WINTER 2024 ~ ISSUE 1 VOL. 38

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

A BENEFIT TO MEMBERS

THE AMERICAN CHESTNUT FOUNDATION

Volunteer Service Awards ~ Chestnut Rowing Boat ~ Chestnut Table ~ Grafting Southern Germplasm

WINTER 2024 ~ ISSUE 1 VOL. 38

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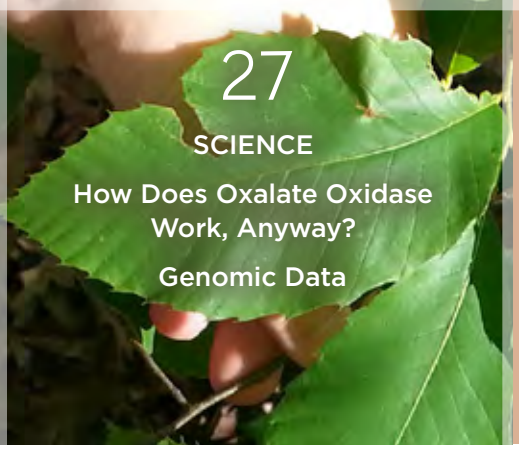

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Will Pitt
President & CEO

JANUARY HAS COME AND GONE, but the energy and fresh start that each new year brings is still present, especially at The American Chestnut Foundation (TACF).

We have several new endeavors to share, starting with, well, me. Although I have passed the six-month mark as TACF's President & CEO, this is my first President's Message in the magazine. I have enjoyed spending the first several months in this new position getting to know board members, staff, volunteers, chapter leaders, and other supporters who make this work possible. Obviously, the strength of TACF lies in the passionate individuals who focus on restoring the American chestnut. I love hearing your stories.

We are also preparing to launch a brand-new look for TACF that will include a logo, tagline, color palette, and even a new magazine design. We have been using our current and only logo for decades, and this versatile rebranding will help us reach a wider audience, especially in the digital age. It will be debuting soon, so be on the lookout for that exciting change.

In 2023 we celebrated 40 years of chestnut restoration work, and this year we turn toward the future. Over the next year, I am excited about seeing you at chapter events, connecting further at our 2024 Annual Fall Meeting in Connecticut, and moving closer to our goal of developing a restoration tree. I am happy to be part of this enthusiastic mission and I know we all look forward to what the next 40 years will bring in our journey to restore this beloved tree.

Will Pitt, President & CEO
The American Chestnut Foundation

American Chestnut Bur in the Snow

This wintry image was taken March 2021 in an American chestnut orchard at The Arboretum at Penn State by TACF's Chief Conservation Officer Sara Fitzsimmons. Sara loves exploring the snowy orchards and capturing the unique beauty of the trees, leaves, and burs during the winter stillness.

CORRECTION

On page 10 of the Spring 2023 issue of *Chestnut*, the photo of Ken Clarkson next to tree 1367 is misidentified as a wild American chestnut. Tree 1367 is a backcross chestnut that still has its original trunk 20 years after planting.

THE AMERICAN CHESTNUT FOUNDATION™

WHAT WE DO

The mission of The American Chestnut Foundation is to return the iconic American chestnut to its native range.

CONTACT US: chestnuts@tacf.org • tacf.org
facebook.com/americanchestnut • twitter.com/chestnut1904 • instagram/tacf1983

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

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

40TH ANNIVERSARY CELEBRATIONS

Last spring, TACF tasked its chapters with an unusual, non-breeding-related request: host a gathering to celebrate the Foundation's 40th anniversary. Some of the chapter celebrations fit right in with normal chapter activities, others spurred the formation of entire party-planning committees. Either way, our volunteers got to display some skills not showcased in our previous 40 years of scientific progress: party planning!

THE AMERICAN CHESTNUT FOUNDATION
40th ANNIVERSARY
1983 – 2023

We are thrilled to highlight some of the diverse 40th Anniversary celebrations our chapters have coordinated over the past year. Additional events are scheduled to take place later this year, so be on the lookout for highlights to come from 2024 celebrations.

From Maine to Alabama, the story of the past forty years was shared and celebrated, underlining the science, partnerships, and volunteers that make TACF the strong organization it is today.

Volunteer Service Awards 2023

Presenting The American Chestnut Foundation's (TACF) annual Volunteer Service Awards is always a highlight of the year, and 2023 did not disappoint! These awards are bestowed on one volunteer from each region who has exceeded expectations and tirelessly aided TACF's mission and vision.

These volunteers received their awards in October 2023, and below are some kind remarks from each presenter about their region's winner.

NEW ENGLAND REGION WINNER:

Mark McCollough, ME Chapter
Presented by Hannah Leeper (pictured) and Catherine Martini, Regional Outreach Coordinators

Mark McCollough retired and immediately threw himself whole-heartedly into the role of President of the ME Chapter of TACF. Mark's professionalism and compassion are perfectly balanced. As Director of Regional Programs, Kendra Collins, put it when she nominated Mark, "He makes time to be part of everything the Chapter is doing; from outreach, following up on wild tree reports, and fieldwork, to the less 'fun' activities like insurance and governance." Mark has also participated in many national activities, including offering prints of his blue jay painting for a TACF fundraiser, then taking the time to pack and ship them to each buyer. On a personal note, as we have settled into our positions this year, Mark has been an invaluable resource for advice and perspective, and he has done it in the most supportive and open ways. Every time we connect with Mark, whether in person or virtually, it is a true gift! He obviously loves this tree and understands the whole picture toward its successful restoration. We are so lucky to have him on TACF's volunteer team.

SOUTHERN REGION WINNER:

Kathy Patrick, GA Chapter
Presented by Marty Cipollini, TACF Board Member

Kathy Patrick, well, what can't you say? As her husband of 25 years, I will certainly be a little biased here, but I think others in the GA Chapter will agree that she is our favorite energizer bunny. There is almost nothing in our Chapter that she has not gotten involved with over the past decade; from pollination, harvesting, and other science-focused work, to organizing events and providing delicious food

for volunteers and event attendees. (You have got to taste her chestnut blondies!) Kathy served as GA Chapter president for four years, helping shepherd us through the COVID crisis and managing to increase membership during that time. As of late 2023, she is in her second year as Chapter vice president and is the vice-chair of TACF's Chapters Committee. As many of us know, changes and unexpected challenges have taken place over the last several years, and Kathy's steady leadership during that time has helped us keep our heads on straight. We do this work "so they may live," she often reminds us. We are very grateful that TACF has recognized her contributions to the Georgia Chapter and to TACF national. Thanks, Kathy!

NORTH CENTRAL REGION WINNER:
Mike Aucott, PA/NJ Chapter
Presented by Stephen Hoy, North Central Regional Science Coordinator

Mike Aucott has been a member of the PA/NJ Chapter of TACF since 2011, but his interest in chestnuts started well before he joined. When Mike was in 5th grade, he found a chestnut stump sprout and was able to take a leaf to add to his leaf collection. His passion for the species persisted during his professional career into 2012 as a member of the PA/NJ Chapter, and in 2020 when he was elected to the Chapter board after his wife Louise's term ended.

He has planted thousands of chestnut trees (American and hybrid), educated the public through school programs, YouTube videos, and presentations; and submitted stacks of Tree Locator Forms from the northern tier of PA and his home in New Jersey. Mike's efforts to elevate the work and awareness of TACF's mission have had a meaningful impact on our organization. Congratulations Mike, and thank you for your continued support!

MID-ATLANTIC REGION WINNER:
Darrell Blankenship, VA Chapter
Presented by Cassie Stark, Mid-Atlantic Regional Science Coordinator

Darrell Blankenship lives in Abingdon, Virginia, where he has been a volunteer at Meadowview Research Farms since 2018. In the 1950s, Darrell was curious about what happened to all the American chestnut trees; however, it wasn't until he retired in 2017 that he finally had the chance to learn about their demise and TACF's ongoing restoration efforts. Since then, he has been persistent in volunteering at our research farm whenever there is an opportunity. He has assisted with plantings in both the greenhouse and field, helped shuck thousands of burs, performed inoculations, collected data on cankers, and much more. He has played a significant role in forming connections with organizations such as Friends of Steele Creek, the Damascus Trail Center, and the Blue Ridge Discovery Center. Darrell is willing to not only table at these events, but also present about the history of the American chestnut. He recently discovered flowering wild chestnuts on his own property and immediately contacted us to collect pollen for breeding and germplasm conservation. Thanks for all you do, Darrell!

The Ark Built by Accident:

A MEMBER OF TACF'S BOARD EXPLORES A FAR-OFF PLACE WHERE AMERICAN CHESTNUTS STILL THRIVE

By Anna Sproul-Latimer, TACF Board Member

Anna Sproul-Latimer hugs a 125' tall *Castanea dentata* specimen in Tervuren Arboretum, March 2022.

The first time I stood there, I reacted how any sane person would: eyes brimming, jaw unhinged, words insufficient to explain to my startled sister what was happening. *Brigadoon. The Galactica. The Ark. I cannot BELIEVE this. How??*

We were standing underneath 14 soaring, columnar, blight-free American chestnuts: an entire Mount Olympus' worth of the old gods, thronged by dozens of babies. We were also in Belgium. How?? Here's how.

In 2022, I met Catherine for a quick sisters' trip: Paris to Copenhagen, via Brussels, in five days. Brussels was at my insistence, as MonumentalTrees.com had told me the tallest American chestnut on Earth grew in an arboretum near there.

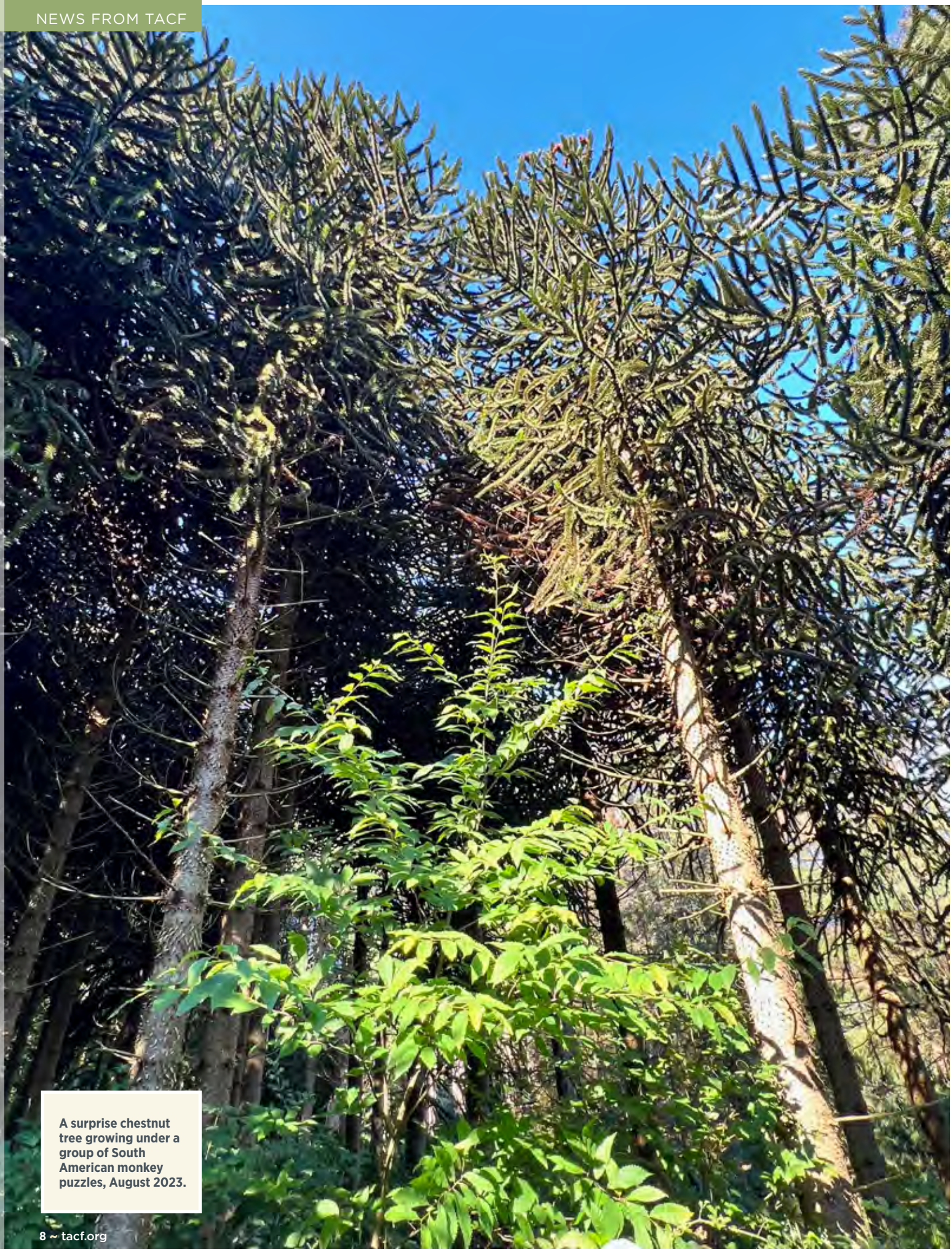
In 1798, French revolutionaries ransacked the countryside outside of Brussels, destroying a nearly 200-year-old Capuchin monastery. For the next 50 years, trees found their footing in those ruins. Then Leopold I, first king of the Belgians, bought the land and chopped them all down.

Before long, Leopold was dead. Fans of the novel *Heart of Darkness* might recall his successor, Leopold II, as one of the cruelest colonizers in history: a king who claimed all Congo for himself and frog-marched its people into forced labor, killing between 1 and 15 million. He set Congo up for unending misery and stole their riches to build lavish public works back home. Belgians called him "The Builder King."

All of Leopold II's buildings had to be built from something – to say nothing of ships to bring rubber home from the Congo and blades to cut off Congolese workers' hands if they didn't harvest the rubber fast enough. Among other things, this made him interested in forestry. In 1902, he approved the Capuchin ruin site for Arboretum Tervuren, the most ambitious forestry project in Belgian history.

Charles Bommer, 46, had pitched the arboretum in the language of nascent progressive management. Let me make a park here, the professor had argued, and our country will benefit many times over: a refuge for the masses; a research facility for timber; a living library, full of exotic wonders to explore.

If I had to guess, I'd say Bommer's real motivation was the last thing. He was the son of two botanists, brimming



A surprise chestnut tree growing under a group of South American monkey puzzles, August 2023.

with wonder, and a polymath: keen photographer; talented landscape designer. With Tervuren, he had a chance to make a masterpiece.

One of Bommer's other fascinations was phytogeography, the study of how topography and time affect plant development. In Tervuren, he wanted to test his hunch that Europe's lack of tree diversity was the product of geographic accident. Mountains and glaciers might have blocked species from spreading there, Bommer reasoned, but surely many would thrive if introduced. Why not source as many trees as I can from every major forest ecosystem in the world that could even theoretically thrive here, plant them all side by side, and see what happens?

Today, 120-plus years later, visitors to his masterpiece can walk in a single afternoon from the Cascades to the Everglades, from Atlas to Andean highlands, straight on into Appalachia, where specimens soaring as high as 125-plus feet and as big around as 11.5′–14′, mature American chestnuts still stand.

Once I saw them, I never wanted to leave. My sister did, though, since it was sort of her birthday. (Rude.)

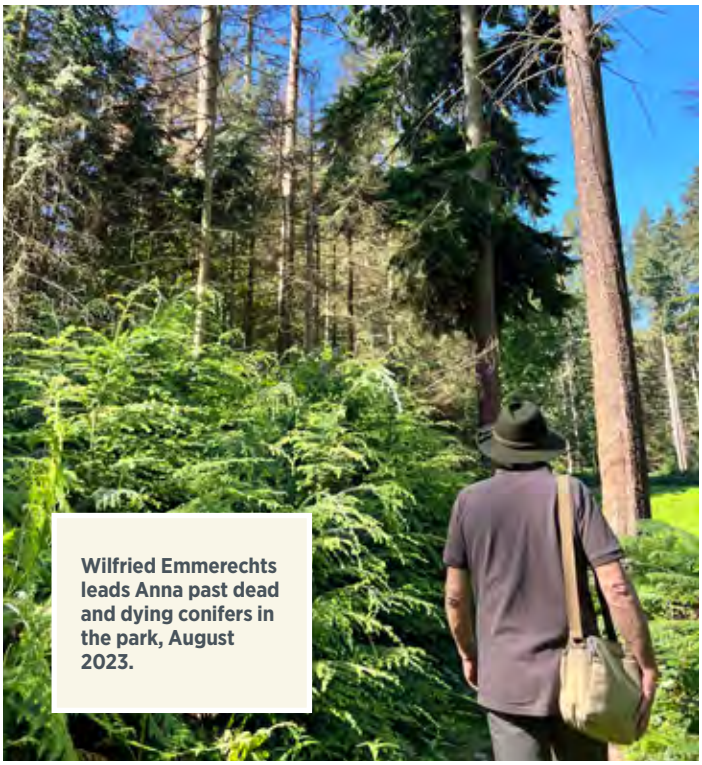
We moved on; my brain did not. For months, I wondered: did The American Chestnut Foundation have any kind of relationship with Tervuren? Should we? Could we? I eventually found contact information for Bommer's successors: Patrick Huvenne, Regisseur, and his deputies, Wilfried Emmerechts and Kevin Knevels. Patrick replied to my email, and then delightful things started happening.

I don't know if Bommer would be thrilled or horrified at how his masterpiece has changed. It is like phytogeographic performance art: Two world wars exhausted the Belgian people's appetite for foreign invasion at home and abroad. There was a little less national interest in timber (and colonialism) and a lot less in the importation of exotic trees.

By the end of the twentieth century, the arboretum was a neglected, weedy mess. In 2005, however, Patrick came in and rolled up his sleeves. He and his colleagues spent 18 years clearing trails, putting up signs, thinning stands, and renovating the once-dilapidated cottage into which Wilfried, Kevin, and their families moved. In short, they resurrected paradise only for climate change to come in and start destroying it again.

By the time I emailed, the deaths were already legion: Douglas firs; Western hemlocks; Sitka spruces. Across the park, magnificent conifers fell, victims of many of the same things killing them over here: drought, heat, bark beetles.

One plague that had not reached the park yet, though, was chestnut blight. An isolated case was destroyed in Brussels in 2015, but to date, that is the closest it has come. As a matter of fact, Wilfried wrote, Tervuren's chestnuts and their



Wilfried Emmerechts leads Anna past dead and dying conifers in the park, August 2023.

Wilfried Emmerechts and Patrick Huvenne discuss their work at Arboretum Tervuren, August 2023.

Some of the park's many enormous *Castanea dentata* specimens, August 2023.

offspring were thriving. He just wished they could tell us where they had come from. He did not even know for sure whether the trees were 100% wild-type *Castanea dentata*.

This, I realized, was a question TACF could help answer. I CCed a few people, and yadda, yadda: live clippings from the Tervuren chestnuts thence flew across the sea to Virginia Tech to Jason Holliday and his genomics lab. Eventually, we were able to send good news back over the Atlantic: yes, they were 100% wild-type American, traceable to somewhere in New England.

In August 2023, my high school sweetheart married his longtime boyfriend in France and invited me to come. On a Thursday morning before the weekend's wedding, Wilfried picked me up from my AirBNB: a tiny house surrounded by goats in the backyard of two lovely people with an artisanal foodstuffs brand. (In addition to the arboretum, Tervuren is apparently the suburb of choice for Belgium's Gwyneth Paltrow types.)

We drove into the park in the kind of beat-up white truck that signals "forestry guy" the world over. Getting out, Wilfried walked with me toward Appalachia, pointing to all the dead conifer stands as we went. He had retired that spring and mourned those trees like old friends: goodbye, goodbye to all our years together.

As we walked along, however, Wilfried and I began spotting chestnut seedlings. Surprise: here were some in Pennsylvania under a massive Eastern hemlock. Double surprise: here was one under Araucaria in the Andes. Baby chestnuts were popping up all over the park.

This makes sense: back at home, before the blight, chestnuts would surge to fill the gaps left by falling giants. Their moment was always in the rebound.

I reeled. Here was hope in a place that had been built up and burnt down by human violence and then redeemed by human love, over and over.

I knew more sadness was coming for Tervuren and us all. But I also knew there would be more Wilfrieds, Patricks, Kevins, Jasons, us.

Hope is like an American chestnut: somehow, despite everything, it smolders through the ages, sustained by love, until the canopy finally opens and the surge begins at last.

Florian Carle on the Housatonic River in Derby, CT. Photo by Corey Morrison.

Charlie, the Chestnut Rowing Boat:

CELEBRATING TACF'S 40TH ANNIVERSARY WITH THE FLOW!

By Florian Carle, CT Chapter Secretary

I HAVE SOME RATHER NICHE PASSIONS.

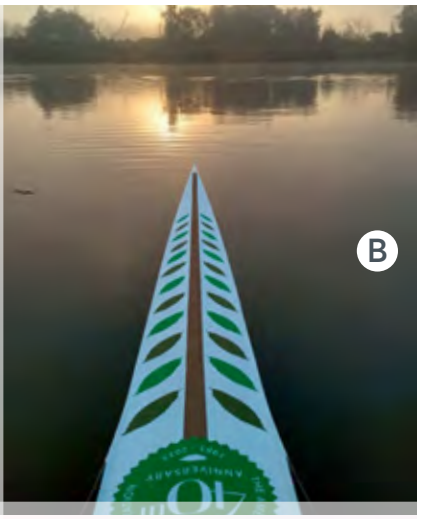
The first one might not seem so niche to you, since you are currently reading a publication called *Chestnut*, but for many, it may seem odd to like one single tree so much. This fondness has led me to both volunteer with the CT Chapter of The American Chestnut Foundation (TACF) and join its board.

My second passion is rowing. I picked up the sport in 2017, and have found it to be an amazing opportunity to get out and about in nature, watching birds fish at dawn, and being surrounded by beautiful sunrises. It is also an extremely challenging sport, requiring early morning practice, rigorous training, and strong dedication.

Over the summer, the CT Chapter had a brainstorming session about ways to celebrate TACF's 40th anniversary. Each chapter in the American chestnut's native range was planning activities and events, and then an idea came to mind: I would be spending every weekend in October racing at regattas in the northeast, much of which is in the native range. This was an obvious sign that my two passions needed to merge for this celebration. What if my boat, which would be traveling from Boston to Philadelphia, displayed the image of Charlie Chestnut, the mascot of TACF's educational youth program? After all, similar to American chestnut trees, my rowing shell is tall, straight, and (mostly) rot-resistant!

With the help of TACF staff members Jules Smith and Catherine Martini, who enthusiastically sent us high resolutions files of Charlie, Kathy McGuire, my designer-extraordinaire crewmate, and I started thinking about a design. Initially playing with ideas of tree branches with leaves and burs wrapping around the boat, we had to be clever with our application. During the fall, I train six times a week, so the vinyl needed to survive the intense use of the boat. It would encounter brackish water splashes during practice, full washes with soap post-practice, winds up to 60 mph on the highway during transport, and on top of that, it had to be removable at the end of the season.

A



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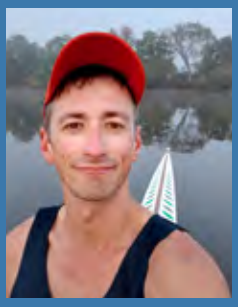


A Kathy (left) and Florian (right) crafting and applying the vinyl to the single shell.

B Sunrise on the Housatonic River in Shelton, CT.

C Introduction of Charlie Chestnut for its first regatta Head of the Riverfront in Hartford, CT on October 1, 2023. Photos by Mike McGannon.

D Charlie Chestnut, Valérie, and Florian at the prestigious and challenging Head of the Charles Regatta in Boston, MA on October 21, 2023. Photos by Dalton Neu.

E Florian sporting Charlie Chestnut tattoo. Photos by Valérie Leindfelder.

2023 Season Stats
(since May 1)

- 1,333,512 meters rowed
- 151 workouts
- 134 hours and 15 minutes

2023 medals

- 1st place at the C.R.A.S.H.-B. World Indoor Rowing Championships (Men's 27-35yo), Boston MA
- 1st place at Head of the Riverfront (Master Men's Single, 27-35), Hartford CT
- 9th place at Head of the Charles (Men's Club Single, open category), Boston MA
- 1st place at Head of the Schuylkill (Master Men's Single, 27-35), Philadelphia PA

We landed on a graphical pattern, with an alternating series of light and dark green American chestnut leaves running along Filippi's (the boat manufacturer) signature blue lines, which we turned brown for the occasion. The stern deck sported the 40th anniversary logo, and Charlie Chestnut stood proudly on the bow deck as the cutest ship figurehead ever created. For the final touch, the boat was temporally renamed Charlie Chestnut. Nightly, my coach Valérie Leinfelder would email the entire Great River Rowing team with Charlie Chestnut listed as one of the boats in the flotilla for the next day's outing; talk about public outreach! One of my teammates even gifted me a jar of chestnut honey, which he noticed at the store for the first time after being introduced to Charlie Chestnut.

Head races, in which the boats are grouped at the starting line and sent racing one by one, are held in the fall. All boats are tracked by a bow number and their time to run the entire course is recorded. Races are about 5,000 meters, but the distance of each race varies. Once every boat in the category has finished, the fastest boat wins. Attendance varies, from a handful of clubs coming together for a friendly scrimmage, to daylong regattas, to the biggest and most prestigious regatta in the world: Head of the Charles Regatta, held in Boston, MA. The competition is three days long and 2,598 boats competing in 2023.

It was exhilarating to race with chestnut leaves on the boat, and it attracted plenty of attention! I travelled with a lot of goodies from TACF, handing out 40th anniversary pins, stickers, and Charlie Chestnut temporary tattoos. Many people stopped me to share that they liked the colors of the boat. A race umpire even used his megaphone mid-race to congratulate me on the paint job. One woman said "Oh, TACF! I've heard of you. Y'all did the documentary with Dolly Parton!" Many others asked about the significance of Charlie Chestnut, which gave me the opportunity to share the work of TACF.

For the last three years, I have taken racing very seriously. In the past, I won a handful of bronze medals and ranked 17th at Head of the Charles. In 2023, my goal was to beat my previous times and places on podium. It was a lot of hard work; I ramped up the intensity and frequency of my workouts and cut down on late-night social activities so I could train at 5:30AM each morning. 2023 has been an incredible success: I won three gold medals and ranked ninth at Head of the Charles. While training was an essential key for this success, I think having Charlie Chestnut on my boat forced me to push harder than I would have if he was not there. I believe I owe him my gold medals.

THANK YOU:

I would like to gratefully thank Pam & Dave Raila for lending me their boat and trusting me to use it for chestnut and non-chestnut related shenanigans!

A big thank you to Kathy McGuire for her vinyl mastery, without which the Charlie Chestnut boat would not have existed.

Thank you to my coach and crewmates in the Great River Rowing club for cheering on Charlie and me, wearing chestnut tattoos, taking photos, and listening to my chestnut stories.

2023

NE-1833 Meeting

By: Stephen Hoy, North Central Regional Science Coordinator

To close out the final year of NE-1833, a group of nearly 30 individuals gathered at the University of Tennessee at Chattanooga in September 2023 to share their progress, challenges, and new areas of research surrounding the relationship between the destructive pathogen, *Cryphonectria parasitica* (formerly *Endothia parasitica*), and American chestnut.

The multistate research program, Northeast Regional Project (NE-140), was formed in 1982 by the USDA to investigate the potential of hypovirulence to control the fungus causing chestnut blight based on promising outcomes in Europe. This invited the opportunity to coordinate chestnut research across universities, non-government organizations (NGOs), and private, state, and federal government agencies. This year marks the end of the most recent proposal period, 2018 to 2023, and the project has been approved for another five-year cycle (NE-2333), building upon previous research areas as well as investigating novel topics related to improving chestnut disease resistance.

Two days of presentations included updates on early backcross material from The American Chestnut Foundation (TACF) planted in forested plots in Virginia over a decade ago, the potential of using CRISPR technology to affect *Cryphonectria parasitica* at the University of Maryland, and the potential effects of controlled burns on chestnut

(l-r) Hill Craddock, University of Tennessee at Chattanooga; Marty Cipollini, Berry College; Angus Dawe, Mississippi State University; Tom Klak, University of New England, Biddeford; and Steve Jeffers, Clemson University, during Fortwood Street Greenhouse tour. All photos by Stephen Hoy.

Hill Craddock stands in the seed and seedling cooler at the Fortwood Street Greenhouse.

Paola Zaninni shows attendees a bag of chinquapin seeds, some with radicles several inches long.

Hill Craddock showing the group a backcross seedling.

seedling establishment and survival at the State University of New York College of Environmental Science and Forestry. Former NE-1833 Secretary Mark Double led attendees down a virtual memory lane with his collection of animated photographs of attendees past and current. Participants also toured the Fortwood Street Greenhouse where the meeting's host Hill Craddock, TN Chapter president and professor at University of Tennessee at Chattanooga, and wife Paola Zaninni, who works alongside Hill, described their chestnut propagation techniques. They highlighted the current crop of control-pollinated backcross seedlings being grown as part of a larger project with TACF's Meadowview Research Farms and Penn State. A second field trip to The Pocket recreation area in Floyd County, GA gave attendees an opportunity to see naturally occurring and regenerating *Castanea alabamensis* (Alabama chinquapin). A recent publication found that specimens of *C. alabamensis* are morphologically distinct from Ozark and Allegheny chinquapin and American chestnut, and that they are not the product of hybridization between chinquapin and chestnut.

The newly funded round of research, NE-2333, proposes to continue the work of developing and evaluating disease-resistant chestnuts using traditional and molecular techniques, assessing biological approaches to control chestnut blight, including the manipulation of the fungus on a molecular level and the deployment of a hypovirus, and investigating chestnut conservation and reestablishment in orchards and forests. The longevity of this project and its many renewals offers an excellent example of collaboration between different organizations for the benefit of chestnut research. The sharing of these projects is an opportunity for formal and informal peer review, conversations with authorities in separate disciplines, and collective decision-making for the long-term benefit of chestnut research and future multistate research projects.

Additional topics presented during this meeting included but were not limited to:

- Nut grafting techniques and improvements by the U.S. Forest Service Southern Research Station to rescue American chestnut germplasm.
- Connecticut Agricultural Experiment Station's research into the relationship between drought, mycorrhizal fungi, and chestnut blight.
- Clemson University's research into the effects of *Phytophthora cinnamomi* on chestnut trees and the early findings of screening candidates for dual resistance to this disease and chestnut blight.
- University of New England's strides in accelerated pollen production on transgenic chestnuts through consistent extended light exposure that resulted in some of the first seeds from control pollinated chestnuts under these conditions. Researchers have improved pollen production seven-fold in the past four years of experimentation.
- TACF's progress towards chestnut restoration, the potential benefits of creating a separate formal cooperative organization to capitalize on the same benefits of the NE-1833 project, and an overview of the current propagation facilities at Meadowview Research Farms, as well as the future construction of a greenhouse to facilitate research areas requiring year-round containment and better environmental control.
- Updates from SUNY-ESF on Darling 58 transgenic chestnut, including some early findings of resistance variation in Darling 58 material, possibly related to the American parent. Additional promoters are being investigated, including a wound inducible promoter.

A complete history and past reports on the USDA CSREES Northeast Regional Projects can be found online at: https://ecosystems.psu.edu/research/chestnut/meetings/crees-ne-projects

2023 Photo Contest Winner

LILY ZEPORAH

"SHINING HOPE"
Blue Ridge Mountains
in Virginia

Thank you to everyone who participated in TACF's 2023 Chestnut Photo Contest!

This year we received a variety of photos, from close-ups of catkins and burs to photos of sunlight streaming through branches and leaves.

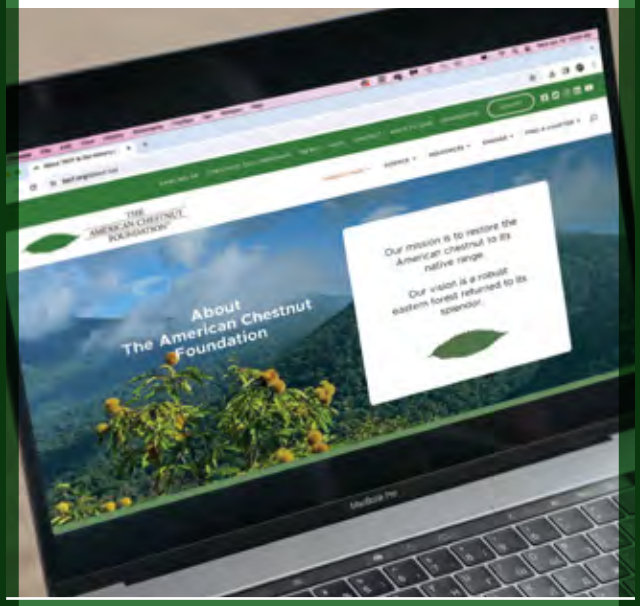
Lily Zeporah captured this year's winning photo, "Shining Hope." Lily came across this special tree in Virginia, in the heart of the Blue Ridge Mountains. Lily said of discovering a wild-type American chestnut that it felt "akin to discovering Black Beard's treasure" and that "finding a young chestnut tree gives us shining hope for the future." Congratulations, Lily!

ABOUT THE JUDGES: Maine Woodland Owners is the only statewide organization that advances stewardship of Maine's small woodland resources through the encouragement of good forest management and the advocating for and supporting of Maine's 86,000 small woodland owners. Since 1975, they have provided regular outreach, education, and guidance from staff and a broad network of forestry experts and resources. The Land Trust program conserves over 11,000 acres in 53 municipalities and offers legacy planning options.

Visit mainewoodlandowners.org or contact Jennifer Hicks at jenn@mainewoodlandowners.org, (207) 626-0005 for more information.

TACF HAS A New Website Address!

If you have visited The American Chestnut Foundation's website lately, you may have noticed a small change. Our website's domain name has officially switched from acf.org to tacf.org!

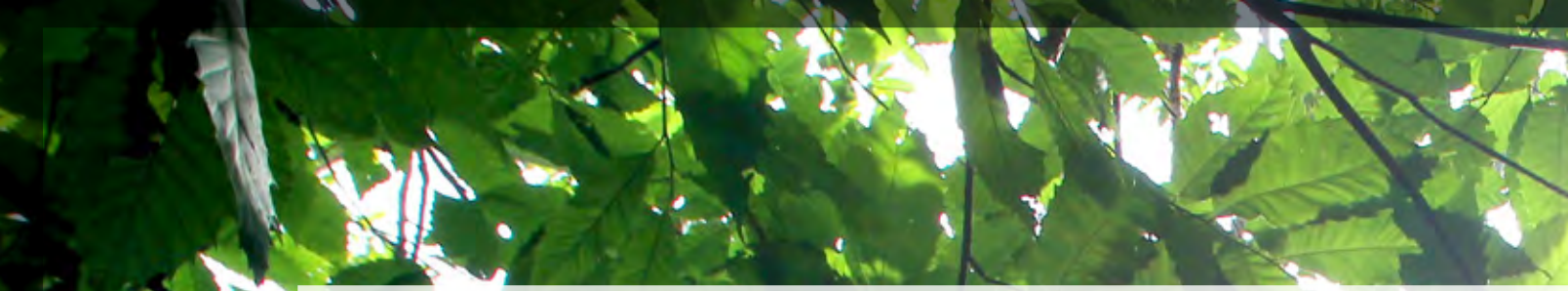


We are pleased to announce that after 27 years as acf.org, our web address now aligns with the organization's acronym, TACF. Any existing links to acf.org will still work in perpetuity, but we encourage you to start using tacf.org.

TACF email addresses now end in tacf.org, so please update your contact list and browser bookmarks. Acf.org email addresses will be deactivated next year.

If you have not visited tacf.org lately, some exciting new features and content have been added, including a new About Us page, Volunteer portal, Science Strategies page, and Partners page, as well as expanded information about our seeds and seedlings.

Please reach out to TACF's website administrator, Hal Brindley, at web@tacf.org with any comments or feedback.



THE PROMISING FUTURE OF AMERICAN CHESTNUT

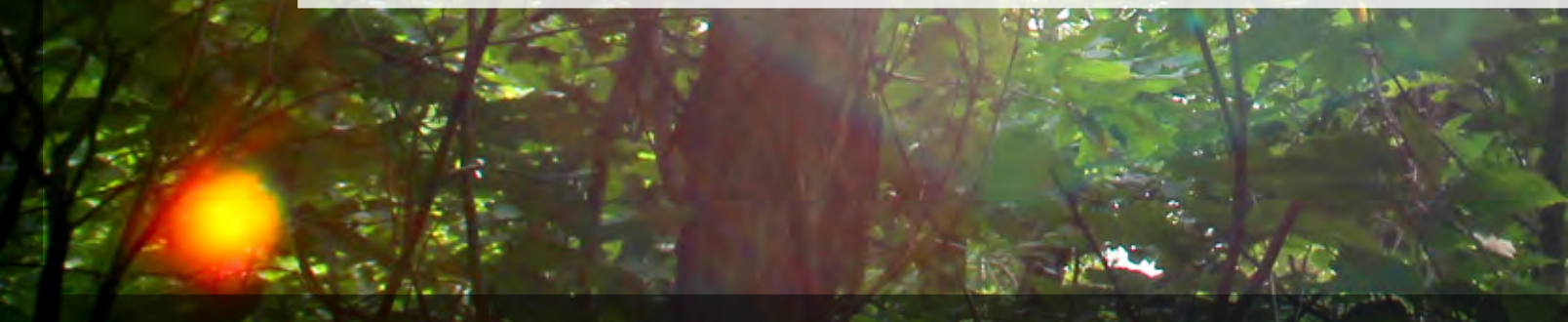
A Mission Rooted in Restoration

2023 marked The American Chestnut Foundation's (TACF) 40th anniversary. As we celebrated four decades of working towards restoring this iconic tree to its native range, we had numerous milestones to reflect upon.

In TACF's first decade, *The Journal of The American Chestnut Foundation* was published, keeping members up to date on the latest science surrounding chestnut restoration. Researchers also began a biological control study using hypoviruses, and the first state chapters were established. Over the next several decades TACF founded its research farm in Meadowview, VA to develop breeding orchards, launched efforts to combat *Phytophthora* root rot, and instituted regional offices. In recent years, support from members like you has allowed Meadowview Research Farms to expand in acreage and facilities.

Your continuing commitment ensures the next forty years look equally bright! Many exciting research initiatives are underway, including transgenic and cisgenic lines, gene editing technologies, further improvement of traditionally bred germplasm, and evolving methods like RNAi which may be able to silence the pathogenic functions of both the blight fungus and other diseases.

Public and private nurseries who partner with TACF stand ready to increase production of the most disease-resistant materials available, and distribute to members and supporters. Restoration is a decades-long process that will integrate many methodologies. Just as the journey of a thousand steps begins with the first, we continue this expedition together toward reestablishing the American chestnut as a foundational tree species for centuries to come.



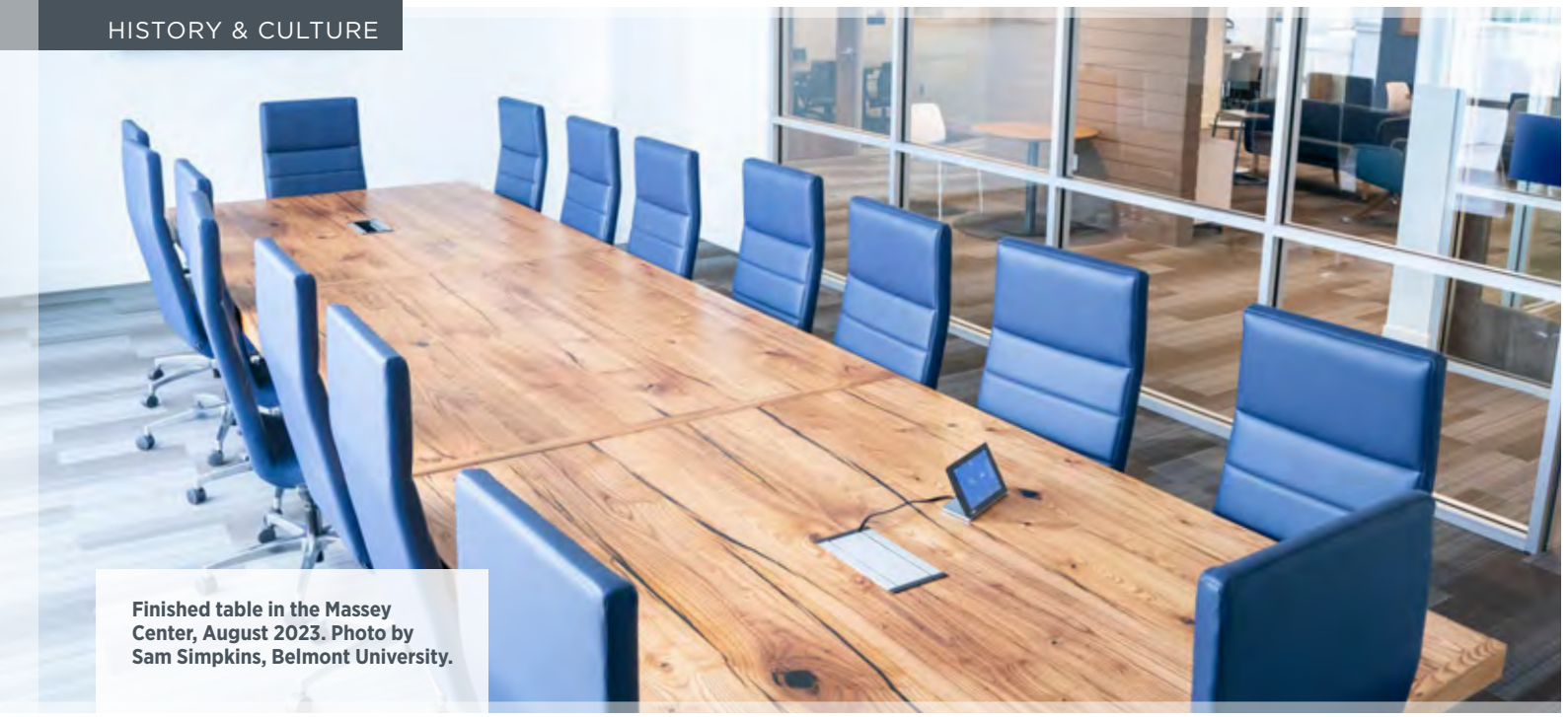
THE
AMERICAN
CHESTNUT
FOUNDATION®


GREEN LACEWINGS
LADY BEETLES
ASSASSIN BUGS

A View

FROM MEADOWVIEW

As you walk down the steps to the nursery, the world gets a little brighter as colorful flowers bloom in all directions. Welcome to the Meadowview Research Farms nursery pollinator patch! This garden was designed as an Integrated Pest Management (IPM) tool which takes a sustainable approach to protecting crop plants by managing interactions between desirable plants and the pests we want to avoid. The pollinator patch helps protect chestnut seedlings in the nursery by creating a habitat for natural enemies that feed on insect pests. Three natural enemies we love seeing around the nursery include Green Lacewings (left), Lady Beetles (middle), and Assassin Bugs (right). These three insects, along with many others, help to control aphids. These pests drain leaves of nutrients and are the most significant pest at the Meadowview nursery. Assassin bugs also go after caterpillars of local moths that defoliate the seedlings. This garden helps reduce the need for chemical insecticides to control pest infestations, which also allows butterflies, bees, and other species to thrive. The addition of this garden to the nursery has created a beautiful ecosystem for insect and human visitors to enjoy.



Finished table in the Massey Center, August 2023. Photo by Sam Simpkins, Belmont University.

PRESERVING HISTORY: BELMONT UNIVERSITY REVIVES AMERICAN CHESTNUT LEGACY WITH

'The Chestnut Table'

By Julia Couch Copeland, Belmont University

Complemented by stunning views of downtown Nashville, Tennessee, "The Chestnut Table" is the star of the sixth-floor conference room in Belmont University's new Jack C. Massey Center. The wood used to make the 19-foot table – American chestnut – was reclaimed in 2022 from column supports used on the front porch of one of the University's oldest buildings, Freeman Hall.

The prolific American chestnut totaled more than four billion trees in its prime at the beginning of the 20th century. As a dominant species in the eastern United States, it was one of the largest, tallest, and fastest-growing trees near campus.

Repairs were made to Freeman Hall's front porch during summer 2022, including the ionic columns that support the porch's roof. Built in 1892, the building is the front-facing part of campus, housing the Office of the President and more than 130 years of Belmont history.

The porch was added later, sometime between 1905 and 1912. As the blight tore through the region – Tennessee included – chestnut trees that had not yet succumbed to the blight were chopped down to use as lumber. In surplus supply, the logs from trees growing nearby were cut down and used as column supports.

"We wanted to make sure we didn't do anything to disrupt the historical integrity of the building," said David Minnigan of the repairs. Minnigan was a principal architect with Earl Swensson Associates, Inc. during the porch restoration and is now the University's architect-in-residence. "There was a bit of a surgical look at how the porch needed to be taken apart so we could put it back together and not lose history."

It was not until working with structural engineers to extract the column supports that the team learned there was something worth saving inside.

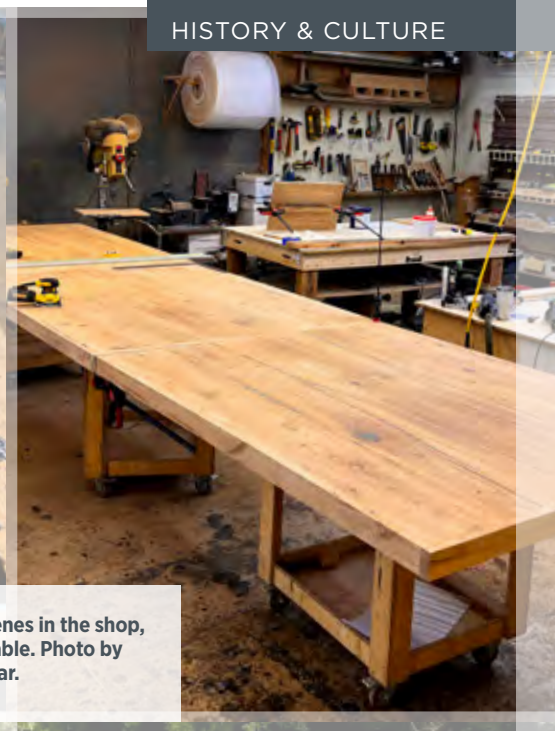
During the porch repair, the internal wood supports were replaced with steel. Of the six logs removed, two of them – though wormy – showed no signs of rot and were reclaimed for another use. Knowing how old the wood was, Minnigan had the foresight to call his friend Dave Puncochar, founder and CEO of the local custom wood studio Good Wood.

"I knew if anyone could do something with them, it would be him," said Minnigan of Puncochar's woodworking.

After taking a piece of the wood back to his shop, Puncochar said most of his team believed it would be chestnut.

"But chestnut is extinct," he said. "And sure enough, when we milled these trees, they were wormy chestnut. We were ecstatic and couldn't believe it."

Extracting chestnut supports from columns on the front porch of Freeman Hall at Belmont University in Nashville, Tennessee, June 2022. Photo by Sam Simpkins, Belmont University.



Behind the scenes in the shop, creating the table. Photo by Dave Puncochar.

Because chestnut is almost exclusively available in a reclaimed state, the wood is hard to find and runs five to ten times more expensive than other varieties. Puncochar offered to use the wood to make a conference room table for Belmont's Jack C. Massey Center, which opened in August 2023.

The wood needed numerous repairs and building was a laborious process. Following milling and drying and cutting panels, the Good Wood team filled each worm hole with epoxy to ensure a smooth surface.

Chestnut darkens in the sunlight, and due to the natural light that floods into the room where the table is housed, it will darken over time, only becoming more beautiful with age.

"We love preserving our past. This piece honors the story that it came from Belmont," said Puncochar.

The table now sits on the sixth floor of the Jack C. Massey Center, which houses collaborative and forward-thinking departments. The groups who use the table will be looking at complex problems from the past and finding creative solutions for the future.

"It was natural that we would take something very old and try to incorporate it into a new building. This is a piece that will be used for years and years, and I can't wait to see the faculty, staff, and students that will surround this table and the ideas formed around this table that will change the future," said Minnigan.

Dave Puncochar of Good Wood Nashville and David Minnigan of Belmont University. Photo by Dave Puncochar.

BIOGRAPHY
Julia Couch Copeland serves as a content writer on the University Marketing and Communications team. She is a double Belmont University alumna and holds a B.S. in public relations and an M.S. in strategic communication and leadership.

Grafting Southern Germplasm

WITH THE FOREST SERVICE'S SOUTHERN RESEARCH STATION

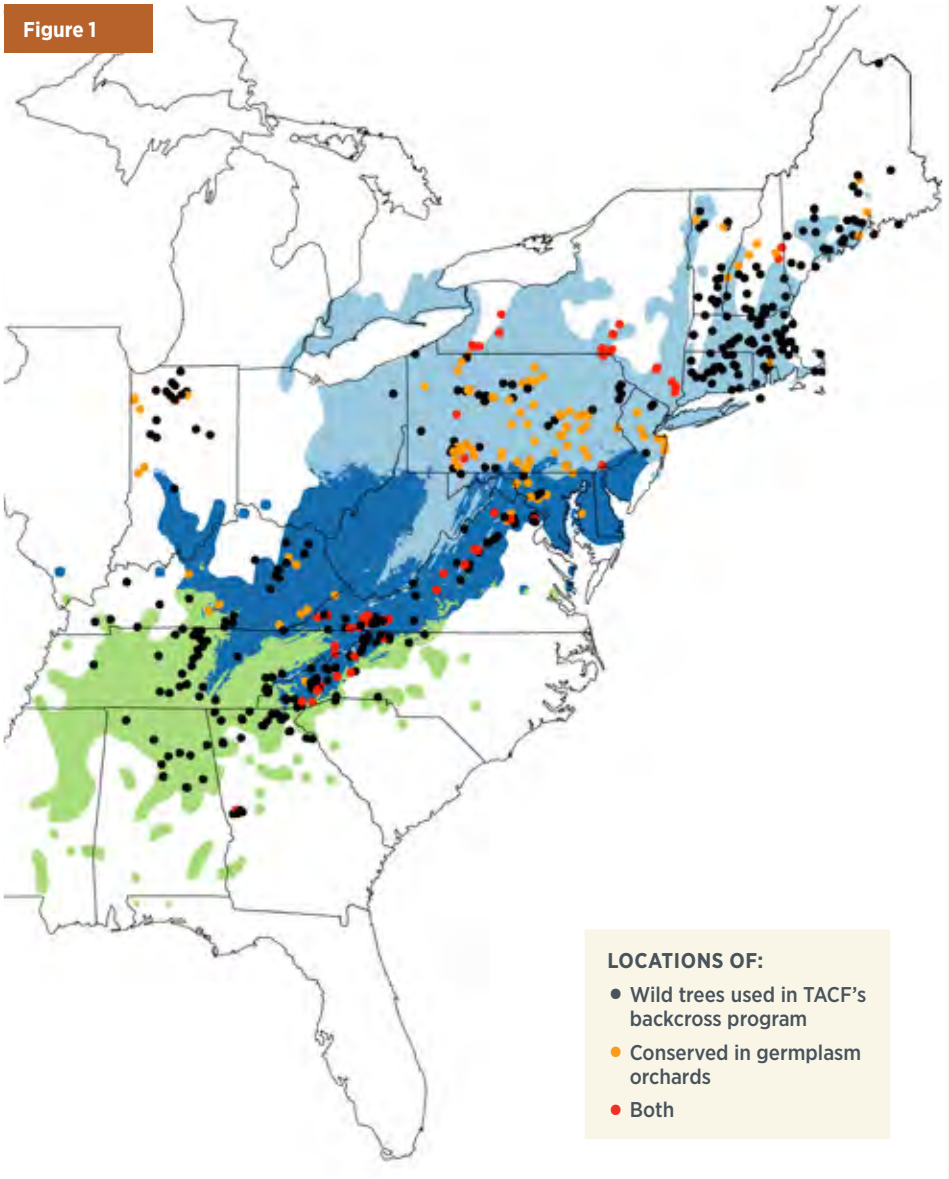
By Jamie Van Clief, Southern Regional Science Coordinator

Background

The American Chestnut Foundation (TACF), in collaboration with Virginia Tech, conducted a comprehensive study of the current population of American chestnuts, which was subdivided into three geographic sub-populations categorizing them into northeastern, central, and southwestern zones (**Figure 1**). This research revealed that the southwestern zone, consisting of western Tennessee (TN) and Kentucky (KY), Georgia (GA), Alabama (AL), and Mississippi (MS), exhibited the highest level of genetic diversity, yet was the least represented, making it a critical focal point for conservation efforts. Coupled with a warming climate, this magnifies the importance of preserving this Southern germplasm, as it holds the key to developing chestnut varieties adapted to hotter climates.

Under the leadership of Dana Nelson, co-director of the Forest Health Research and Education Center at the U.S. Forest Service (USFS) Southern Research Station (SRS), a new initiative was launched to locate, graft, and conserve chestnut germplasm sourced from the southwestern zone. Notably, Warren Nance, a retired SRS forest geneticist, played a pivotal role in this endeavor. His exhaustive efforts over several years led him to develop an improved modified nut-grafting technique, which he then used on 25 American chestnuts native to Mississippi and Alabama that he located. These states, situated in the southwestern-

Figure 1



Three seed zones of the American chestnut population, northeastern, central, and southwestern. Photo made in collaboration with Virginia Tech.

most part of the native range of American chestnuts, had received less attention in previous TACF conservation initiatives. Beginning last year (2022-23), SRS and TACF have been collaborating to collect scions and graft trees of high conservation value across the southwestern zone of American chestnut.

First attempts

In 2021-22, the efforts of Nance and Chance Parker (SRS forestry technician), yielded a remarkable 63% success rate with 1,290 graft attempts. Meticulous control of humidity, light, and temperature, along with acclimation strategies, were pivotal in achieving this success. Five grafts from each source were successfully delivered to TACF's Meadowview Research Farms in spring of 2022 (**Figure 2**).

Expanded Scion Collection

After a successful first year, the SRS-TACF collaboration was initiated in

Figure 2

Grafts and pots placed in pierced gallon-sized bag to hang in a window with partial sun to maintain high humidity. Photo by Chance Parker.

Bags are opened slowly as the grafts acclimate to the grow room's humidity, where they will be kept until they are able to move to the shade house. Photo by Chance Parker.

Successful grafts moved into the station's greenhouse.

Figure 3

After reviewing past biodiversity studies, the staff at Alabama A&M were able to locate surviving wild Americans in and around Bankhead National Forest. Pictured: Patience Knight (r), field coordinator/wildlife research technician, Helen A. Czech (l), wildlife research associate, and Jamie Van Clief (ctr), TACF southern regional science coordinator.

winter 2022-23 with a call to a handful of seasoned southern volunteers. The response was overwhelming, with more than 90 mother trees collected from across Kentucky, Georgia, Alabama, and Mississippi. Seventy-six of these trees became sources for the next round of grafting, reflecting the strength of these TACF chapters and partnerships with notable highlights including both The Land Between Lakes National Recreation Area and Alabama A&M University (**Figure 3**). These efforts helped with the resulting 61% take rate. The detailed breakdown of the 2022-23 success rates and genotypes across states is outlined in **Table 1** and the map below (**Figure 4**).

Next Steps

For now, the grafts are over-wintering in the care of SRS staff member Chuck Burdine. In the spring, these seedlings are slated for distribution to a handful of germplasm conservation orchards and Meadowview Research Farms, where we hope to grow them in our high-light growth chambers, expediting pollen collection and the preservation of this underrepresented diverse population.

Origin	Number of takes	Attempts	Percent take	Number of genotypes
Alabama	157	224	70.1	20
Georgia	165	215	76.7	19
Kentucky	126	187	67.4	14
Mississippi	248	406	61.1	23
SUM	**696**	**1,032**	**—**	**76**
AVERAGE	**—**	**—**	**61.1**	**76**

Table 1: 696 successful takes out of 1,032 attempts, with an average success rate of 61.1% sourced from 76 mother trees. Table by Dana Nelson and Gay Flurry, SRS forestry technician.

Figure 4

Map showing the distribution of all the successfully grafted scion wood that has been collected from the four states. Made by Geocodio.

Anticipating the 2023-2024 season, a new call for scion wood was shared in November, urging individuals to join in this effort! For more information, interested parties are encouraged to reach out to Jamie Van Clief.

Thank you to the continued dedication of chapter leaders including David Morris, Marty Cipollini, Jack Rogers, and Ken Darnell, to our many volunteers who sent in scion, and our numerous partners, most notably our colleagues at the Forest Service's Southern Research Station.

HOW DOES Oxalate Oxidase (OxO) WORK, ANYWAY?

By John Hempel, Research Associate Professor, University of Pittsburgh (ret); TACF Science and Technology Committee

Had Sandra Anagnostakis, of the Connecticut Agricultural Experiment Station, not found oxalic acid (OAA) to be the toxic agent of the chestnut blight fungus,[1] nor the late Bill Powell, of SUNY's College of Environmental Science and Forestry, not found her paper in the library and seized on the idea that genetic introduction of OxO might provide relief to the tree from the fungus,[2] there would be no discussion of oxalate oxidase (OxO) here.

Despite setbacks with the Darling 58 effort, the logic of introducing OxO into the American chestnut genome remains valid. So while a new recombinant is developed, with a better promoter and perhaps targeted location in the genome, one might ask what we know of OxO itself? A lot, if you ask a biochemist.

Proteins and Enzymes

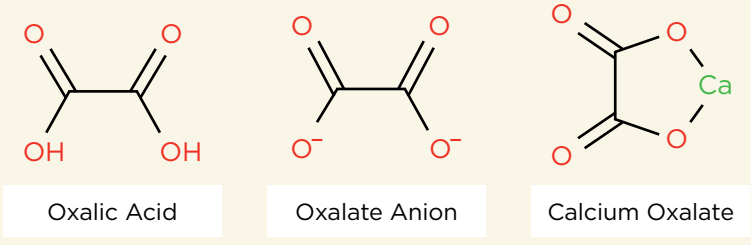
Proteins are linear polymers of amino acids. One of just 20 amino acids which only differ in their side-chains is found at each position of the protein chain, which can be any length. The occurrence of each is independent of the adjacent one. The twenty side-chains offer a variety of chemical properties. The linear chain folds into a functional, three-dimensional arrangement, directed by the sequence. All of this yields endless possibilities.

Enzymes are proteins that promote rapid chemical rearrangement (catalysis) of one or more compound – the substrate(s) – into product(s). They accomplish this by a combination of atomic forces that transiently bind the substrate into a pocket in the enzyme and exert forces on the atomic orbitals of the relevant parts of the substrate(s), bringing them into proximity and resulting in a rearrangement of the atoms of the reactants to yield the products. The reactions promoted by enzymes are not impossible in their absence, but rate enhancements afforded by them can be staggering. The fastest, catalase, converts 2.8 million molecules of hydrogen peroxide into water and oxygen per second.[3] These rates are brought about through interactions with amino acid residues and often additional components in the enzyme's "active site." In a very general sense, catalysis occurs through induction of a well-choreographed ballet of electrons in the substrate(s) that results in rearrangement of their atoms to yield the product(s).

All enzymes can be classified into one of six categories, including the Oxidoreductases. Many enzymes also require one of a variety of small "cofactors." Some also require metal atoms, which are far better at donating or accepting electrons than the purely organic chemical amino acid sidechains, and are referred to as metalloenzymes.(Footnote A)

About one third of all metalloenzymes with known three-dimensional structures have a Magnesium (Mg) ion at their catalytic center, followed

Figure 1



Oxalic Acid | Oxalate Anion | Calcium Oxalate

Standard representations of (non-ionized) oxalic acid, in its ionized (oxalate) form, and chelated with a divalent metal ion (example, calcium).

at 19-15% each by Zinc (Zn), Iron (Fe), and Manganese (Mn) atoms, and then in single-digit percentages by Calcium (Ca), Vanadium (V), Cobalt (Co), Copper (Cu), Molybdenum (Mb) and Tungsten (W).[4] Over 125 enzymes contain Mn. OxO, an Oxidoreductase, was discovered to contain Mn in the 1990s. Catalytic function comes from reaching the +3 (MnIII) oxidation state as detailed below.

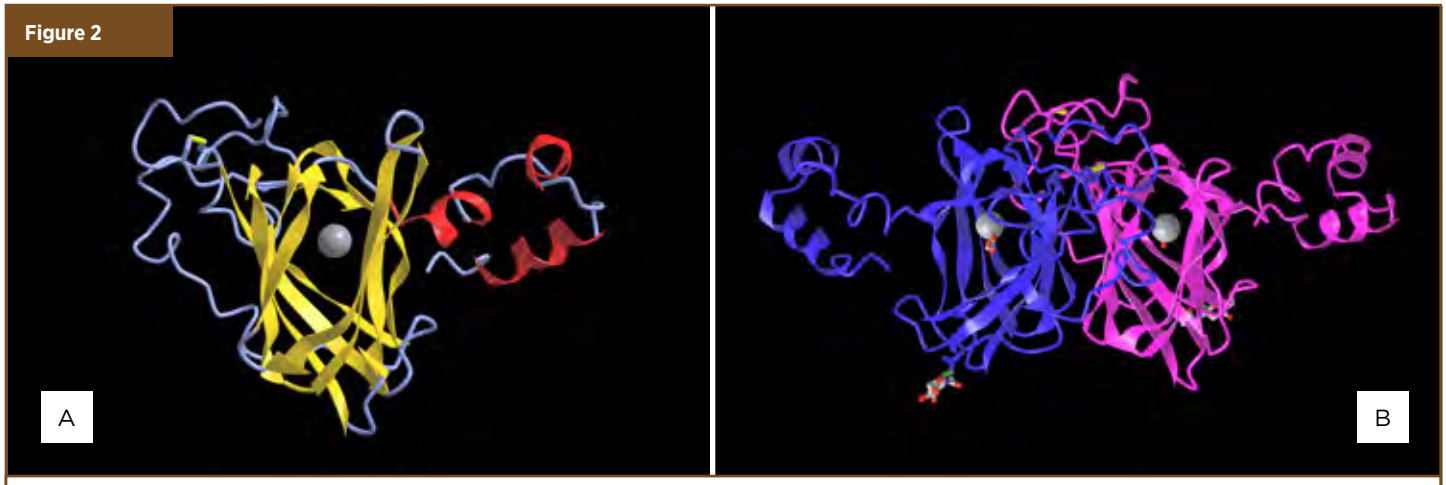
By virtue of its atomic structure with each of its five d orbitals incompletely filled with electrons, Mn is a potent oxidizing agent. Oxidation involves loss of electrons, so an oxidizing agent is one that has a potent ability to remove electronegative electrons from other compounds, making them more electropositive, or oxidized. But, for an oxidizing agent to be able to oxidize anything, it must first become electron-deficient itself, and few agents are able to remove electrons from Mn atoms. One of those is oxygen – O_2 – itself.(Footnote B)

Great Oxygenation Event

Manganese is relatively common in the Earth's crust at about 0.1%. The oxidation state of Mn in Earth's minerals reflects the availability of oxygen at the time of formation. It is hard to imagine that oxygen was not initially present in the Earth's atmosphere, and only arrived with the Great Oxygenation Event (GOE) some 2.35 Billion years ago, with the advent of cyanobacteria and photosynthesis, which itself depends on a complex of Ca, O and Mn atoms at the catalytic core of the photosynthetic complex.

Prior to the GOE, there was no atmospheric oxygen (O_2); Earth's oxygen was tied up as H_2O in the oceans, or mineralized in geologic strata as various oxides [e.g. carbonate, sulfate and silicate] In calcium carbonate strata, manganous (II) carbonate is a minor yet significant component, suggesting much higher concentrations of dissolved Mn(II) in seawater at the time, as soluble complexes of hydroxide, carbonate, and chloride. Without O_2 to oxidize it to higher oxidation states, Mn (II) could not promote electronic rearrangements in enzymes. The advent of O_2 enabled Mn to achieve higher oxidation states (Mn+3 and Mn+4) to then become an oxidant itself, providing an advantage for its incorporation in enzymes. With only one exception, of some 125 presently-

Figure 2



A. Schematic "ribbon diagram" of the monomer (subunit, the basic repeating unit) of barley OxO. The "regular secondary structure" of the subunits is mostly "Beta-strand" (yellow ribbons with arrowheads), with less "alpha-helical" structure (red spirals). "Random" structure is blue. This folding pattern is a "beta sandwich". The Mn atom (grey) is positioned in the middle of the sandwich.

B. Two monomers (blue, purple) form a dimer through association of the two outboard beta sheets of each monomer. Three dimers associate through their alpha-helical domains to form the active hexamer (Figure 3).

FOOTNOTES

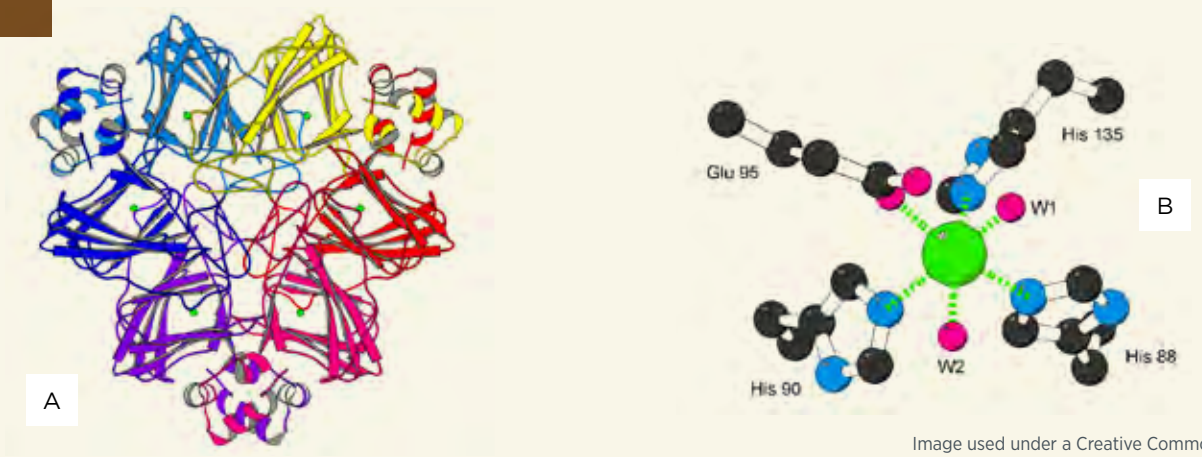
A Sometimes the metal atom only serves in a structural role, like a brace in a building. Alcohol dehydrogenase, an Oxidoreductase in our livers that initiates metabolism of beverage alcohol, contains two Zn(II) ions per subunit. One stabilizes the enzyme structure while the other participates in catalysis.

B A visual example of manganese oxidation can be seen in old purple-ish glass bottles or Victorian doorknobs. The green of some glass (eg. Coke bottles) is due to iron (Fe2+) impurities in the sand used to make it. If Fe2+ is oxidized to the Fe3+ state, the glass becomes a nearly-indetectable light yellow. Salts of Mn in the 3+ state are used for this. Although incompletely understood, it is generally believed that over time, exposure to ultraviolet (sun) light promotes reverse electron exchange that turns Mn compounds into ones with a purple color.[14]

C In domestic life, the strong cation-binding capacity of OAA anion2- with Fe3+ is the basis of its use as a household rust remover, while relative to our health most kidney stones are composed of the calcium(II) oxalate.

D Research on the enzymatic properties of OxO is complicated by the fact that the "resting state" of the enzyme as purified in the laboratory is inactive, with Mn in the +2 state. Only through oxidation of the metal to the +4 state by artificial means (sodium periodate), and then reducing it to the +3 state (with ascorbic acid) is maximal activity achieved.

Figure 3



A. Three dimers (Figure 2B) assemble into the functional hexameric protein, with the Mn atom (green) tethered in the approximate center of each sandwich. The dimers bind each other largely through interactions of their alpha-helices, visualized since each monomer is a different color.
B. The coordination of the Mn atom (green) in the beta-sandwich by the side-chains of three Histidines, Glutamic acid, plus two water molecules.[12]

known Mn-containing enzymes are taken to have arisen after the GOE.[5]

The GOE also enabled formation of insoluble minerals containing Mn ions, resulting in depletion of manganese levels in average seawater by 1,000 fold. Nevertheless, underwater "black smoker" thermal vents continued to release water rich in iron, nickel, magnesium and manganese. Some have speculated that the earliest life forms originated at these black smokers. A more reasoned view argues that their high temperatures (250-400°C) and acidic pH rule this out, with attention turning to non-volcanic alkaline hydrothermal vents with milder temperatures (60-90°C) some 10mi from the smokers, where their warmth is due to the exothermal reaction of newly-formed rock called olivine with water.[6]

Oxalic Acid and Germin Proteins

Oxalic acid (**Figure 1**, pg. 27) is the simplest dicarboxylic acid – an organic (carbon-based) molecule with two carboxylic acid (COOH) groups. OAA was long regarded as a metabolic waste product, since in its ionized (COO-) form it appears to be two CO_2 molecules joined together.

OxO catalyzes conversion of one molecule of OAA plus O_2 into two molecules of CO_2, and one molecule of hydrogen peroxide (H_2O_2).

More recently the "Germin" proteins that arise during seed germination were discovered to be oxalate oxidases, prompting their scrutiny. OAA, with its closely-spaced carboxylate groups, has a strong attraction to doubly-charged metal cations (positively-charged ions) such as Ca2+ ions (**Figure 1**, pg. 27). Calcium fluxes are involved in plant development, so the discovery that OxO can act on calcium(II) oxalate, releasing Ca++ in the process, suggested a role in development.[7] Peroxide from OxO action may also have developmental roles.[8 (Footnote C)]

OxO activity was first described in 1912, before any knowledge of protein structure.[9] Three-dimensional structures of OxOs from four sources have since been determined by X-ray crystallography and are available from the Protein Data Bank. All show the Mn atom tethered (liganded) to the side-chains of four amino acid residues – three Histidines and a Glutamic acid – plus two water molecules (**Figure 2A** & **3**). These ligands serve to stabilize the transient +3 oxidation state necessary for initiation of the reaction.

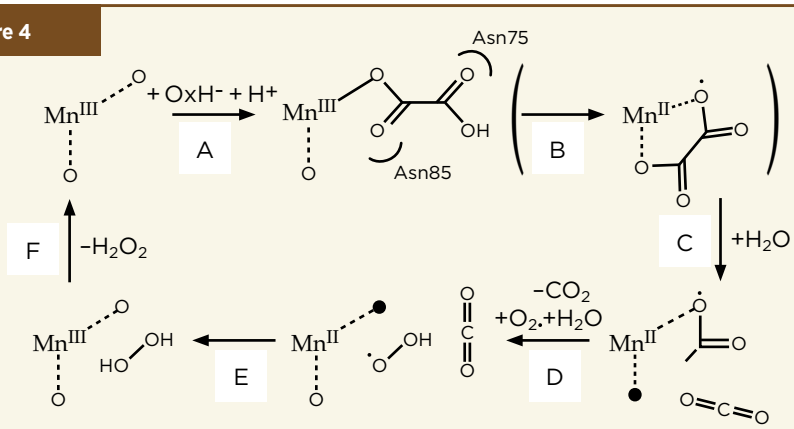
The relatively ordinary-sized enzyme consists of three pairs of identical protein chains of some 200+ (depending on species) amino acid residues – six altogether – to form the functional enzyme. It has the unusual property, for its occurrence in mesophilic organisms (existing in ordinary temperatures) of being exceptionally thermostable. OxO from the mold *Fusarium* attains maximal activity at 80°C (176°F).[10] Many proteins begin to denature (unfold) with loss of activity above 41°C – not much higher than normal body temperature. Similar extreme stability has been noted in OxO from other sources.[11] It is thus tempting to speculate that this reflects an origin in thermal vents where Mn ions were abundant.

OxO Catalytic Mechanism

A catalytic mechanism of OxO has been proposed based on evidence gained through a variety of advanced spectroscopic techniques that are beyond the scope here, but may be summarized as follows.[13 (Footnote D)]

The cycle begins with OAA coordinating Mn III as a doubly-negatively-charged substrate ionically bound to a triply-positively-charged Mn atom. The charge-imbalance is satisfied by the negatively-charged carboxyl from a Glutamic acid in the enzyme (**Figure 4:A**). OAA is positioned with help from two asparagine (Asn) residues, and displaces the water molecules shown in **Figure 3B**. Next, electron flow from one carboxylate of the substrate to the other and then to MnIII, reducing it to the MnII state, creates an unpaired electron at the first carboxylate – a highly reactive oxalate radical (**Figure 4:B**). Oxalate radicals are unstable and rapidly decompose into CO_2 and formic acid (formate), or in this case a CO_2 and a formate radical, still coordinated to MnII and charge-balanced from the Glutamic acid residue (**Figure 4:C**). The formate radical then loses a proton (H+) to become a CO_2 radical. Next, an oxygen and water molecule bind, with the O_2 picking up the proton and an electron from the CO_2 radical, yielding a peroxy radical and CO_2 (**Figure 4:D**). Electron transfer from MnII to the peroxy radical re-oxidizes Mn back to the +3 state, and transfer of a proton from water to the radical yields hydrogen peroxide (**Figure 4:E**). The remnant hydroxide from the water now serves as a MnIII ligand again. Peroxide is released as the remaining product (**Figure 4:F**), and OxO is ready for another reaction cycle to occur. Note that at every step of this reaction, charges were balanced and electrons were accounted for.

Figure 4



Catalytic mechanism of oxalate oxidase.[13]

Understanding the details of this reaction may not help fight *C parasitica*, but it should be satisfying to know that the way OxO converts OAA to CO_2 and H_2O_2 is known in fine detail, which is the intent here. No magic, just biochemistry.

THANKS

The author thanks Joan Valentine for her critical review of this manuscript and suggestions.

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DEDICATION

Dedicated to my uncle, Loren C Hurd (PhD 1929, U Wisconsin Madison), a metallurgical chemist who as a post-doc in Germany was close to the chemists who discovered Rhenium (Ref 15), located in the Periodic Table in the same column as Manganese, two rows below it.

Genomic Data

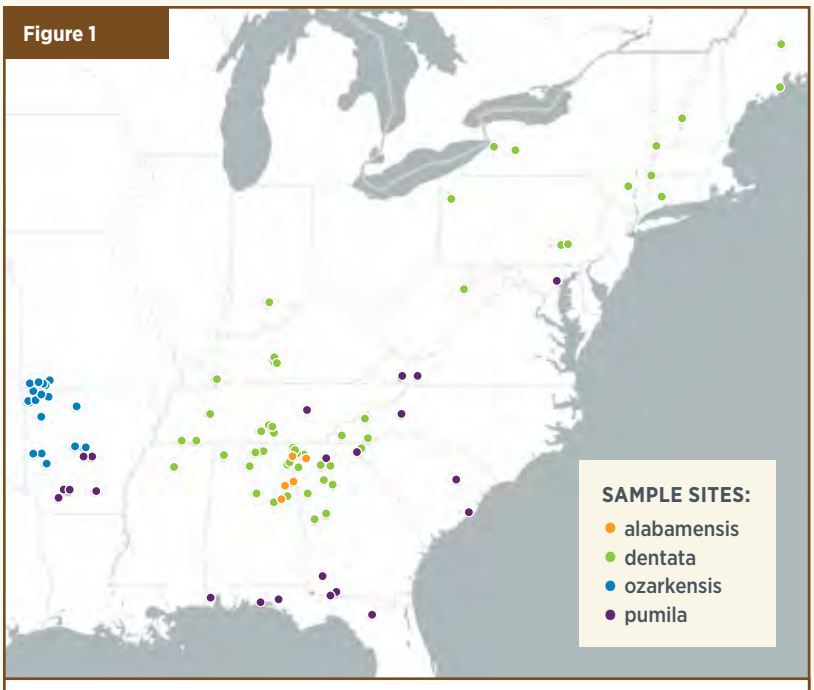
SUPPORT RECOGNITION OF THREE NORTH AMERICAN CHINQUAPIN SPECIES AND LACK OF NATURAL HYBRIDIZATION WITH AMERICAN CHESTNUT

By M. Taylor Perkins, The University of Tennessee at Chattanooga; Alexander M. Sandercock, Virginia Tech; Paul H. Sisco, Carolinas-TACF Chapter; Frederick L. Paillet, University of Arkansas; Jared W. Westbrook, The American Chestnut Foundation; Ronald S. Revord, University of Missouri; Jason A. Holliday, Virginia Tech; J. Hill Craddock, The University of Tennessee at Chattanooga

Background

For over a century, researchers have hypothesized that species boundaries between American chestnut and its closest evolutionary relatives, the North American chinquapins, are highly porous. In the early 20th century, botanists described hybrid taxa thought to descend from natural hybridization between American chestnut and chinquapins (Dode 1908; Camus 1929). More recent studies have postulated that American chestnuts in the southeastern United States contain additional genetic and morphological diversity due to past and ongoing hybridization and introgression with chinquapins (Shaw et al. 2012; Li and Dane 2013). If southern American chestnut populations do indeed contain alleles introgressed from chinquapin, then these alleles may provide adaptive advantages to American chestnuts in certain environmental conditions. The population genetic structure of American chestnut has been extensively studied, yet we know little about the genetic structure of the chinquapins and whether they have exchanged potentially adaptive genetic material with American chestnut. To investigate these topics, we generated and analyzed whole-genome sequencing data from 262 plants representing American chestnut (*Castanea dentata*), Allegheny chinquapin (*C. pumila*), Ozark chinquapin (*C. ozarkensis*), Chinese chestnut (*C. mollissima*), Japanese chestnut (*C. crenata*), and the recently rediscovered taxon *C. alabamensis* (**Figure 1**).

Figure 1



Sample sites of North American *Castanea* species used in this study.

Results and Discussion

We inferred a phylogenetic tree using 5,769,022 single nucleotide polymorphisms (SNPs) generated from our samples (**Figure 2**). The phylogenetic tree showed that the three chinquapin taxa form a clade that is sister to American chestnut, consistent with the results of a recent study of these species (Perkins et al. 2021).

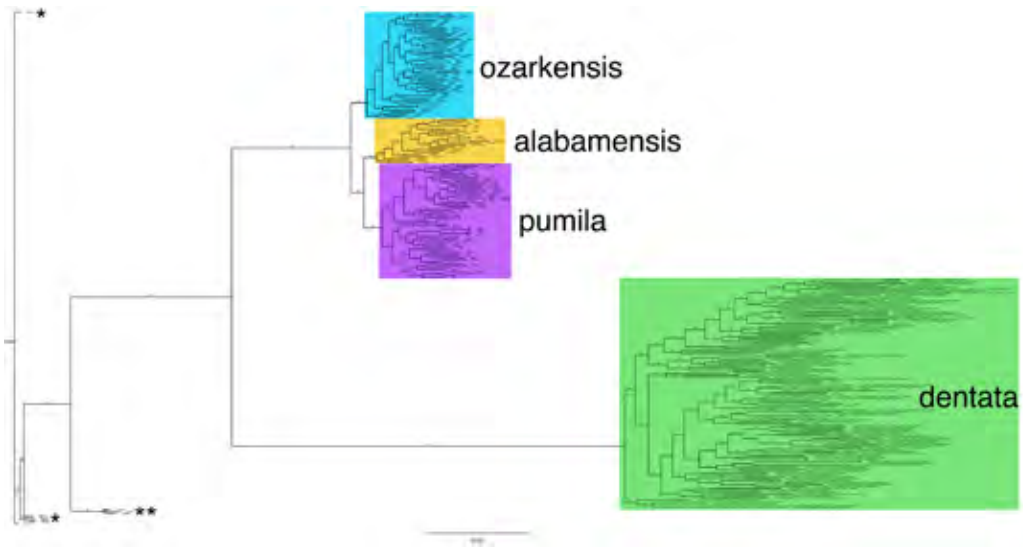
Population structure analysis revealed fine-scale genetic structure within the North American *Castanea* species that varied across large geographic regions (**Figure 3**). This analysis did not support the hypothesis of introgression of alleles from chinquapins into *C. dentata* (**Figure 3A**).

We found evidence for three genetic clusters in *C. dentata*. This is consistent with a recent study by Sandercock et al. (2022), who concluded that American chestnut population structure has been shaped by a combination of Pleistocene glacial refugia, postglacial recolonization, and contemporary gene flow. One cluster occurs at highest frequency in the northeastern part of American chestnut's distribution (**Figure 3A**). From Pennsylvania to the northernmost extent of our sampling in Maine, plants had 100% ancestry from this source. From Kentucky southward, two other clusters predominate: one occurs more frequently in the eastern portion of the species' distribution and a second occurs more frequently in the western portion of the distribution. American chestnuts in the southern Appalachians commonly have genomes that contain a mixture of these two ancestry sources. Gailing and Nelson (2017) previously documented similar allele frequency differences between samples from the east and west of the Appalachians. The "northeastern" cluster occurs as the minority ancestry in slightly under half of all American chestnuts sampled from Tennessee and North Carolina southward. We observed an exception to this pattern in montane sites of North Carolina, Tennessee, and South Carolina, where "northeastern" ancestry is present as the majority source.

Population structure analysis identified low to moderate levels of admixture between the different chinquapin species (**Figure 3B**). 13% (18/135) of all chinquapin samples had hybrid ancestry from a different chinquapin taxon. We detected *C. pumila* ancestry in 10 of 54 *C. ozarkensis* samples. In these admixed plants, *C. pumila* ancestry was never more than 27% of a plant's total ancestry. Introgression from *C. ozarkensis* to *C. pumila* was limited to two plants at one sample site, at the interface of the Ouachita Mountains and Coastal Plain in Arkansas. Introgression from *C. pumila* to *C. alabamensis* was detected in six plants from one sample site in northern Alabama.

We detected two genetic clusters within *C. pumila* (**Figure 3B**). One occurs at highest frequency in the Appalachian Mountains and at our northernmost chinquapin site near Baltimore, Maryland. The second occurs at highest frequency in Coastal Plain sites of Arkansas, Alabama, Florida, Georgia, and South Carolina. From Florida to coastal Georgia and South Carolina, plants display a cline of ancestry with increasing levels of "Appalachian" ancestry as sampling increases in latitude and elevation. Interestingly, the "Appalachian" ancestry type corresponds to plants matching the description of *C. pumila sensu stricto*, which is characterized by pubescent twigs and shrubby habit, while the "Coastal Plain" ancestry type corresponds to both stoloniferous and arborescent plants that match the descriptions of the formerly recognized taxa *C. alnifolia* (Nuttall 1818) and *C. floridana* (Ashe 1922).

Figure 2



Phylogeny of the North American *Castanea* taxa. Phylogenetic tree was inferred using IQ-Tree software (Nguyen et al. 2015). North American *Castanea* samples are highlighted. Samples representing the Asian outgroup species are denoted with asterisks (* = *C. mollissima*, ** = *C. crenata*).

To more rigorously test for introgression from chinquapin species into *C. dentata*, we inferred admixture events using an alternative method, with TreeMix software (Pickrell and Pritchard 2012), and performed ABBA-BABA tests (Patterson et al. 2012) between *C. dentata* and each chinquapin species (figures not shown, for brevity). These analyses revealed two admixture events between *C. dentata* and chinquapins that were not apparent in our population structure results. In each case introgression occurred in only one direction, from *C. dentata* into chinquapin populations. In one case, a population of *C. ozarkensis* from

Arkansas was descended from an admixture event with *C. dentata*, followed by backcrossing to *C. ozarkensis*. This was likely an ancient admixture event that occurred when the distributions of *C. dentata* and *C. ozarkensis* overlapped. In the second case, some *C. alabamensis* individuals were descended from an admixture event with *C. dentata*, followed by backcrossing to *C. alabamensis*. The current distributions of *C. dentata* and *C. alabamensis*, combined with the placement of this admixture event on an evolutionary tree, suggest that this genetic exchange was more recent.

We have shown that hybridization between the different chinquapin taxa is infrequent. Additionally, *C. alabamensis* was distinct from other chinquapin taxa in all analyses. These findings, along with key differences in morphology and ecology, lead us to advocate recognition of three chinquapin species: *C. pumila*, *C. ozarkensis*, and *C. alabamensis*. Finally, our results have allowed us to reject the hypothesis of past and ongoing introgression from chinquapin into *C. dentata* populations. Therefore, American chestnut restoration in the southeastern United States can proceed without special consideration for the existence of uniquely adapted hybrids between *C. dentata* and chinquapin.

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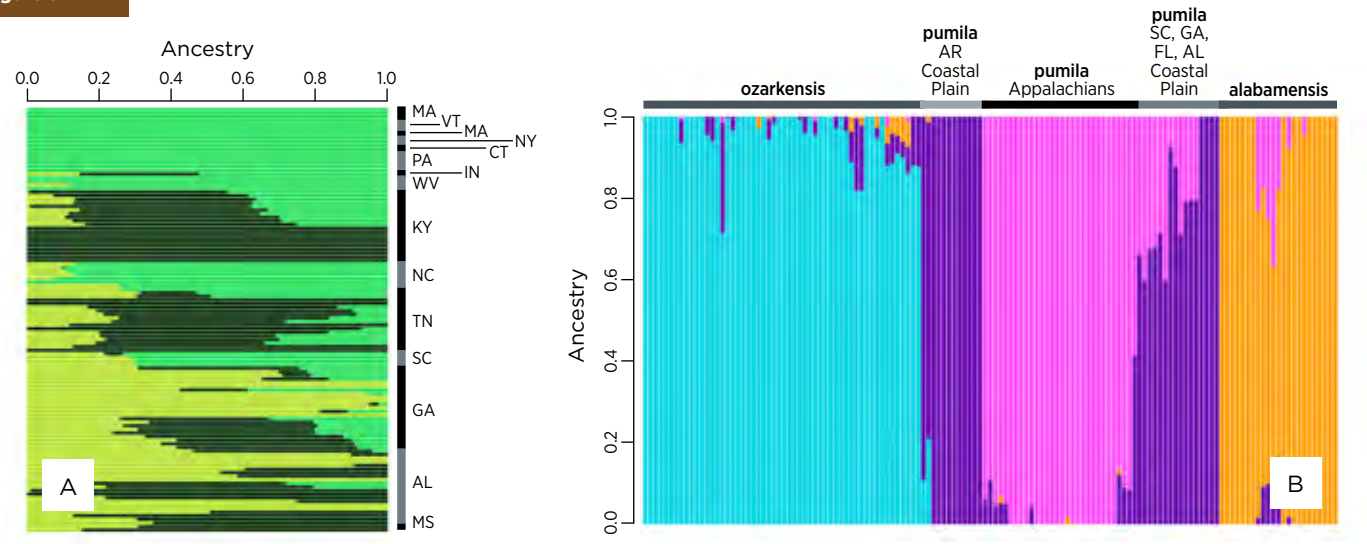
Figure 3



Ancestry proportions estimated from population structure analysis of the North American *Castanea* species. Ancestry proportions were estimated using ADMIXTURE software (Alexander et al. 2009). Samples are considered hybrids if they contain 10% or more ancestry from a second species. **A**: Results for American chestnut, arranged by approximate latitude (north to south) and labelled by state. Rows correspond to individual plants and ancestry proportions for each plant are depicted along the x-axis. Light green = northeastern type; dark green = southwestern type; yellow-green = southeastern type. **B**: Results for the chinquapin species, arranged by species and then by approximate longitude within species (west to east). Columns correspond to individual plants and ancestry proportions for each plant are depicted along the y-axis. Blue = *C. ozarkensis*; purple = Coastal Plain *C. pumila*; pink = Appalachian *C. pumila*; yellow = *C. alabamensis*.

Prosciutto-Wrapped Chestnuts with Maple Glaze

By David Ruberti, MA/RI Chapter

For the past few years, my wife and I have attended the MA/RI Chapter's annual meeting. The event is full of interesting speakers and shares Chapter business updates, but my favorite part is the chestnut-themed potluck dinner!

David pours maple sap tapped from his tree.

The first years we attended, we scrambled to produce chestnut recipes. We brought different soups, a salad, and I believe stuffing. In 2022 I found a prosciutto-wrapped chestnut recipe online but could not locate it when I looked again in 2023, though I did see many recipes using bacon and thought that might be it. After giving it more thought, I remembered that the recipe used prosciutto to wrap the chestnut, securing it with a toothpick, frying both in a pan, then coating with honey and an herb. I practiced making a few with honey, and they were good, but I decided it would be so much easier to coat with maple syrup. I have maple syrup from trees I tap each year, so I poured some on a plate, dipped the wrapped chestnuts, and placed fresh rosemary on top. It tasted much better, in my opinion, than the online recipe that called for honey. It did not take long for them to disappear during the Chapter's annual meeting in December 2023!

Recipe

Wrap one strip of prosciutto around each chestnut (20 or so), securing each with a toothpick.

Heat frying pan to medium, adding 2-3 tablespoons of olive oil.

Place prosciutto-wrapped chestnuts in a heated pan, turning as they start to fry to include each side. This takes about two minutes.

Pour desired amount of maple syrup on a plate.

Spin the prosciutto chestnuts in the maple syrup, then sprinkle fresh rosemary on top.

A Tribute to Carl Absher

What kind of person becomes obsessed with American chestnut restoration? Who spends so much time blowing on the embers of loss, hoping against hope to rekindle one little source of warmth and safety on this planet?

Who does that knowing that even in a best-case scenario – if they successfully get the fire going again – they won't live long enough to enjoy it themselves? Knowing that they are only here to help bridge the gap between their ancestors' warm, safe past and their children's warm, safe future?

Hopeful people do this. Faithful people do this. Above all else, people whose love is stronger than their fear, pride, common sense, and even their mortal bodies.

Carl Absher, 72, longtime Virginia board member, was one of those people. He loved tectonically. As an arborist and naturalist, he sowed that love into the planet with precision and practicality. And he loved well: he was married to his college sweetheart, Becky, for more than 50 years; he talked often with a twinkle in his eye about his kids, B.J. and Amanda, and his grandsons, Jameson and Lucas.

All of us in the Virginia Chapter were huge Carl fans. Retired to his hometown of Blacksburg, VA from life as a tree-care business owner and vegetation manager in Tennessee, he remained one of our most dedicated field volunteers until a couple weeks before his death.

In February 2023, Carl went into the woods with several board members to assess our (thriving!) restoration planting on Johns Creek Mountain. Before that, he did the most and stayed the longest when we planted our RESMAP study planting in Matthews State Forest near Galax.

Even after Carl could no longer do much outside, he weighed in on all our online Chapter discussions and golf-clapped for our Instagram posts. He refused to let a pesky nuisance like *two competing, advanced terminal cancer diagnoses* get in between him and what he loved. For heaven's sake, he even scaled a giant sequoia at age 69 and got a Tasmanian Devil tattoo with "NEVER GIVE UP" underneath.

I joked with Carl once that he was unkillable like Rasputin. He responded that no, he was more like an American chestnut: one cancer was the blight, the other was *Phytophthora*, and here he was, chugging along, a little cankered. Hope never leaves when you are a chestnut person, even when you are dying. Even when you are dead.

Your people will keep nursing the fire along until we ourselves are gone and new friends take our place. Together with TACF's national office, the Virginia Chapter recently established the Carl Absher Memorial Internship, raising $10,000 against a matching $10,000 from Chapter funds. This internship will help train the next generation of scientists dedicated to the work of world-mending. For more information, email vachestnut@gmail.com.

We love you, Carl.

In Memory of our TACF Members

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Catawba Community Club
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Barry Hypes
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We regret any errors or omissions and hope you will bring them to our attention.

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SAVE THE DATE FOR TACF'S 2024

Wild-Type American Chestnut Seedling Sale

TUESDAY, MARCH 19 AT 8:00AM

ARE YOU READY TO GROW SOME AMERICAN CHESTNUT TREES?

As a special membership benefit, The American Chestnut Foundation (TACF) will be selling wild-type American chestnut seedlings on Tuesday, March 19, 2024. This is a member exclusive sale and is very popular. Seedlings are expected to sell out quickly.

Online and phone sales will open at 8:00AM on Tuesday, March 19. A private website link will be emailed to active members on Sunday, March 17 and again when the sale goes live. Members must be active as of March 1, 2024 to receive the link.

To accommodate demand and meet nursery bundle requirements, TACF is offering one bundle of 10 bareroot seedlings at $90 while supplies last (shipping early to mid-April included.)

Growing wild-type American seedlings is a wonderful learning experience and helps preserve genetic diversity. While wild-type American chestnuts are not blight-resistant, they can thrive for many years and produce seed for consumption and future breeding.

NOTE: In order to prevent the spread of contagions from the native range, no orders will be shipped to states west of the Mississippi River or outside the continental U.S.