving genetics from wild trees has been a primary focus. The 2021 harvest included 5,052 wild nuts from 23 different wild mother trees.
- The Burrillville Land Trust in RI has established a forest-type Germplasm Conservation Orchard (GCO)

## NEW ENGLAND REGION



CT



MA/RI



on the Edward D. Vock Conservation Area and planted American chestnut trees on Earth Day 2022 with a group of volunteers from Amica Insurance. The GCO at Flint Fields in Lincoln, MA was expanded as planned.

- Four interns were hired in the 2021 field season. Altogether they logged in 480+ hours, which equates to 12 weeks of mowing, weeding, planting, roguing, and other tasks.
- Participation in an American chestnut bee survey was organized by entomologist Michael Veit.
- The South Kingstown Land Trust received a new kiosk constructed by Cameron Gillette of Boy Scout Troop 1 Kingston as part of his Eagle Scout Project.
- The Chapter continued to have hybrid meetings when possible in order to allow more folks to attend.

#### ME Chapter

- In the past, the ME Chapter has largely accomplished its goals with a “working board,” who have done most of the physical work involving breeding and seed orchards. To address the advancements of TACF’s science plan, volunteer coordinator Eva Butler spent considerable time in fiscal year ‘22 on member outreach to increase participation in these types of events and activities.
- Significant efforts have been made to solicit members to invigorate the Outreach Committee toward renewing the biannual newsletter, creating more press releases, and meeting the demand for public speaking engagements.
- Actions to appreciate volunteers have been implemented, such as offering lunches to those who participate at events, hosting a volunteer appreciation day at the Maine Coastal Botanical Garden, and giving volunteers seedlings.

- Some restoration activities increase the risk of physical injury. The Chapter is reviewing its insurance coverage to ensure volunteers receive the proper training and safety equipment.

#### VT/NH Chapter

- VT/NH Chapter President Doug McLane and ME Chapter Vice President Tom Klak combined efforts once again to help advance American chestnut restoration. They pollinated hundreds of chestnut flowers in Cape Elizabeth, ME with transgenic pollen. This is part of the research underway at the University of New England (UNE), led by Klak, in collaboration with SUNY’s College of Environmental Science and Forestry.
  - When the nuts mature, each will be harvested and analyzed to determine the inheritance of the oxidase enzyme that will allow the next generation to tolerate chestnut blight.
  - Nuts produced by wild-type American chestnut trees in most of TACF’s Germplasm Conservation Orchards will be pollinated with transgenic pollen, once approved for distribution by federal agencies.
  - Pollinating and harvesting hundreds of chestnuts is labor-intensive work. The efforts of Doug and Tom are greatly appreciated!



IN



PA/NJ



PA/NJ

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

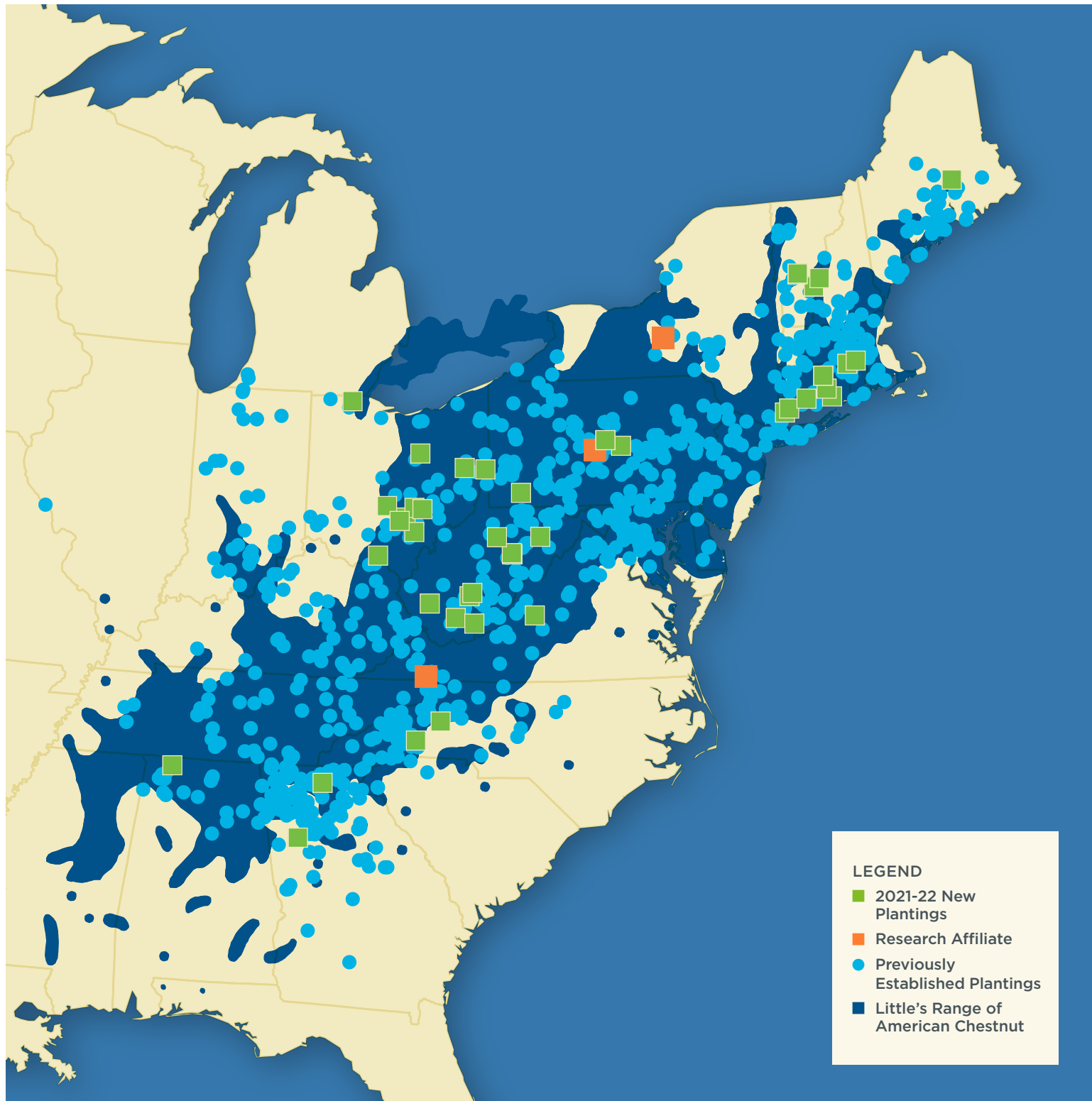


NY

### PA/NJ Chapter

- PA/NJ Chapter members gathered to dedicate the Codorus Orchard to the memory of David J. Armstrong; long-time member, volunteer, and mighty friend of the American chestnut. New interpretive signage was installed at the orchard and his fellow chestnut enthusiasts shared memories and tributes.
- To help achieve its wild American conservation objectives, Chapter board member Lake Graboski began setting up targeted "American Chestnut Conservation Missions." Each mission will identify areas of special interest to the breeding program. Hikers and local conservation groups are encouraged to search for American chestnuts.
- Connor McInerney, an alumnus of Penn State and the Chapter's Orchard Intern Program, created a project to collect all observations of American chestnuts on the iNaturalist app. Now, American chestnut observations in PA and NJ is uploaded to iNaturalist, they will be imported into the project page. Here, the observations can be further identified and analyzed.

NORTH CENTRAL REGION



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

Number of Chestnuts  
Planted: 19,762

Number of Volunteers: 329

Number of Attendees/  
Beneficiaries: 107,822

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

Number of Chestnuts  
Planted: 3,082

Number of Volunteers: 833

Number of Attendees/  
Beneficiaries: 1,496

**MID ATLANTIC REGION:  
KY, MD, VA, WV**

Number of Chestnuts  
Planted: 2,471

Number of  
Volunteers: 3,683

Number of Attendees/  
Beneficiaries: 4,436

**SOUTHERN REGION:  
AL, Carolinas, GA, TN**

Number of Chestnuts  
Planted: 4,115

Number of Volunteers: 356

Number of Attendees/  
Beneficiaries: 496



KY



MD



WV

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

### KY Chapter

- KY Chapter leaders continued relationship-building with a major supporter in Louisville who, thanks to their generous donation in 2012, worked with the Parklands of Floyds Fork and the Chapter to establish an American chestnut orchard there in the 2010s. The Parklands welcomes more than two million visitors a year, creating an incredible opportunity for this orchard to educate the public about the tree's restoration. Sadly, the Chapter's supporter and friend passed away last year.
- Veteran KY Board Member Anne Bobigian developed a site suitability map for the geographic regions of American chestnut in KY to assist in searching for surviving trees, as well as the best areas for restoration plantings.
- Board Members Rex Mann and Lynn Garrison, along with Chapter volunteer Jacob Pease, continue to develop a positive relationship with

U.S. Forest Service leaders of Land Between the Lakes National Recreation Area. This acreage contains wild American chestnuts, west and north of their original range, and their unique genomics.

### MD Chapter

- The MD Chapter prepared spaces for and planted 405 seedlings, representing dozens of hybrid families, that were screened for resistance to *Phytophthora cinnamomi* at TACF's Bent Creek Resistance Screening Center in Asheville, NC. The Chapter found PRR at two orchards and has lost a significant number of promising hybrid trees to the disease. TACF screened family lines from several states at Bent Creek using *Phytophthora* originally isolated from one of the MD orchard's to determine whether they have inherited resistance to PRR. The infected but possibly resistant seedlings were planted back into that orchard. They will be observed over several years and could provide TACF and the Chapter with a valuable source of

## MID-ATLANTIC REGION



KY



PRR-resistant hybrids for the backcross breeding program. The Chapter is grateful for the volunteers who participated in this endeavor, and to Southern Regional Science Coordinator (RSC) Jamie Van Clief and former Mid-Atlantic RSC Tom Saielli, who devoted countless hours of their time and labor to the project.

#### VA Chapter

- The VA Chapter launched two social media accounts, Instagram and Facebook, in an effort to engage with a more broad and diverse audience, and it is already paying off. Together, these accounts are closing in on 1,000 followers and continue to grow!

#### WV Chapter

- The WV Chapter sent eight newsletters between July 2021 and June 2022, not only to current members (about 150), but 90 past members who let their memberships expire. The newsletters include updates and stories about Chapter plantings and presentations as well as news relative to the national office.
- The Chapter's newest board member Lewis Cook not only supervised three plantings in Fayette County, he also spoke to a group of students at Oceana High School in Wyoming County and assisted with a planting in Upshur County.
- A \$1,000 grant, given by the Chapter to West Virginia University's Science Adventure School (SAS), provided funds to develop a 4-hour curriculum on American chestnut for 6th grade students. Among other activities, the school's programs on outdoor education featured the American chestnut tree. Several students commented that this program was their favorite.



### SOUTHERN: AL, CAROLINAS, GA, TN

#### AL Chapter

- Nearly 400 seedlings were planted at a TVA orchard in Mussel Shoals, AL.

#### Carolinas Chapter

- Carolinas Chapter volunteers potted 2,200 seeds in February 2022 at the U.S. Forest Service Resistance Screening Center in Asheville, NC producing 160 survivors that were then planted in Waleska, GA.
- In addition to the 350 seeds planted in April 2022 at the Pryor Orchard near Edneyville, NC, seeds were sent to the GA Chapter for planting in its conservation orchard. Other seed were sent to Meadowview Research Farms for research and to supply nuts for TACF's annual Wild-Type American Chestnut Seedling Sale.
- Chapter member Paul Sisco created a spreadsheet that provides data for 1,667 wild American seeds. Also collected and listed are 89 seeds from Alabama and North Carolina Chinquapin trees. Paul, along other volunteers, made crosses of 14 female parent trees at five different orchards. Crosses made at the Pryor and Cliffs of Glassy orchards produced seed to test for Phytophthora root rot (PRR) resistance. Additional crosses were also produced, some of which will be used in trial plantings.
- A total of 121 open-pollinated seeds were collected from the Pryor and Biltmore Orchards, and 80 open-pollinated seeds were collected from the Cliffs of Glassy Orchard.
- Chapter member Jon Taylor collected 1,667 wild American chestnut seeds from 90 different trees in GA, VA, MD, PA, and WV. Jon, a master woodworker,



crafted a handmade frame from salvaged American chestnut wood that encased an historical photo of the tree which was presented to Dolly Parton in honor of her late uncle, Bill Owens, a champion of American chestnut restoration.

- Carolinas Chapter President Doug Gillis crafted a frame and posts from salvaged American chestnut wood, which he installed by two American chestnut hybrids at TACF's national office in Asheville, NC.
- In June 2022, Elizabeth Clark, representing the James K. McClure Education and Development Fund, contacted Doug Gillis through the Carolinas Chapter with interest in supporting the Chapter and the Foundation. Doug connected her with TACF President & CEO Lisa Thomson and, through that relationship building, the McClure Fund awarded the Foundation a grant to help market and distribute TACF's documentary film.
- Carolinas Chapter member Joe James continues performing PRR resistance research at his farm near Seneca, SC. In spring 2022, with help from Clemson University students, 40 PRR-resistant trees were pollinated with transgenic pollen. Open-pollinated PRR-resistant nuts were harvested in fall 2021. They were planted and inoculated in screening tubs. Survivors that showed resistance were planted on Joe's farm.

#### GA Chapter

- The GA Chapter grafted 62 scions of pure *C. dentata* on *C. mollissima* root stock. The material will be sent to the U.S. Forest Service Regional Screening Center in Asheville, NC for nut grafting.
- Blight evaluations, orchard inoculations, and culling took place at the Roy Richards and Flint backcross seed orchards.

- Several new *Castanea* trees were catalogued and mapped, including one *C. dentata* in Oconee County and about 27 new *C. dentata* from recent TreeSnap app observations.
- Testing of protocol is underway for locating new *C. dentata* using drones or satellite imagery in collaboration with the University of North Georgia-Gainesville's Environmental Spatial Analysis program. This partnership was initiated by GA Chapter president Jack Rogers.
- Pollen from 27 trees was collected, processed, and frozen (10 *C. dentata*, 14 "Best" backcross trees, and three F<sub>1</sub>s).
- While the Chapter's Best x Best hand pollinations at the University of Georgia were not as successful as hoped, GA pollen was collected, shipped to other chapters, and is being stored at TACF's Meadowview Research Farms.
- Small Stem Assays (SSA) were completed on nearly 350 seedlings at Berry College (11 backcross lines plus controls). An SSA manuscript was published in "Plant Disease" (Conn, et al.). This alternate SSA method is becoming the standard for early blight screening at TACF.

SUCCESS STORY

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.

\$25,000+ DONORS

Our conservation  
success story  
continues with  
your support

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DEEP APPRECIATION

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		<b>% of Income</b>
Contributions	\$2,262,112	76.5%
Membership	507,844	17.2%
Federal Grants	75,000	2.5%
Investment Income	57,373	1.9%
Merchandise Sales	27,243	0.9%
All Other Income	29,016	1.0%
<b>TOTAL REVENUE</b>	<b>\$2,958,588</b>	

**EXPENSES**

<b>PROGRAM SERVICES</b>		<b>% of Expense</b>
External Research	\$288,675	10.1%
National Office Operations	428,423	14.9%
Marketing & Communications	146,801	5.1%
Science & Research	906,072	31.6%
Chapter & Membership	280,821	9.8%
Research Farm Operations	515,615	18.0%
<b>TOTAL PROGRAM SERVICES</b>	<b>\$2,566,407</b>	<b>89.4%</b>
<b>SUPPORTING SERVICES</b>		
Management & General	\$262,256	9.1%
Fundraising	\$41,302	1.4%
<b>TOTAL SUPPORTING SERVICES</b>	<b>\$303,558</b>	<b>10.6%</b>
<b>TOTAL EXPENSES</b>	<b>\$2,869,965</b>	

**8**  
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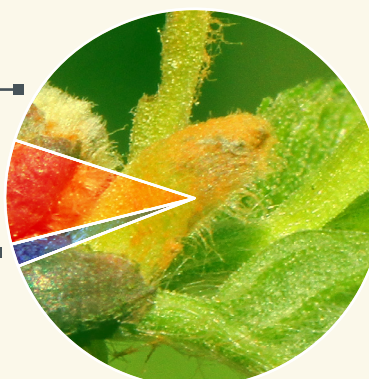


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