FALL 2021 ~ ISSUE 3 VOL. 35

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

A BENEFIT TO MEMBERS

THE AMERICAN CHESTNUT FOUNDATION

Cherokee Indians ~ Herbicide Translocation ~ Price Farm Plantings ~ Lost American Chestnut Relative

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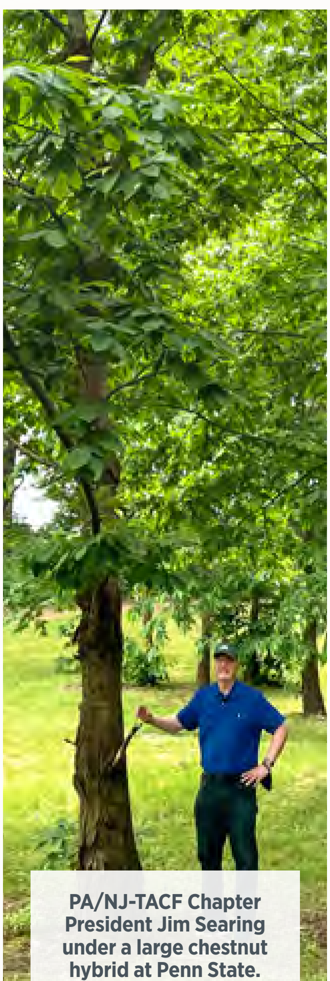
Seven Years as a:

CHARITY NAVIGATOR
Four Star Charity

living wage CERTIFIED

Lisa Thomson

President and CEO



PA/NJ-TACF Chapter President Jim Searing under a large chestnut hybrid at Penn State.

GREETINGS RESILIENT CHESTNUTTERS!

At press time, we are still addressing COVID concerns as an organization. After a brief taste of freedom, thanks to a fully vaccinated staff and lifting of travel and in-person restrictions, we are now monitoring the Delta variant surge closely. We continue to implement CDC and individual state guidelines to ensure the safety of our staff and volunteers. We hope it will abate quickly but this pandemic continues to be unchartered waters. Thanks to a flurry of travel in June and July to address the backlog of field work, we are grateful to science staff and chapter leaders for their support and energy this summer.

Throughout the strange year that was 2020, TACF's 16 chapters continued to do important work, both safely in the field with social distancing and conducting virtual board and annual meetings. The flexibility and determination of these volunteers is truly remarkable. There is far more work to be done than any single staff person or organization can do, and the strength of the chapters lies in extending the vast network of partners and individuals who share their own unique backgrounds and skills to further the mission of TACF.

The importance of having this broad network of partners and citizen scientists cannot be understated. Please look for future articles on the breadth and impact of chapter work to highlight this remarkable army of talented chestnut enthusiasts who leverage the work of TACF exponentially. Chapters not only cheerfully adapt to new science direction and activities, but also provide important innovation for the organization as a whole. Perhaps most importantly, our chapter volunteers are often master storytellers who eagerly share our hopeful, bold mission with a variety of audiences and partners.

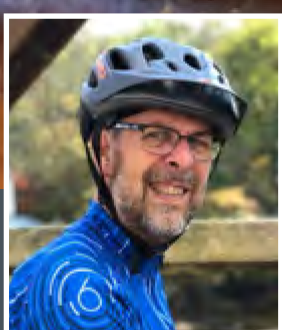
We salute these stalwart volunteers, who constitute a dedicated cadre of hardworking chestnutters who are part of the Grand Experiment of rescuing an iconic tree. This network is unique in the conservation movement and a model for other organizations, and is one of the reasons TACF's mission is so enduring, decades from its inception.

Lisa Thomson

Lisa Thomson, President and CEO
The American Chestnut Foundation

Chestnuts

This stack of American chestnuts was harvested at Meadowview Research Farms. The photo highlights the detail and color of this precious seed.



TACF BOARD CHAIR DR. BRIAN MCCARTHY has stepped down to recover from a serious spinal cord injury due to a mountain bike accident in early July. We are grateful that Vice Chair Jay Cude immediately assumed his duties. While the full extent of Brian's recovery is not yet known, please send healing thoughts or good wishes to Brian, c/o Lisa Thomson at lisa.thomson@acf.org, as he continues to recover.

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.

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Bringing the Chestnut Home:

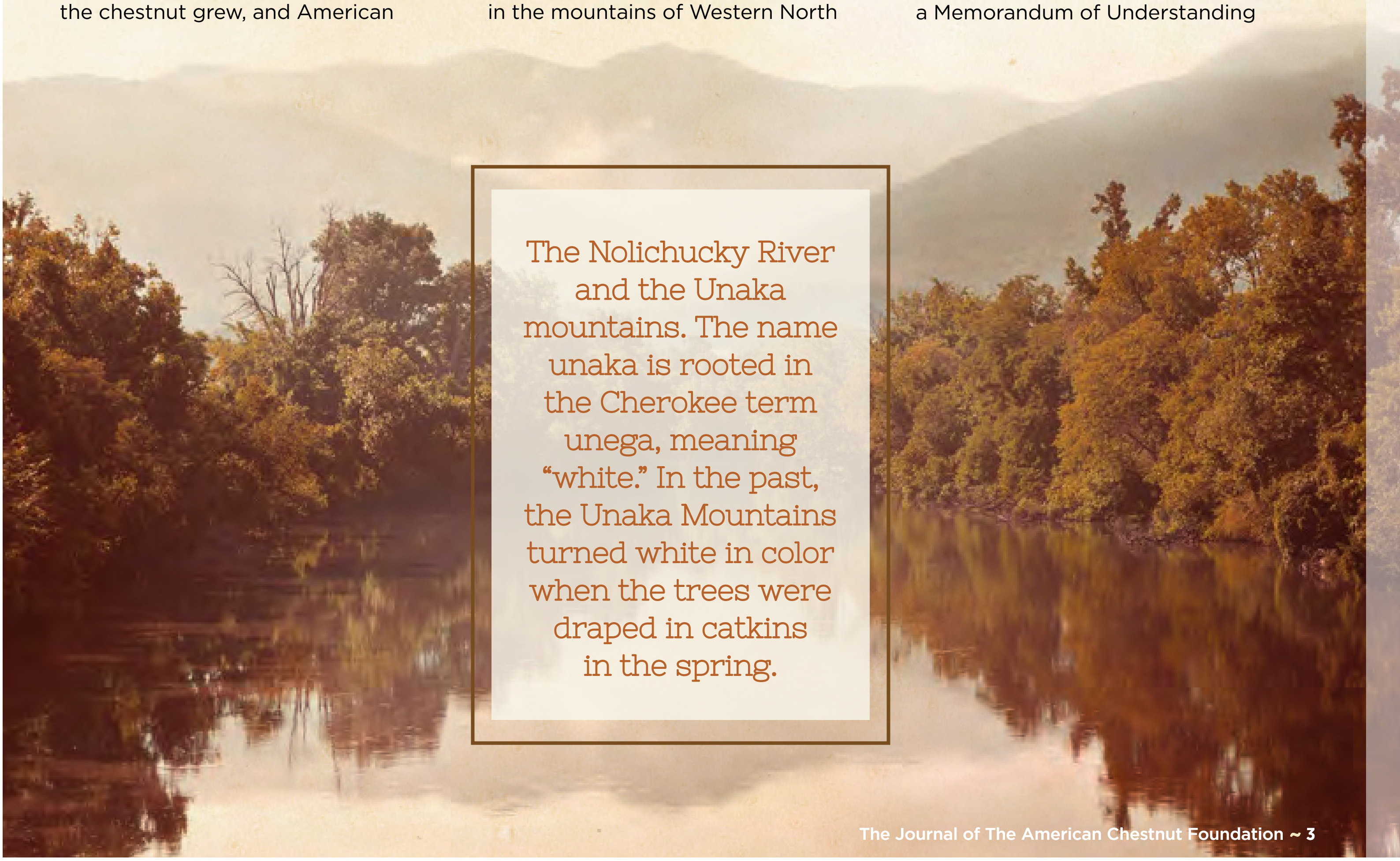
EASTERN BAND OF CHEROKEE INDIANS

By Samantha Bowers, TACF Director of Philanthropy and External Affairs

For thousands of years, before globalization and colonialism brought the invasive chestnut blight pathogen to American soils, the Cherokee made a cough syrup from the leaves of the American chestnut tree. They also took an infusion of year-old chestnut for heart trouble. Today, the knowledge of this culturally significant tree is fading for two reasons: The Indian Removal Act displaced Cherokee people from their home lands where the chestnut grew, and American chestnuts have been reduced to stump sprouts by the introduction of chestnut blight. Thanks to a special friendship, a partnership has formed to return the American chestnut to tribal, sovereign lands.

Two important members of our communities, the Eastern Band of Cherokee Indians (EBCI) tribal elder Jimbo Sneed and the late father of TACF's Director Emeritus and USFS Forester Rex Mann, who grew up in the mountains of Western North Carolina, began this sincere outreach. Rex was interested in sharing with Jimbo, his wife Hazel, and his son Dike, the importance of his life's work to restore the American chestnut back to eastern U.S. forests.

Nearly six months later, atop a pristine mountain in the American chestnut's native range on April 21, 2021, the EBCI and The American Chestnut Foundation (TACF) celebrated a new partnership with a Memorandum of Understanding



The Nolichucky River and the Unaka mountains. The name unaka is rooted in the Cherokee term unega, meaning "white." In the past, the Unaka Mountains turned white in color when the trees were draped in catkins in the spring.

(MOU) signing ceremony with Vice Chief Alan B. Ensley and TACF's President & CEO Lisa Thomson. The purpose of this MOU is to establish a demonstration orchard for the restoration of the American chestnut tree using local genetics, as well as collaborate on the management requirements for sustaining repopulation of the American chestnut tree. The signing site was at the location of a chestnut orchard to evaluate scientific progress on developing blight-resistant American chestnuts and to begin producing seed for distribution across tribal lands.

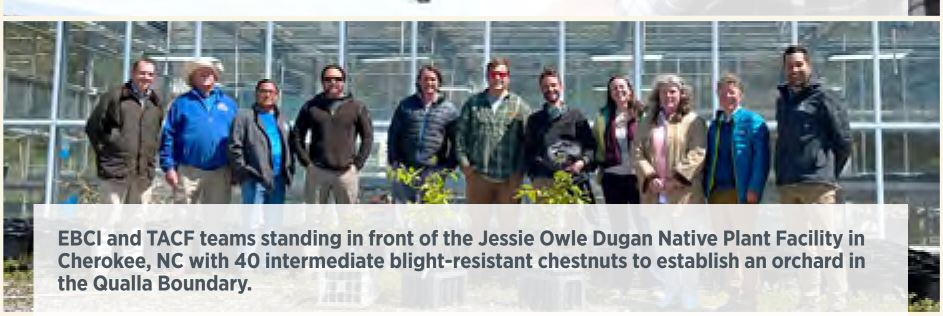
At the signing ceremony, Lisa Thomson said, "The formalization of our trusted partnership was built on this premise of returning something to the forests important to the Cherokees of yesteryear and hoping that other tribes within the former range of the tree might also be interested in participating in this long-term, hopeful rescue mission." The EBCI Secretary of Agriculture and Natural Resources Joey Owle stated, "I hope that one day in the future, 200, 500, a thousand years from now, those generations can stand next to a 6- or 8-foot diameter chestnut tree in our mountains and be able to trace the story of that tree back to today."

Now, the real work begins. When the EBCI Natural Resource team visited Meadowview Research Farms on June 30, 2021, they shared with TACF staff how American chestnut restoration fits into their Forest Management Plan and cultural preservation within the Tribe. Conversely, TACF staff gave a tour of the facilities, including training with lift pollinations and inoculations, so that this initial exercise can be put into practice on tribal lands.

For a chance to eat traditional Cherokee chestnut bread, attend the 107th Annual Cherokee Indian Fair, October 8-12, 2021. Learn more at: visitcherokeenc.com.

See page 35 in this issue for a throwback recipe of chestnut bread from the July/August 2011 *Journal*.

(L-R) EBCI Secretary of Agriculture and Natural Resources, Joey Owle; EBCI Vice Chief, Alan B. Ensley; TACF President and CEO, Lisa Thomson; and retired USFS Forester & TACF Director Emeritus, Rex Mann at the MOU signing ceremony.



EBCI and TACF teams standing in front of the Jessie Owle Dugan Native Plant Facility in Cherokee, NC with 40 intermediate blight-resistant chestnuts to establish an orchard in the Qualla Boundary.

During the EBCI staff tour at Meadowview Research Farms, Nursery Manager Lily Kingsolver describes the integrated pest management measures she has implemented.

The Cherokee language has preserved their word for chestnut: *tili* (chestnut) and *tlugvi* (tree). Additionally, the Unaka Range is a mountain range stretching from the Nolichucky River in the south to the Watauga River in the north. The name *unaka* is rooted in the Cherokee term *unega*, meaning "white." With up to one in four trees in the Unaka Range being American chestnut prior to the blight, and because the chestnut has long white blossoms, the Unaka Mountains turned white in color when the trees were draped by catkins in spring.

Furthermore, the Institute for American Indian Studies has archived several Iroquois uses of chestnut. They ground the wood of this tree into a powder to use on the chafed skin of babies. The meat of the nut was ground and combined with bear grease and used to treat hair. The bark of this tree was mixed into dog food to treat worms. The Mohegans made tea from the leaves to treat rheumatism and colds. An infusion of leaves was also used to treat whooping cough. Chestnuts were roasted, ground into flour for cakes and bread, and stewed into puddings and soups.

Next steps for the partnership include: 1) prepping an orchard site within the Tribal Reserve for intermediate blight-resistant American chestnut trees to be grown for seed, 2) locating surviving populations of American chestnut within EBCI land to be used for cross-pollination efforts, and 3) gathering information about the traditional uses of American chestnut, whether that be for food, medicine, craft, stories, songs, or other ways in which the Tribe utilized, and will once again embrace, this culturally significant plant.

If you know of any flowering American chestnut populations within the Qualla Boundary, please reach out to the EBCI Natural Resources Department (jaimvanl@ebci-nsn.gov, tommcabe@nc-cherokee.com, or (828) 359-6225).

A small group gathered for the MOU signing ceremony, which took place at the location of the chestnut orchard to be planted this fall.

EBCI and TACF teams pose for a picture in front of the newly constructed high light growth building at Meadowview Research Farms.

Tree Breeding Coordinator Eric Jenkins trains EBCI staff how to use a lift to pollinate chestnut trees at Meadowview Research Farms.

PART 2
OF A 2-PART SERIES

The Bradford American Chestnut Tree

By Robert S. Seymour, ME-TACF Chapter

Part 1 of this story, "Old and New Chestnut Lumber," was published in the 2021 spring issue of *Chestnut*.

I first saw the Bradford tree in 2003 during a forest management audit of the Maine Bureau of Parks and Lands (BPL). BPL forester George Ritz (**Figure 1**) had just managed an extensive partial harvest of the public lot in Bradford, where he found this and several other small, sawlog-sized American chestnut trees near the western property line. Every year since then, until my retirement from the University of Maine forestry faculty in 2017, a visit to this tree was the highlight of an afternoon field lab with George. Although students learn to identify various species in their dendrology class, none had ever seen such a large specimen in the wild, so this was indeed a special treat for all.

Before the blight, chestnut was, of course, the iconic tree of the eastern deciduous forest, reaching immense sizes on highly fertile cove sites in the central Appalachians. This stand in Bradford, ME, was about as different from those sites as one could imagine: somewhat poorly drained soil, growing in mixture with balsam fir, hemlock, and red maple. This land was never in agriculture, so how this tree got its start will forever remain a mystery. Bradford is over 50 miles from the closest native range in Maine, though many nearby towns now have known occurrences of the species.

In the fall of 2016, my last trip with students, the tree (**Figure 2**) was in failing health and died soon thereafter. During the 13 years of observation, it grew from 15″ diameter at breast height (dbh) to well over 22″ dbh. Note the wide rings in the end grain cutoff of the board (**Figure 3**) used to fashion the top of the blanket chest, which shows an inch of diameter growth every two years. During January 2018, the tree was salvaged by Prentiss and Carlisle (a forest resource and timberland management service), bucked into five 8′ logs, and trucked to Lumbra Hardwoods in Milo, ME where it was sawn and kiln dried. Remarkably, a ring count on the stump revealed less than 60 years of age, suggesting the tree was established after a heavy harvest of the area circa 1960 before the land was in public ownership. Later in 2018, Ritz and I retrieved a couple of boards from the basement of Glen Rae, president of the ME Chapter of The American Chestnut Foundation (TACF), in recognition of our efforts on behalf of this tree and TACF.

Fast forward to March 2020, when I went to Chris Becksvoort's shop in New Gloucester, ME, to pick up two Haystack chairs we had ordered. As a woodworker myself, I had long admired Chris' work as contributing editor of *Fine Woodworking* magazine, but it was not until I read his book *Shaker Inspirations* that I realized he was an alumnus of our program in the early 1970s. While touring his shop, aware of my great interest in wood, he showed me the salvaged church pews while lamenting he didn't have enough material for the top of a planned heirloom blanket chest. Serendipity indeed! In June I delivered the

BIOGRAPHY

Bob Seymour is Professor Emeritus of Silviculture at the University of Maine's School of Forest Resources, who retired in 2017 after a 40-year career on the faculty. He writes a monthly column on silviculture for *Maine Woodlands* newsletter and, along with his wife Jessica Leahy, was the 2020 winner of Maine's Outstanding Tree Farm, Wicopy Woods in Sebec, ME.

perfect board, just enough for the top of the chest. Given its rarity, we agreed to $20 per board foot – $240 for one 12″ wide 8′ long 6/4 plank, which I donated to TACF. By late August, Chris emailed a picture of the completed chest, with his plans for a large donation to TACF. It didn't take my wife and I long to decide that this chest should stay in Maine where it has already become a treasured heirloom. This is indeed a one-off, museum-quality piece, which is now displayed prominently in our home office (**Figure 4**).

Although this tree is gone, other mature individuals remain alive in the stand, and there are many large saplings up to 25′ tall in harvest gaps created in 2003, obviously growing from seeds produced by the specimen tree. Members of the ME-TACF Chapter also visited the tree repeatedly while alive, collecting nuts for our local breeding program, so the tree lives on.

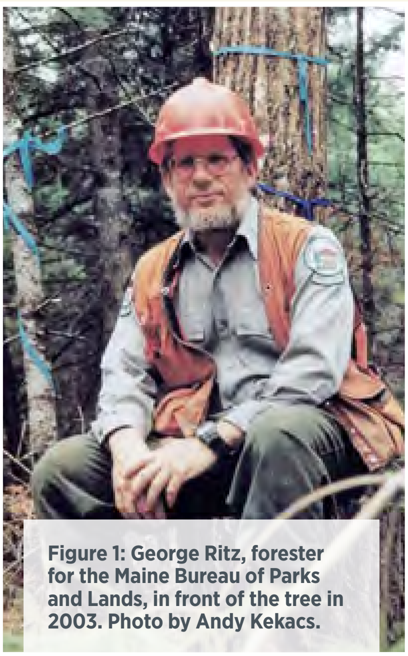


Figure 1: George Ritz, forester for the Maine Bureau of Parks and Lands, in front of the tree in 2003. Photo by Andy Kekacs.

Figure 2: Shawn Fraver, Associate Professor of Forest Ecology at the UMaine School of Forest Resources, standing by the tree in 2014.

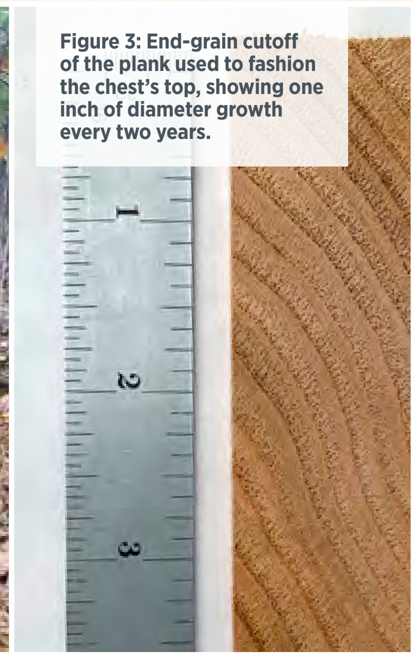


Figure 3: End-grain cutoff of the plank used to fashion the chest's top, showing one inch of diameter growth every two years.

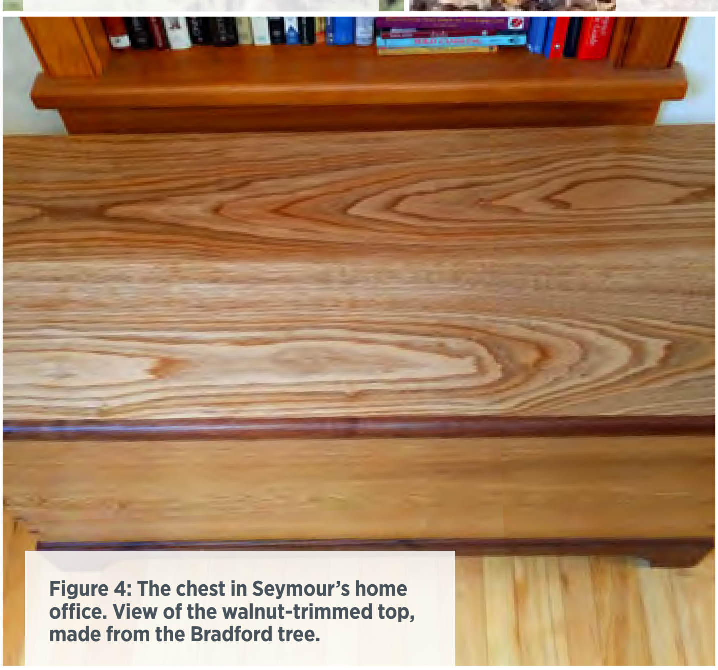


Figure 4: The chest in Seymour's home office. View of the walnut-trimmed top, made from the Bradford tree.

Figure 5: Authors Chris Becksvoort (left) and Bob Seymour in Becksvoort's showroom, New Gloucester, ME.

Timbers and Trees

By Jacob R. Pease and John B. Hewlett, Murray State University Biology Department

Western Kentucky summers are, to put it lightly, sultry: fueled by myriad rivers, wetlands, and crops. The lowland nature of Kentucky's Jackson Purchase region belies a landscape dotted with intriguing terrain and geological mosaics. Sandstone and limestone soils mix with interesting conglomerates and loess and are cut by small, ephemeral tributaries. Ridgelines are frequented with chestnut, blackjack, and southern red oaks that complement sourwoods and sassafras. Drier sites are juxtaposed by bald cypress and locusts that call the bottomlands home. The region is varied and breathtaking.

Traversing the aforementioned ridgelines, even under a dense, shady canopy, is deceptively hot. In summer, the air is so thick with humidity that sweat is seldom able to carry heat from one's body. We found ourselves traveling north to the crest of a narrow ridge under such conditions one day in 2019.

Even in the morning, the oppressive heat began to make its presence known. Nevertheless, we, along with our field techs, were loaded with the gear necessary to track and handle some of our friends in the woodline above.

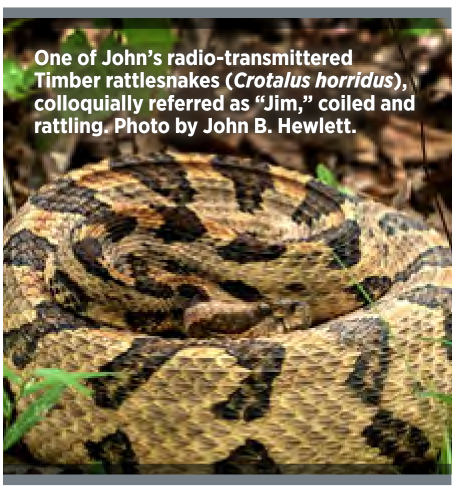
For in these woods, there be dragons. Regal, keeled-scaled beauties adorned with chevrons and a brilliant rusty mid-dorsal stripe that breaks up their outlines with the underlying leaf litter. This particular specimen was part of a long-term monitoring study investigating physiology and disease in the species – *Crotalus horridus* – so named by Carolus Linnaeus in 1758 for its (horridus) "scaly texture." However, these snakes are anything but horrid. Commonly known as the Timber rattlesnake, this charismatic large-bodied pit viper has experienced declines across its range for the better part of a century. In many ways, it has mirrored another, now functionally extinct staple of the American landscape: the American chestnut.

Both organisms hold great significance to American culture. The Timber rattlesnake adorns Christopher Gadsden's famous flag – one that served as a rallying symbol for American revolutionaries. The snake's disposition was later coined by Benjamin Franklin to be a perfect

An American chestnut's beautifully serrated leaves at Land Between the Lakes. Photo by Jacob R. Pease.

allegory for America, in the December 27th, 1775 issue of the *Pennsylvania Journal*, a year after the assembly of the First Continental Congress. The American chestnut was instrumental to early American culture for its wood, nuts, bark, and medicinal uses. Even today, "Chestnut" adorns street signs across the country; a testament to its historic importance.

Chestnuts are integral for nutrient cycling and as a food source for forest species. Timber rattlesnakes



One of John's radio-transmittered Timber rattlesnakes (*Crotalus horridus*), colloquially referred as "Jim," coiled and rattling. Photo by John B. Hewlett.

consume rodents and other prey that harbor diseases and can help stem the onslaught of modern day pestilences such as rickettsia, lyme disease, and hantavirus, among others. As a result, both are major contributors to their ecosystems.

Similarities in the decline of the American chestnut and Timber rattlesnakes have only recently become more clear, as a relatively newly-described fungal pathogen, *Ophidiomyces ophiodiicola*, has been found across eastern North America and resulted in marked declines of some populations. Human activity

John Hewlett and Jacob Pease standing with their respective study organisms on the last sampling day of the year when study sites for both species fortuitously overlapped. Photo by Brianna Gibbons.

led chestnut blight to the U.S. Anthropogenic climate change likely increases *O. ophiodiicola* presence and prevalence in the environment. Evidence suggests that altered rainfall patterns as a result of anthropogenic climate change may be resulting in vegetative shifts from xeric to mesic and thus potentially being more conducive to fungal growth. Likewise, alterations in climate and ecological conditions will impact the future of our beloved chestnuts.

Furthermore, recruitment for the two species is complicated by life histories. Timber rattlesnakes are characterized by slow maturation (7-8 years for males, 11-13 years for females), and low overall reproductive output. A single female will likely have one litter in her life averaging 7-12 pups; however, if she does reproduce a second or even third time, it is likely on two to three year intervals. Comparatively, modern chestnuts harbor similar reproductive shortfalls, as blight eliminates the dominant stem of most specimens before they reach maturity.

Ultimately, when we finally tracked down our rattler on that muggy day, the snake happened to be within a few dozen yards of an American chestnut. We sampled, checked for lesions, did a site survey, and began the process of releasing the snake back to its leaf litter dwellings. We walked the tubed Crotalid to the tree and posed, knowing full well that an opportunity like that does not come about often.

Conservation biology is so often a field of tragedy, struggle, triage, and extinction. However, on the day our photo was taken, two testaments to nature's resolve to not go gentle into that good night were brought together. Two ardent conservationists, united by similar ideals and hopes for the future, stood beneath the shade of a small chestnut and walked away as better men.

There will always be those who care enough to make a difference. For that reason, our descendants will be able to walk the woods one autumn morning and pause. The slight chill of an

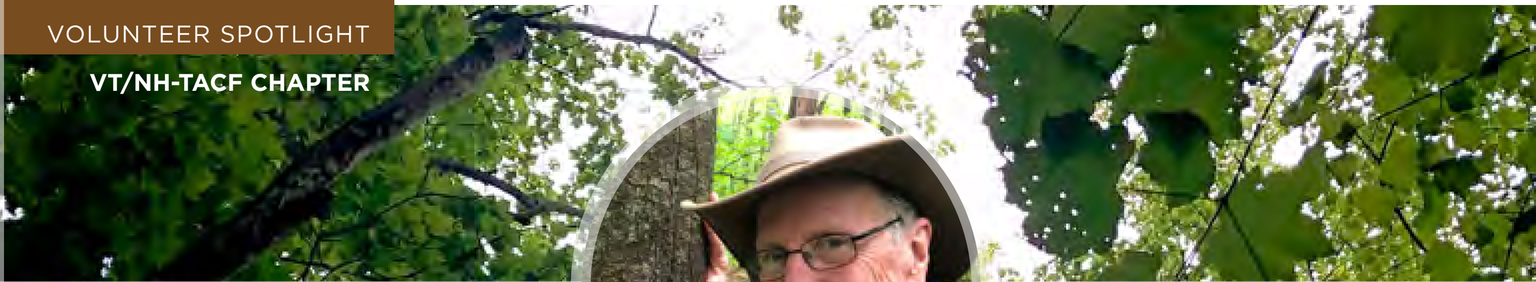
Another of John's tagged specimens – "Gordo" – coiled in the leaf litter on a cool fall afternoon as he was making his way back to his hibernaculum for a 5-month snooze. Photo by John B. Hewlett.

October wind will shuffle the hints of gold and scarlet as they look upon the base of a mighty chestnut. A Timber rattlesnake, perhaps, at the base of the tree, sits in wait as the squirrels above fatten themselves on the plentiful mast. Scenes such as those are why we live; that is why we fight.

BIOGRAPHY

Jacob Pease's research pertains to the multidisciplinary reintroduction of the American chestnut on Kentucky's public lands. This project focuses on combining principles of GIS, conservation, forestry, remote sensing, and forest ecology to streamline the reintroduction process for native tree species that face extirpation or extinction by anthropogenic factors.

John Hewlett's research is at the interface of endocrinology, wildlife disease epidemiology, and ecology. Specifically, he studies how stress physiology affects disease susceptibility and behavioral ecology with methods ranging from radio-telemetry to molecular techniques.



Tom Estill

CHESTNUT VOLUNTEER HAS STARS IN HIS EYES

By Scott Carlberg, Contributing Author

In clear, crisp New Mexico nights Tom Estill and his grandfather scanned the heavens for Russia's Sputnik satellite. It was 1958. The space age and Tom's love of science blasted off about the same time. He wanted to be some part of the U.S. space program.

This guy with stars in his eyes also digs deep in Mother Earth. He plants and tends chestnuts. It's a match made in heaven because it is all about the educational journey.

A 66-foot chestnut discovered in Pine Hill Park in Rutland.

Not much could stop Tom from learning, even as a child. "I remember a new mall in Albuquerque – Arrowhead Mall – a strip mall with a big arrow for the sign. There was a drawing at a store for a telescope." Tom was obsessed. "I made all my family fill out a ticket for the drawing, but that was not good enough for me."

Tom figured someone in the crowd would be asked to reach in the fishbowl for the winning ticket. "I put on a tie and coat. Stood right up front, smiling." Tom was picked to draw. He stood in front of the fishbowl and spied a ticket with his mother's name. "I reached in and grabbed that ticket then swirled my hand in the bowl."

From the heavens to the tiniest elements – Tom and his first microscope in 1963.

Eighth graders planting an American chestnut at their school in Rutland, VT.

His mother came up to claim the telescope. "I pretended like I didn't know the lady."

That boy became a teacher. Tom Estill, or as his students call him, Mr. E, has taught for 47 of his 70 years. Still is, in a multitude of places. After his degree from San Jose State University, he taught at a private California school. Teaching can be done most anywhere, though, so he asked his wife, "If you could live anywhere in the country, where would you like to live?"

The answer was clear. "As far from your mother as possible."

"Well, that would be New Hampshire," he said. They moved.

Tom's teaching career flourished. First, at Vermont's Oxbow High School on the Connecticut River – the school is on an oxbow bend – for 13 years. Then Lyme Elementary in Lyme, NH, for nine.

The bottom dropped out of his world in 1998. His wife Peggy was diagnosed with cancer. They moved to Rutland, VT to be closer to the hospital for treatment. A few months later Peggy was gone. Tom moved to a new career and fresh start back to California and family.

The Jet Propulsion Laboratory tapped Tom as an experiential educator. Then, the dream job: NASA's Goddard Space Flight Center as an aerospace education specialist on the east coast. "I brought space and science to people across the region. After all, our engineers were busy doing their work."

The job's challenges were more mundane. He remembered being at the New York Museum of Natural History. "I had a truck full of space stuff, including a moon rock." Tom was in the back of the truck gathering his presentation materials. "The back door closed behind me. It locked. There was no handle inside." Tom pounded on the back door. Had a janitor not come out for a smoke break and heard the pounding, Tom would have missed his show.

"When working at NASA I volunteered on Sundays at Maryland's Patuxent National Wildlife Refuge. I led nature walks and was asked to drive the tourist tram." Turns out that Tom was tram-trained by Ron Clemons, a MD-TACF Chapter volunteer. "On the property Ron pointed out ancient telegraph poles, made from American chestnut, that helped spread the news of Lincoln's assassination. "Still in use! Old poles that still looked good."

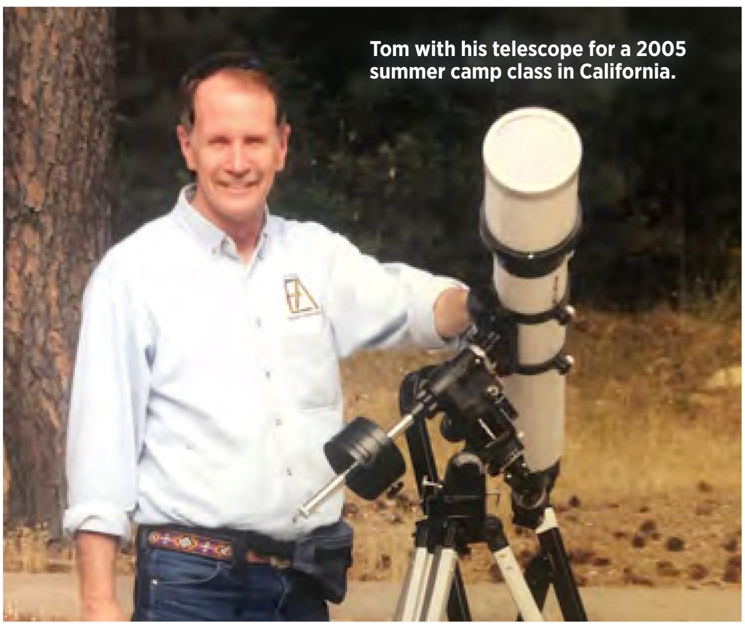
That history and staying-power impressed Tom. "Behind the visitors center there were new chestnut trees, the F_1 cross." That impressed Tom even more.

When the NASA project ended, Tom went back to teaching in Rutland. He needed a community activity for students. The answer: Plant chestnuts as an 8th grade project.

Like the trees, that idea grew. “TACF wanted another germplasm conservation orchard, and I responded,” says Tom. He connected with Rutland’s Allen Street School. “Lots of land there.” Members of the VT/NH-TACF Chapter guided the planting. Tom says, “We expanded from 15 to 40 trees. The kids have done a great job. They water, feed, and weed. What is important is that they feel like they are part of an important movement, and they are.”

Every school in Rutland now has chestnut trees. Students in every school have learned about chestnuts – history, biology, the environment. One of the students told a reporter he thinks the chestnut project is, “Pretty cool because not many people can say they saved a species of tree.”

After 47 years of teaching, Tom’s Rutland work is his personal capstone project. “I have no doubt about the environmental movement going across the globe. Kids are aware of the environment and that they can make a difference. I’ve had former students go into Environmental Science with the Peace Corps, or into biology” Tom says with stars in his eyes.



Tom with his telescope for a 2005 summer camp class in California.

One of the 20 American chestnuts maintained by the advanced biology class at Mount St. Joseph Academy in Rutland, VT.

Students and teachers at Allen Street School in Rutland, VT helped establish this germplasm conservation orchard in 2020.

PART 1
OF A 2-PART SERIES

Nontarget Injury Due to Herbicide Translocation

VIA ROOT GRAFTS IN CLOSELY PLANTED AMERICAN CHESTNUT ORCHARD PLOTS

By Sara Fern Fitzsimmons, TACF Director of Restoration and Stephen Hoy, Penn State Orchard Manager

Spacing in TACF's chestnut research orchards varies considerably depending on location and type of planting established. In some orchard types, most notably what we refer to as a "seed orchard," a very close spacing of 1' between trees and 7' between rows was implemented (Fitzsimmons et al 2014).

Figure 1: A selection from plot 16-16 stands between multiple herbicide-treated cut-stumps, signified by white paint.

Only 2-3 years after planting, trees in seed orchards undergo an initial round of inoculation and selection using a weak strain of the blight fungus. Twelve months after inoculation, 50% or fewer of the trees are retained, while the other trees are cut and removed. As any observer of chestnut can attest, the species can strongly resprout, and removing unselected trees completely can be quite the chore.

One of the easiest, least labor-intensive, and most effective methods of ensuring a hardwood tree dies entirely (and does not resprout), is the cut-stump method of herbicide application. Many publications warn about the potential of herbicide translocation across root grafts of the same species (Kochenderfer et al 2012). That warning is also prominently noted in manuals for most herbicide products (Loveland Products, Undated).

"RESTRICTIONS: DO NOT MAKE CUT STUMP APPLICATIONS WHEN THE ROOTS OF ADJACENT DESIRABLE TREES MAY BE GRAFTED TO THE ROOTS OF THE CUT STUMP. INJURY RESULTING FROM ROOT GRAFTING MAY OCCUR IN ADJACENT TREES."

Heeding those warnings, TACF has opted to manually remove stumps, using a stump grinder in orchards where tight spacing is implemented. This machinery is expensive and grinding out stumps can be very time-consuming. While the warnings of root-graft herbicide transference are bold and sound dire, there is little documentation of that occurring (Borman and Graham 1960; Lewis and McCarthy 2007). Most available literature describes a significant lack of non-target injury in high-concentration, individual stem herbicide applications (Chalker-Scott 2015). For this reason, a study of four, closely-spaced seed orchard plots were used to investigate the risk of nontarget injury in chestnut due to glyphosate treatment of adjacent stumps.

Definition of the Study

In 2014, four seed orchard plots were established in the "East Orchard" at the Arboretum at Penn State. Trees were planted with 1′ between trees, 7′ between rows, and 10′ between the plots. Rows are 30 trees long and, when full, have five rows totaling 150 trees. These plots, with a total of 564 trees, were then used for an investigation into the possibility of herbicide translocation with treatments starting in August 2019.

Two of the adjacent plots (16-15 and 16-16) had undergone an initial round of inoculation on June 19, 2018 using the SG2,3 strain of the fungus. The other two plots (16-8 and 16-9) had not yet been inoculated. Each plot had a different genetic background but were all from the B_3F_2 generation of backcross hybrid.

For plots 16-15 and 16-16, trees which had the most blight-resistant phenotype were selected for retention. Around each selected tree, all surrounding trees were cut and removed, but a random number of trees were stump-treated with a glyphosate-containing herbicide (**Figure 1**), while others remained untreated. In plots 16-8 and 16-9, where inoculations had not been performed, a random number of trees were selected to be cut and stump-treated with herbicide. All cut trees in these plots were treated with herbicide.

The cut-stump treatment for all treated trees was completed August 23, 2019. A concentrated product containing 41% glyphosate, Makaze, was applied with a paintbrush immediately after cutting to treatment trees. The remaining stump was covered entirely with the undiluted herbicide product (**Figure 2**).

Ground line diameter (GLD) and survival were taken at 11 (7/17/20) and 22 months (6/24/21) following treatment in all four plots (**Figure 3**). During the measurements at 11 months, eight total individuals were observed to have extreme leaf affliction, evident of herbicide damage. Leaf and stem tissue samples of four of those eight individuals were collected and submitted to Bartlett Diagnostic Laboratories to test for presence of glyphosate.

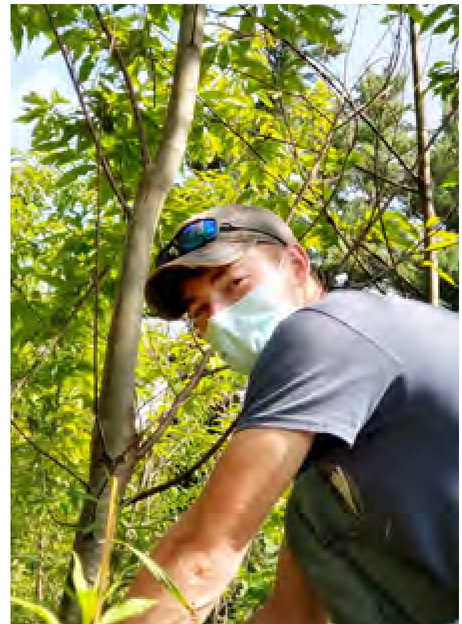


Figure 3: PA/NJ-TACF Chapter Intern and PSU Alum Sean Freidhof measures ground line diameter (GLD) and survival in the herbicide trial plots.

Full results and discussion of this study will be presented in Part 2, which will be published in the next issue of *Chestnut* magazine.

Figure 2: Cut stumps and spacing in plot 16-15. The cut stump surrounded by white paint was treated immediately following cutting.

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MEADOWVIEW RESEARCH FARMS

Land Prep for 2021 Price Farm Plantings

By Dan Mckinnon, Meadowview Research Farms Director of Land Management

It is well before spring, when the first seedlings are placed in the ground, that we start preparing for several large plantings on the Glenn C. Price Farm. There is an immense amount of planning and discussion regarding how to distribute seedlings amongst Meadowview Research Farms, state chapters, and collaborators. Once we have decided what to plant, Meadowview Tree Breeding Coordinator Eric Jenkins and I decide where to plant. When determining location, we consider the size of each orchard and the soil suitability of the area. By early spring, we decided what type, how many, and where on the Price Farm the orchards and seedlings would be planted.

In long-used agricultural lands, it is common for the ground to be compacted to varying degrees. This is not good for chestnut roots that need to expand in search of water and nutrients. The scope of compaction is dependent primarily on how the land has been utilized in the past and the type of soil in the area. When selecting a site and considering soil types, we look for a

Southwest Virginia Branch members Darrell Blankenship (left) and Stan Tucker (right) are hard at work.

MEADOWVIEW

MEADOW

"loam," or soil that has an even content of sand, silt, and clay, and an acceptable nutrient content for hardwood tree growth. While much of this can be determined by digging a small hole and observing the soil to see if it is too rocky or "duffy," sending soil samples to an analysis lab is recommended before preparing a planting site.

After confirmation of soil suitability, Eric determined row spacing and lengths and marked the locations where the rows will be subsoiled, a/k/a: ripped. "Ripping the rows" is the practice of dragging a large shank of steel through the earth that literally rips the ground open. The shank, called a subsoiler, is attached to the rear of the tractor and is dragged through the top 30+ inches of ground. Purposes of subsoiling include visible definition of planting rows, loosening soil for ease of root expansion, and revealing underground obstructions that might interfere with plant growth, such as bedrock or long-buried irrigation tube.

With the subsoiling complete and the obstacles removed or marked to avoid, we begin prepping the rows to receive the seedlings. A few weeks prior to planting, we return to spray non-soil-active herbicide to eliminate competing vegetation. After the herbicide has taken effect, we return again to subsoil once more (many areas of the farms are highly compacted due to the long history of agricultural use, so we subsoil twice to further enhance water permeability). With nice, even rows, vetted and well-prepared for transplanting seedlings, we put a labeled pin flag at each planting position a few days prior to the planting.

It takes soliciting a large workforce to conduct massive field plantings, a difficult proposition to make during a global pandemic. During the planning process, we understood that many people might be cautious to return to a close-interaction environment. Ensuring the safety and comfort of everyone, we requested two days of assistance from vaccinated staff at TACF's national office and volunteers from the Washington County Master Gardeners. We were glad to have the help of more than a dozen staff, interns, and volunteers over the two-day planting.

Some folks arrived early on the first morning to begin hauling the seedlings to their respective planting sites. It is imperative to keep the seedlings in order so that they can be easily and

Rows have been ripped and flagged in preparation for staff to prepare holes for the plantings.

efficiently unloaded and taken to their permanent location. While one crew loads, hauls, unloads, and delivers the individual containers to their specific place, another crew uses gas-powered earth augers to dig the holes that will soon be home to the seedlings. As planters arrived, Regional Science Coordinator Tom Saielli graciously gave a short demonstration on best planting practices.

The first day went very well; there were little-to-no problems and the weather was beautiful. The second day, however, Mother Nature was less cooperative, bringing cold, wet weather that eventually involved a somewhat painful sleet storm! After taking a welcome lunch and discussion break to avoid the inclement weather, the crew returned to afternoon sunshine, perfect for finishing the plantings and conducting post-planting cleanup.

Southwest Virginia Branch Vice President Beth Merz carefully removes a tree from its pot.

DOUBLE YOUR IMPACT

Did you know you can double your impact to restore the iconic American chestnut? Whether renewing your membership or supporting TACF's 2021 End of Year Appeal, you may qualify to have your employer match your generosity. Corporate matching gifts are a type of philanthropy in which companies financially match donations that their employees make to nonprofit organizations. On top of that, even if you are retired, your place of past employment may still grow your giving.

Corporate matches bring in an additional $10,000 each year to enhance American chestnut research and advancements. Microsoft, Apple, Google, and Duke Energy are just a handful of the many companies offering a matching gifts program. Ask your employer today about the ability to expand your giving.

For more information, contact Donor Relations Manager Shana Zimnoch at shana@acf.org or call our national office (828) 281-0047.


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Your commitment shapes the environment that makes the work of The American Chestnut Foundation possible. With 16 state chapters, thousands of volunteers, millions of acres, and your dedicated support, the eastern United States will experience thriving stands of American chestnut. Your generosity paves the way to new understandings of a complex organism through genomic research, diversifying transgenic material, and combining resistance to Phytophthora root rot. To rescue this iconic tree with such a rich history, we need your help. Contribute today to ensure healthier forests for a better tomorrow.

2021 END OF YEAR APPEAL

This sample, taken from Mark's collection, shows how TACF's *Journal* has changed over the years.

Sharing My Journal Collection

By Mark P. Widrlechner, TACF Member

Maybe I felt a desire to hold on to magazines and scientific journals subliminally from my mother, who spent her career working at a public library in the Chicago suburb where I grew up, or perhaps it was from my stint staffing a small research library while a student at Michigan State University. In any case, by the time I moved out of a home that my late wife and I lived in for nearly 20 years, my magazine collection was massive. At that time, I parted with many, but had numerous connections with The American Chestnut Foundation (TACF) that I've maintained my *Journal* collection, until now. As I gradually move toward retirement, I have been downsizing my office and felt that the time was right to donate my collection of past issues to the Parks Library at Iowa State University so it can be more broadly used.

That collection goes back to the beginning – Volume 1, Issue 1, a modest issue by its cover, but one that really set the stage for the organization that has bloomed from it. Volume 1, Issue 2 briefly noted my election to the Board of Directors along with four others, two of whom I got to know quite well, Al Ellingboe and Dennis Fulbright (whose passing was marked in a nice tribute in Volume 34, Issue 1). For those who are interested in chestnut-breeding history, I suggest reading Charles Burnham's historical overview in the third issue, Volume 2, Issue 1.

Putting a more practical spin on how to make crosses and grow the hybrid seedlings was well addressed for the first time in Volume 6, Issue 2. One paper in that issue (confirming the location of the stigmatic surface in American chestnut) was written by Rick Harrison, whom I got to know when he was an undergraduate assistant at the North Central Regional Plant Introduction Station where I worked as Horticulturist. Rick was among the brightest and best organized Iowa State University students who ever worked on my crew.

Perusing the back issues, it's been fascinating to see how quickly Charles Burnham's basic breeding plan could be refined and expanded through rapid progress in genetic

Mark presenting the journals to Dawn Mick, head of the Access Services Department of the Iowa State University Library. Photo by Stephani Scherbart.

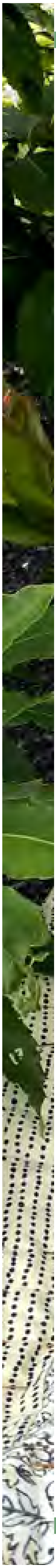
A box of Mark's archived *Journals* he packed to take to the library.

research. This can be seen in Volume 7, Issue 1, where David Mulcahy and Robert Bernatzky proposed the use of Marker-Assisted Selection to increase breeding efficiency and in Volume 9, Issue 1, where Charles Maynard gave us a preview of how genetic transformation could be applied to chestnut.

Other articles that I've found exciting include early insights on patterns of genetic diversity across the range of American chestnut presented by Hongwen Huang in Volume 12, Issue 2, and the 2008 TACF expedition to China to gain insights into the disease pressures, ecology, and diversity of chestnuts in that land, as reported in Volume 23, Issue 1. And I have always admired Fred Paillet's line drawings, such as those found in Volume 10, Issue 1. There are not many scientists with that sort of artistry.

There was a big switch in the *Journal* in July 2010 when it became a color magazine. The new format allows stories to be told in different ways, appealing to broader audiences. Perhaps that has helped to foster the growth of citizen science that has always been a part of TACF, but has become more diverse over time, as can be seen in recent issues. As our organization matures, one last thing I've enjoyed when new issues arrive is checking the Chestnut Pioneer articles. So many of my colleagues from the 1980s and 90s when I was most active have made great contributions, such as Don Willeke (Volume 31, Issue 2), Bill MacDonald (Volume 31, Issue 3), and Sandra Anagnostakis (Volume 33, Issue 2).

The evolution of the *Journal* reflects the great progress of our organization. With rise of digital communications and social media, I wonder how long people will continue to hold physical collections of journals in their personal libraries. I look forward to the upcoming issues (perhaps even in some new format) to keep abreast of chestnut restoration and the realization of the dreams and plans of our founders.



Orchard Pathways

QUILT RAFFLE

$20.00 per ticket

Purchase online now through November 14

Winning ticket to be drawn Tuesday, November 16

We are grateful to have received this stunning, handmade original quilt entitled “Orchard Pathways.” Generously donated by artist Sharon Carrier, this quilt is made from cotton fabric and measures 42″ x 61.5″.

Sharon’s quilts reflect the patterns and rhythms of the natural world. Living in North Carolina’s Outer Banks, she explores themes of environmental vulnerability and resilience. Along with the quilt, she also gifted TACF an original poem that will accompany this lovingly crafted work of art. An excerpt from the poem:

“Walk with me on orchard pathways with saplings arranged in neat, hopeful rows.”

Purchase your tickets now for your chance to win the “Orchard Pathways” heirloom quilt and poem package, Sharon’s artistic celebration of TACF’s hopeful mission.

TACF employees and their family members are excluded from this raffle.

The Marriage of Biotechnology and Breeding

ENHANCING DISEASE RESISTANCE AND GENETIC DIVERSITY IN THE AMERICAN CHESTNUT RESTORATION POPULATION

By Jared Westbrook, TACF Director of Science; Vasiliy Lakoba, TACF Director of Research, Meadowview Research Farms; and Israel Golden, TACF Stanback Intern, Duke University

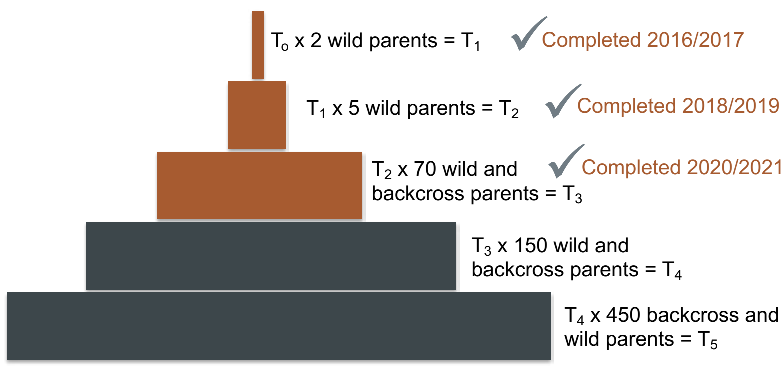
The American Chestnut Foundation's (TACF) breeding program and the State University of New York College of Environmental Science and Forestry (SUNY-ESF) biotechnology program have historically been pursued in parallel to enhance blight resistance in American chestnut. There are synergistic advantages in merging these programs including accelerated diversification of blight-resistant populations, potential for higher levels of blight resistance, and the ability to generate American chestnut trees with resistance to both chestnut blight and Phytophthora root rot. We summarize our plans to merge biotech with breeding while also continuing to pursue these approaches in parallel.

Accelerated production of genetically diverse blight-resistant seed:

Our collaborators at ESF have created the blight-tolerant Darling 58 variety of American chestnut by inserting an oxalate oxidase (OxO) gene from wheat into the genome of a single American chestnut tree from New York. Through breeding simulations, we learned that Darling 58 and its offspring will need to be bred with a genetically diverse sample of approximately 700 American chestnut trees over five generations to enhance the blight-tolerant population's capacity to adapt to a large geographic range and a changing climate (Westbrook et al. 2020). We have now completed the third generation of breeding Darling 58 with other American chestnuts (**Figure 1**). Our collaborators at ESF and the University of New England have accelerated the generation time to one to two years by inducing seedlings to produce pollen in their first growing season with long day treatments (Baier et al. 2012; also see Klak et al. in this issue). With the advent of speed breeding and efficient pollen production, the major bottleneck has become the accessibility of a genetically diverse collection of flowering American chestnut mother trees.

Using backcross hybrids and the current population of wild trees conserved in orchards alleviates this bottleneck and accelerates the diversification of the blight-tolerant population. For the past 35 years, TACF has rescued genetic diversity from wild populations via backcross breeding or collecting wild (non-hybrid) seed from trees ranging from Maine to Alabama (**Figure 2**; Fitzsimmons, 2017). TACF volunteers have planted seed from these trees in orchards and many are now flowering. The orchard accessibility of flowering mother trees enabled us to begin breeding to diversify the blight-tolerant Darling 58 population in 2019. Since then, we have bred second generation Darling 58 offspring with over 75 backcross hybrids and wild American chestnut trees conserved in TACF orchards. We have now planted thousands of transgenic blight-tolerant seedling progeny in multiple orchard locations. The blight-tolerant offspring are currently regulated by the USDA and cannot be distributed to the public at this time; however, we are optimistic that regulatory approval will be granted to distribute Darling 58 offspring in the next few years. The seedlings planted this year will likely take decades or more to grow large enough to produce substantial quantities of blight-tolerant seed via open pollination. As the Chinese proverb wisely states, "The best time to plant a tree was 20 years ago, the second best time is now."

Figure 1



Five generation breeding plan to increase the genetic diversity and minimize inbreeding in the blight-tolerant population derived from the Darling 58 founder. As of 2021, ESF and TACF completed the first three (T_1, T_2, and T_3) generations of breeding and are beginning the fourth generation.

Synergies from crossing 'Darling 58' with backcross trees:

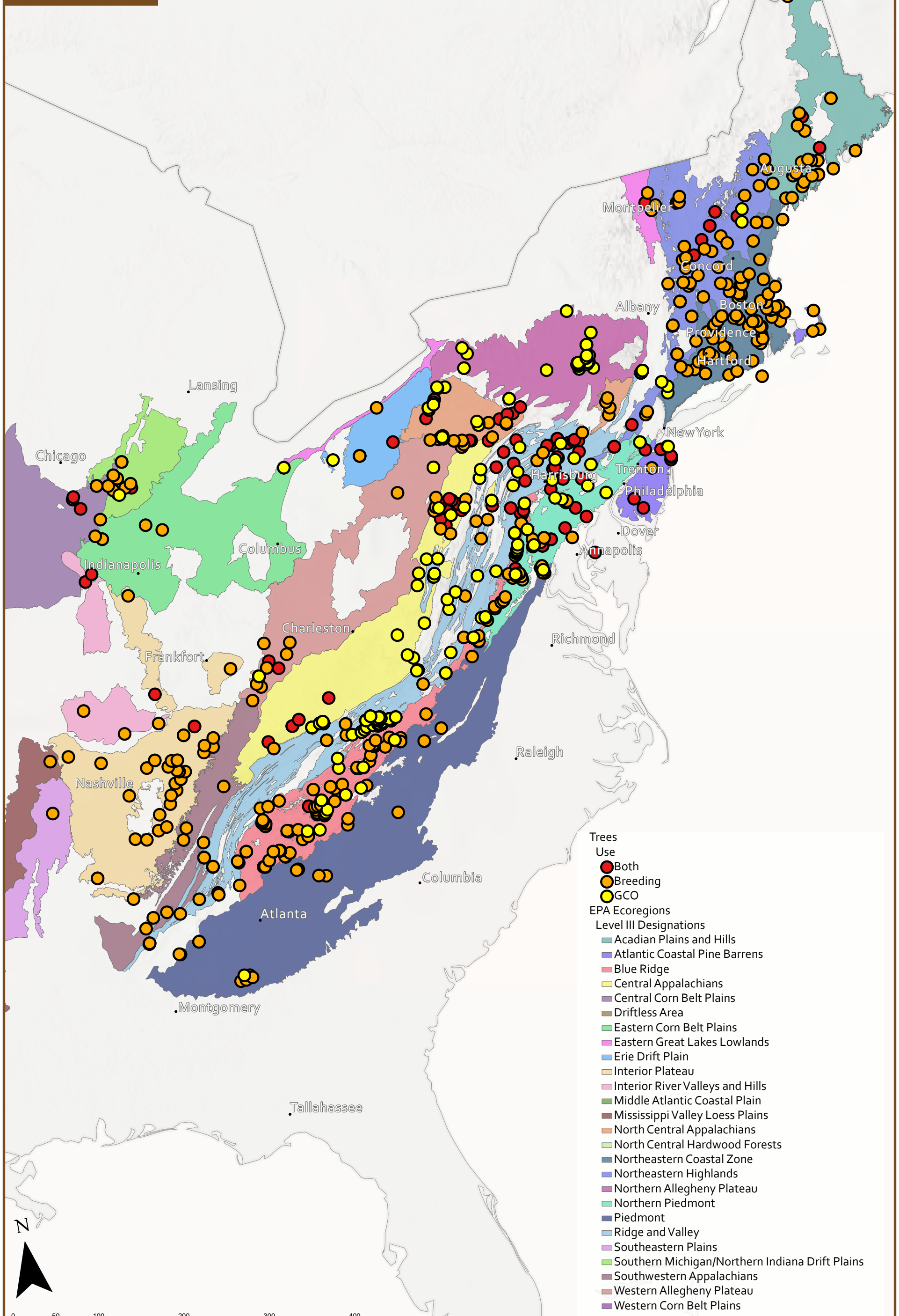
We are currently performing three types of crosses between American chestnut backcross trees and Darling 58 offspring to capture additional genetic diversity, enhance blight resistance, and combine blight and root rot resistance (**Figure 3**).

❶ **Darling 58 x high percentage American hybrids:** Using backcross hybrids as mother trees for Darling 58 crosses enables us to rescue a wider base of genetic diversity as compared to using only the smaller subset of wild trees in our germplasm conservation orchards (GCOs; Figure 2). There are potential ecological tradeoffs from introducing Chinese chestnut genes (via hybrids parents) into the population, including reduced height growth and reduced cold tolerance (Thomas Van Gundy, 2016; Gurney et al. 2011). To minimize potential tradeoffs, we are restricting a subset of our Darling 58 crosses to hybrids that inherited > 95% of their genome from American chestnut. The offspring will inherit negligible percentages of their genome from Chinese chestnut (< 2.5%) and are expected to be indistinguishable from American chestnut. Through a collaborative genotyping effort with Virginia Tech, we identified 440 hybrids with >95% American chestnut genome inheritance that are offspring of 174 wild trees. We are also crossing Darling 58 with wild trees conserved in orchards and we are encouraging our chapters to help us find and propagate additional wild American chestnuts so we can further diversify the blight-tolerant population.

❷ **Darling 58 x blight-resistant hybrids:** Trees that we reintroduce into the forest will need to have high levels of blight resistance to be competitive. The blight resistance of Darling 58 offspring has not yet been assessed over long time periods and in multiple environments. It is possible that we will need to further improve blight resistance. Therefore, we are breeding Darling 58 offspring with a subset of our most blight-resistant backcross trees to combine OxO with Chinese chestnut genes for blight resistance. We have identified approximately 50 potential mother trees from TACF's chapters that have exceptional blight resistance and that inherited 75% to 90% of their genome from American chestnut (**Figure 4**). In the subset of stacked resistance Darling 58 lines, we will aim select offspring with enhanced blight resistance relative to progeny of crosses with wild trees, while also increasing American chestnut genome inheritance relative to the hybrid parents.

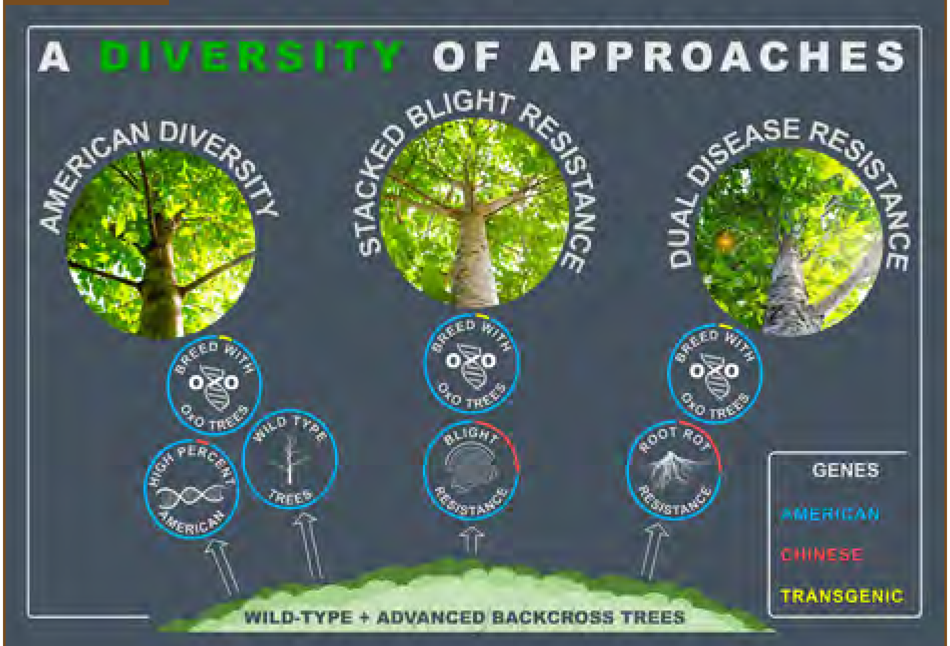
❸ **Darling 58 x root rot-resistant hybrids:** Phytophthora root rot (PRR), caused by the water mold *Phytophthora cinnamomi*, is a threat to American chestnut populations in previously cultivated soils where this pathogen has been introduced. Historically, *P. cinnamomi* has been limited to the Southern U.S. but it is expanding northward due to warming winter temperatures (Burgess et al. 2017). Through collaboration with the U.S. Forest Service and Clemson University, we have identified approximately 100 hybrids from TACF's breeding program that inherited resistance to PRR from Chinese chestnut and between 80% and 95% of their genome from American chestnut. We have begun breeding Darling 58 offspring with our PRR-resistant backcross hybrid to combine resistance to blight and root rot. We will likely need two generations of breeding and selection to generate trees with high levels of resistance to both diseases. The first generation will at best, inherit intermediate resistance to root rot because the Darling 58 parent is susceptible to PRR. Resistance to root rot will be enhanced by intercrossing first generation progeny and intensively selecting a subset of second generation trees that inherited OxO and PRR resistance genes from both parents.

Figure 2



Map of wild American chestnut trees that TACF has used as parents for backcross breeding and germplasm conservation orchards. Wild trees were plotted onto ecoregions from the Environmental Protection Agency by Israel Golden.

Figure 3



Crosses between Darling 58 and wild type or backcross trees (A) represent genetic diversity from wild-type populations, (B) stack OxO with Chinese chestnut genes for blight resistance, and (C) combine resistance to chestnut blight and Phytophthora root rot.

The development of the Darling 58 trees by ESF researchers represents the most promising approach to date for enhancing the blight tolerance of American chestnut. TACF's efforts in backcrossing and conserving wild trees in orchards has positioned us to introduce the OxO blight tolerance trait into a genetically diverse population within the next decade. Using backcross hybrids as mother trees also enables us to enhance disease resistance potential by combining OxO with Chinese chestnut genes for blight and root rot resistance. We are also interbreeding select chestnut hybrids that combine blight resistance and American chestnut traits so that we can continue to offer traditionally bred trees for restoration. We are hopeful that in pursuing a diversity of approaches, we will ultimately be successful in restoring a genetically diverse and disease-resistant population of American chestnut to Eastern U.S. forests.

Figure 4

Examples of backcross trees with exceptional blight resistance. Pictured from top to bottom: David Morris (TVA orchard, Alabama), Martin Cipollini (Berry Orchard, Georgia), and Jamie Van Clief and Tom Saielli (Cochran orchard, Tennessee).

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A wild-type American chestnut sapling in a Germplasm Conservation Orchard in Saco, Maine is pollinated with T3 transgenic pollen.

BREAKTHROUGH:

Transgenic Pollen in Less Than a Year

By Thomas Klak and Ellen Spiers, University of New England (UNE), Biddeford ME; and William Powell, SUNY College of Environmental Science and Forestry (ESF), Syracuse NY

Transformation of the American chestnut for enhanced blight tolerance has been led by scientists at ESF. The project's history, and its plan going forward, is detailed in a 289-page petition submitted to the USDA that seeks deregulation of the Darling 58 transgenic tree (Powell, Newhouse et al. 2020). Darling 58 began as a clone in 2012. In recent years, ESF has engaged a growing number of collaborators, including those at UNE, in the essential effort to genetically-diversify Darling 58. This transgenic diversification project has been guided by the modeling and breeding plan outlined by Westbrook and colleagues (2018; 2020).

These documents emphasize that future generations of Darling 58 must minimize the founder effect and embody the American chestnut's genetics across the native range. To generate blight-tolerant trees with enough genetic diversity to adapt to a large native range and to climate change, the plan is to outcross Darling 58 to a range-wide sample of 700 American chestnuts over three to five generations.

Westbrook (2018 p.35) cautions that it would take many years – even decades – to outplant, raise to maturity, and breed the necessary generations of trees in field settings: "Assuming it will take approximately seven years to complete one generation of breeding growing trees under standard orchard conditions, it may take up to 35 years from the time of regulatory approval to complete three outcross generations plus two intercross generations." This generation time has been substantially shortened at ESF and UNE through indoor, year-round production of transgenic pollen on young trees (known as "speed breeding"). This procedure was first published by Baier et al. (2012) in this journal, and detailed in a recent TACF Chestnut Chat: bit.ly/chestnutspeedbreeding.

There are two ways to genetically-diversify Darling 58 progeny: through increasing the number and geographical range of wild-type mother trees bred each year with transgenic pollen (this contributes the most genetic diversity, and therefore finding and growing wild-type chestnut trees is something everyone can help with); and through advancing the transgenic pollen itself generationally through indoor speed breeding.

Speed breeding labs at ESF and UNE have been producing and freezing pollen from transgenic seedlings. Each June during the past two years, the labs have then shipped transgenic pollen to a growing number of collaborators across the native range (from Maine, Indiana, Georgia, and in-between; all under USDA permits). This collaboration incorporates a growing number of geographically-diverse mother trees. Sites have then shared their transgenic offspring for outplanting in permitted experimental orchards in Maine, New York, Pennsylvania, and Virginia.

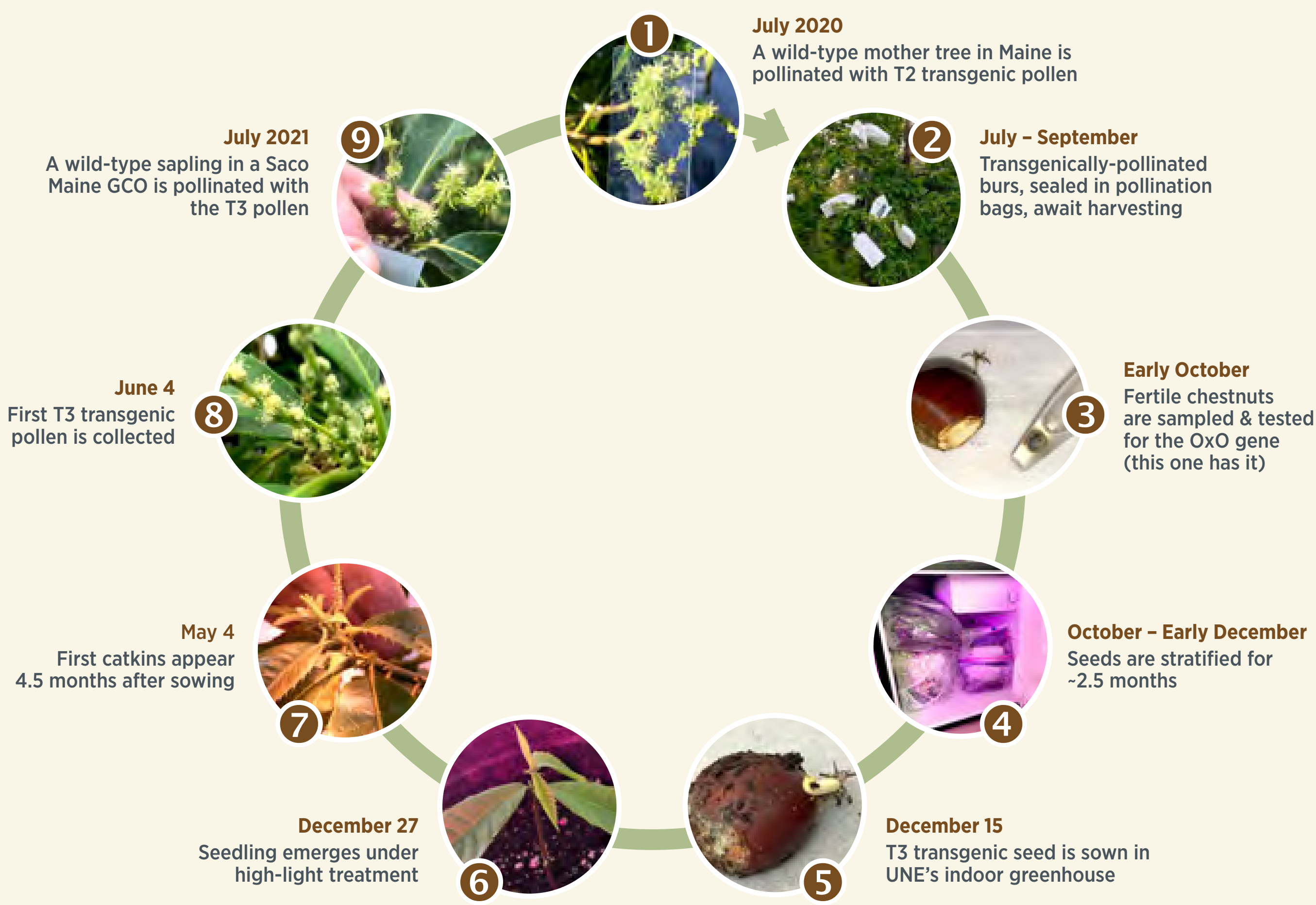
Westbrook (2018 p.35) projected that "[g]rowing transgenic trees under high light to stimulate the development of catkins in the first growing season could shorten the seed-to-seed generation time to two years." ESF's generational progress to date has followed that pattern, beginning with pollen from initial (T0) Darling 58 clonal lines first applied in the 2016 breeding season, then T1 pollen for 2018, and T2 pollen for 2020 (Powell 2020 p.82-3).

Building on ESF's progress, UNE ventured to speed up the generational process and advance an entire generation within the same "chestnut year." We define a chestnut year as the time when female flowers on local trees are ripe for pollination, until the same time next year (in Maine, this is typically early July to the following July). Under ideal conditions this could represent one chestnut generation.

In June 2021, UNE produced T3 transgenic pollen in time to pollinate flowers this year, and shared it with our collaborators in other states. Figure 1 explains how it unfolded. Our results are a "proof of concept:" one successful pollen-producing seedling (and many more that failed!). The plan for the next chestnut year is to improve on our singular positive result using a variety of genetically-diverse seeds. We could potentially produce

Figure 1

From Pollination to Pollen-Producing Seedling in One Chestnut Year



Increasing transgenic genetic diversity by pollinating a flower and then producing pollen from that nut in the same chestnut year. Graphic by Ellen Speirs; photos by Tom Klak.

T4 pollen from pollinations conducted in July 2021 (**Figure 1** #9), in time for the 2022 breeding season.

Once speed breeding is able to produce a sufficiently genetically-diverse generation of Darling 58 pollen sources, it is still a long-term project due to the need to grow up large trees for large-scale seed production. We need to keep diverse populations wild-type chestnut trees alive and reasonably healthy (i.e. bearing lots of female flowers) so that we can apply the transgenic pollen.

We recognize that selecting for early pollen development may produce offspring that inherit that tendency. We see our precociously-mature transgenic chestnuts as representing one end of a maturation continuum. On the other end will be slow-growing blight-tolerant chestnuts that may have many virtues other than speed of flower development. This difference represents some of the multiple directions of transgenic breeding. Historically the American chestnut had both environmental and agricultural importance. An agricultural chestnut and one for forest restoration have many differences and would benefit from multiple breeding tracks (cf Powell, Newhouse et al. 2020 p.186-7). For example, forest restoration trees need to grow quickly and reach the canopy to compete with other wild tree species. They need to be genetically diverse to be able to fill all their previous niches within the forest ecosystem. The population must be adaptable, through natural selection, to future environmental changes. Also, the nuts should be on average smaller, typical of American chestnuts, to provide mast to the greatest diversity of wildlife. On the other hand, an agricultural chestnut tree should mature early, produce an abundant crop of larger, easily harvested, good tasting, and nutritious nuts, and have more uniform characteristics to lessen farm inputs and enhance marketing. The rapid breeding methods described here will help the development of multiple outcomes to fully realize all aspects of American chestnut restoration.

ACKNOWLEDGMENT

We thank Jared Westbrook and Andy Newhouse for helpful suggestions on an earlier draft.

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Castanea alabamensis:

REDISCOVERY OF A LOST AMERICAN CHESTNUT RELATIVE

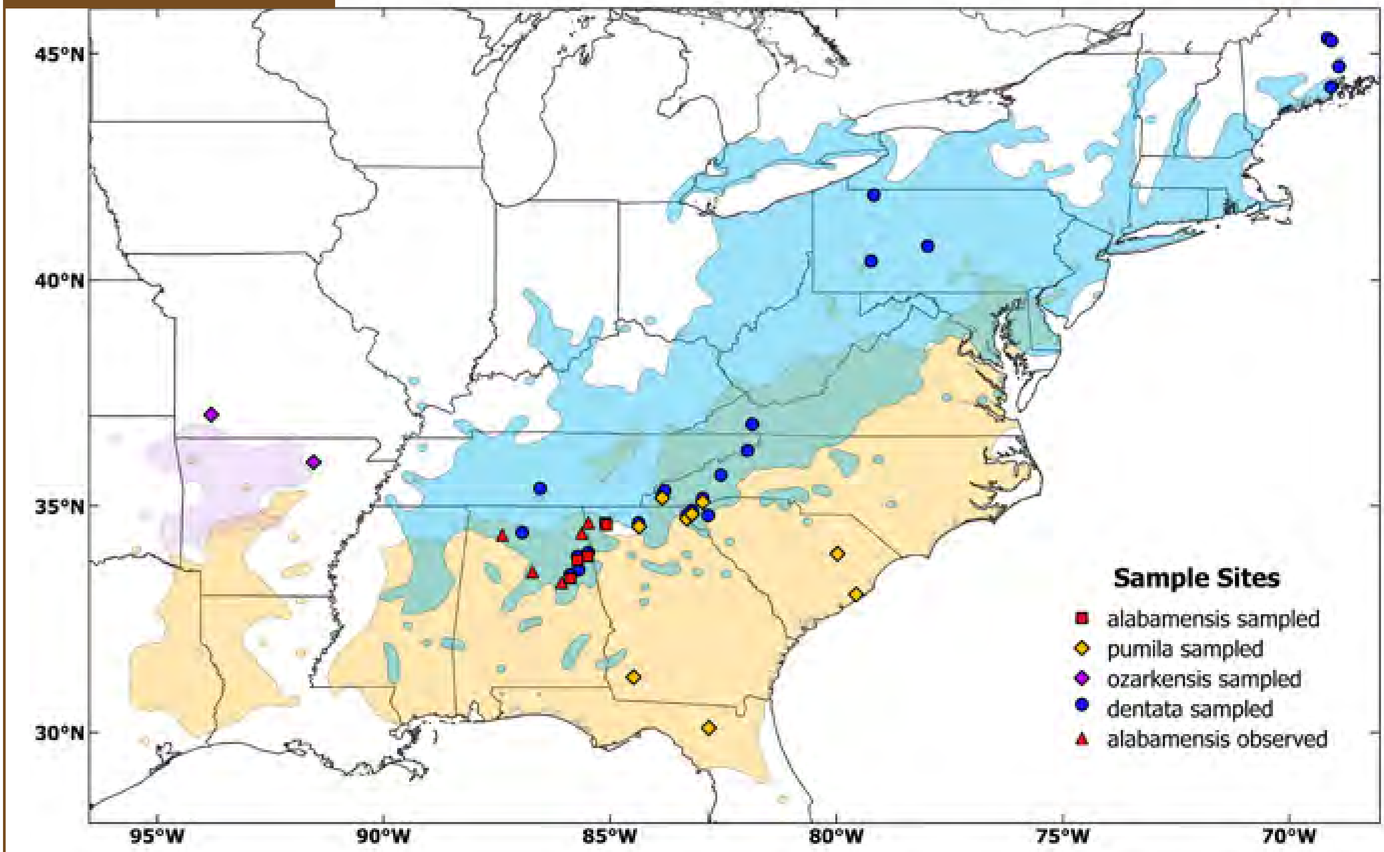
**By M. Taylor Perkins, Research Associate, The University of Tennessee at Chattanooga;
Tatyana Zhebentyayeva, Associate Research Professor, The Pennsylvania State University;
Paul H. Sisco, Staff Geneticist (retired), The American Chestnut Foundation; and
J. Hill Craddock, UC Foundation Davenport Professor, The University of Tennessee at Chattanooga**

[This article is a synopsis of our full-length paper in the fall issue of the journal *Systematic Botany*. Figures and portions of the text are reproduced with permission from the American Society of Plant Taxonomists.]

When you hear the word "chinquapin" what comes to mind? If you are familiar with chinquapins and live in the eastern U.S., you probably picture a shrub with small burs containing a single nut (**Figure 1A**). This is the Allegheny chinquapin of the Appalachians and southeastern Coastal Plain. You may have heard of the Ozark chinquapins of Arkansas, Missouri, and Oklahoma that were medium-sized trees before the blight arrived (**Figure 1B**). Recently, however, we've rediscovered another tree-sized chinquapin, the Alabama chinquapin, originally described in 1925 but long thought extinct (**Figure 1C**). It resembles an American chestnut tree so much that knowledgeable botanists have assumed it was *Castanea dentata* (**Figure 1D**). But when Alabama chinquapins bloom, they produce a single nut per bur, just like the Allegheny and Ozark chinquapins. What are Alabama chinquapins and how are they related to the other members of *Castanea* in North America? We now know the answer.

Figure 1 Herbarium specimens of (A) Allegheny chinquapin, (B) Ozark chinquapin, (C) *Castanea alabamensis*, and (D) American chestnut. SERNEC data portal: https://sernecportal.org/portal/.

Figure 2



Sample sites of *Castanea alabamensis*, American chestnut (*dentata*), Allegheny chinquapin (*pumila*), and Ozark chinquapin (*ozarkensis*), plus one hybrid of mainly Ozark chinquapin ancestry (from Missouri). Species' ranges are from Little (1977).

There exists remarkable morphological diversity within the North American *Castanea*, a group thought by many to be comprised of a small number of species. Within the chinquapins alone, plant form at maturity varies from dwarf shrub (<1 meter tall) to canopy tree among different populations (Johnson 1988; Nixon 1997). These variations in form, as well as variation in leaf, twig, and fruit traits led botanists at one point to recognize as many as six species of North American chinquapin (e.g., Camus 1929). Others have speculated that hybridization between American chestnut and chinquapin caused further variation in populations of these species (Camus 1929; Shaw et al. 2012; Li and Dane 2013). In modern botanical literature, several chinquapin taxa recognized in the early 20th century have been presumed extinct or reduced to taxonomic synonyms of Allegheny and Ozark chinquapins. Currently, most botanists agree that the genus *Castanea* in North America contains three members: American chestnut, Allegheny chinquapin, and Ozark chinquapin (e.g., Nixon 1997; Weakley 2015). Yet, questions about the evolutionary relationships among these three taxa and whether they hybridized in the wild are unresolved.

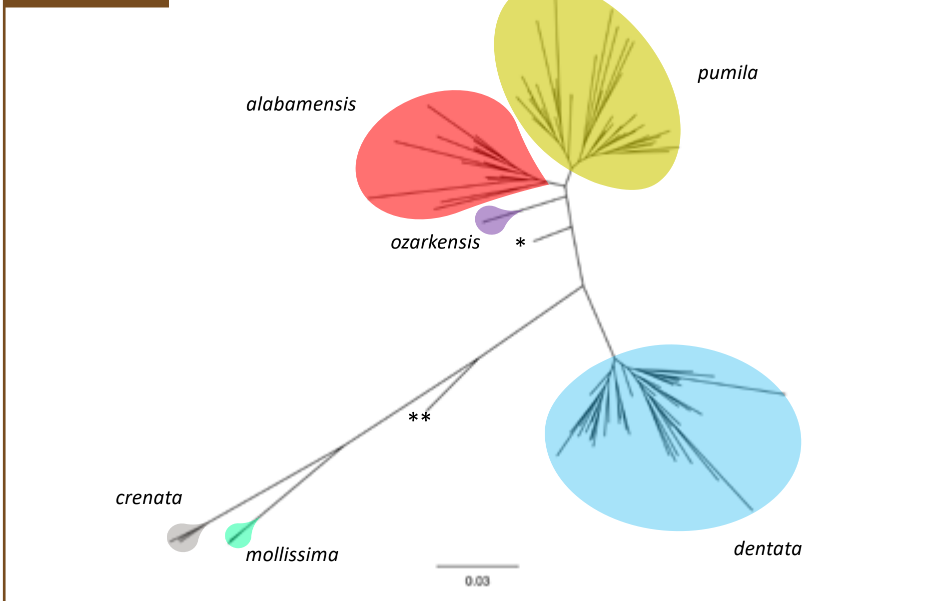
In 2015, we sought to clarify the evolutionary relationships of American chestnut to the different chinquapin taxa by analyzing chloroplast DNA sequences. In northern Alabama and northwestern Georgia, we found plants that appeared to be American chestnut, but had some atypical traits, like simple trichomes (hairs) projecting from the leaf margins. Because of how chloroplast DNA is inherited in chestnut, this preliminary study was inconclusive regarding the species identity of these unusual plants (Perkins 2016).

Review of the historical botanical literature shed some light on this issue. We found that the unusual plants were identical to the taxonomic description of *Castanea alabamensis*, a chinquapin species discovered in 1925 that was thought to have been driven into extinction by blight decades ago (Ashe 1925; Johnson 1988). Camus (1929) hypothesized that *C. alabamensis* is a naturally occurring hybrid of American chestnut and chinquapin. Shaw et al. (2012) and Li and Dane (2013) studied these same trees in Alabama and Georgia and – not making the connection to the species *C. alabamensis* – also hypothesized that they were naturally occurring American chestnut × Allegheny chinquapin hybrids.

To determine whether *C. alabamensis* is a distinct branch on the Castanea evolutionary tree or whether it represents hybrid progeny of American chestnut and Allegheny chinquapin, we sampled more trees for DNA extraction in 2017 (**Figure 2**). We used a new genotyping-by-sequencing method recently adapted for *Castanea* species (Zhebentyayeva et al. 2019) to obtain genome-wide DNA sequences from 103 plants of *C. alabamensis*, American chestnut, Allegheny chinquapin, Ozark chinquapin, Chinese chestnut, and Japanese chestnut. We aligned our sequencing reads to v.1.1 of the Chinese chestnut reference genome (Staton et al. 2015) and identified genomic locations where single nucleotide differences exist between the reference genome and samples. This process identified 190,656 such differences, referred to as single nucleotide polymorphisms (SNPs), which we used for analyses.

We inferred a phylogenetic tree to visualize evolutionary relationships among our samples; *C. alabamensis* clustered as a distinct group among the other chinquapin taxa (**Figure 3**). Because standard phylogenetic trees cannot identify hybrid individuals present in a dataset, we performed population structure analysis using the software STRUCTURE (Pritchard et al. 2000).

Figure 3

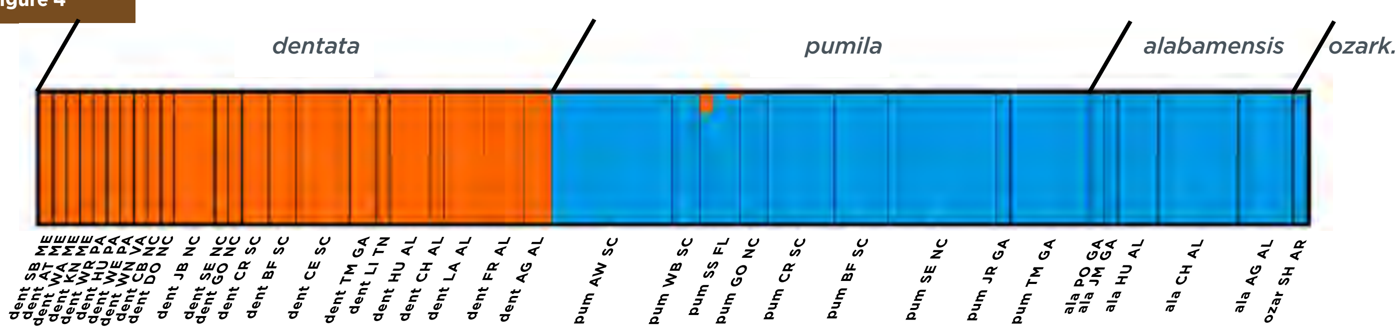


Phylogenetic tree of North America and Asian *Castanea* samples. Asterisks indicate hybrids identified by STRUCTURE. One asterisk (*) corresponds to a hybrid derived from Ozark chinquapin and an unidentified Asian species; double asterisks (**) correspond to a first-backcross descendant of *C. dentata* and *C. mollissima*.

Population structure analysis identifies distinct genetic groups in a dataset, detects admixed individuals that have ancestry from multiple genetic groups, and estimates ancestry proportions in admixed individuals. STRUCTURE showed no evidence of American chestnut ancestry in *C. alabamensis* (**Figure 4**).

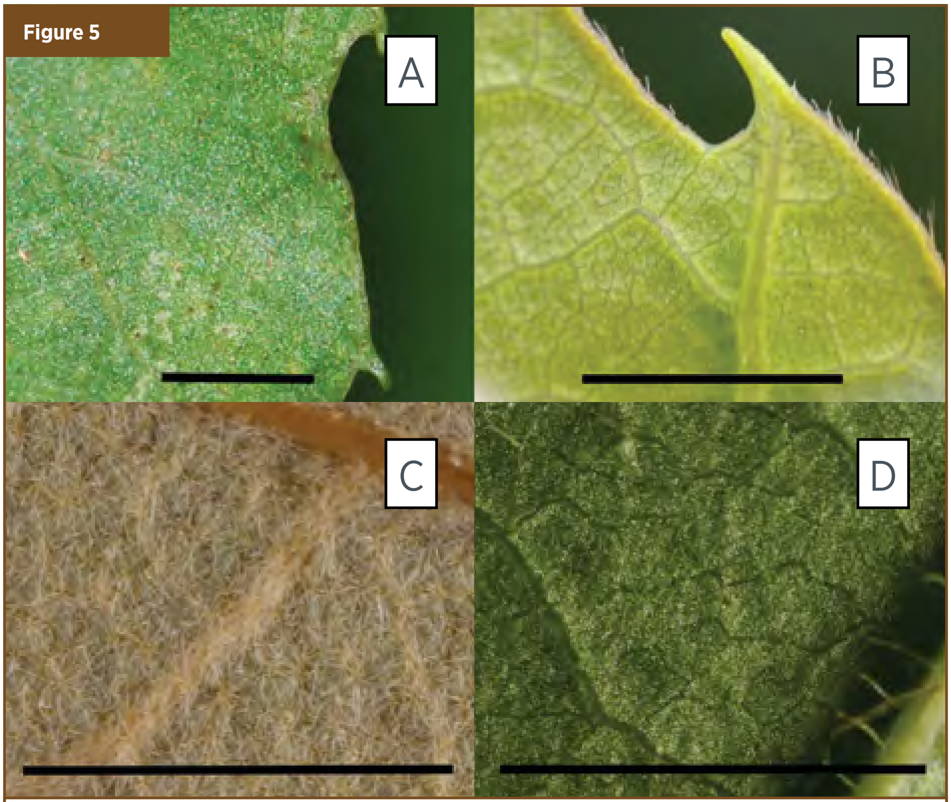
Next, we studied leaf, twig, and flower/bur traits of ~1100 *Castanea* specimens from our field collections and the herbaria of UNC-Chapel Hill and UT-Chattanooga. While *C. alabamensis* closely resembles American chestnut in leaf shape, plant habit, and smooth leaf undersides, the two taxa can be distinguished by the simple trichomes

Figure 4



STRUCTURE results for North American samples. The first genetic group (orange) corresponds to American chestnut. The second genetic group (blue) corresponds to the chinquapins. Individual samples are represented as columns in the STRUCTURE bar plot, and vertical black lines are used to demarcate plants from different sample sites. As shown for two Allegheny chinquapins from Florida (labeled "pum SS FL"), an admixed sample is represented by a column that contains multiple colors, in this case blue and orange. Sample site information along the lower edge of the plot indicates species, site code, and state (e.g., pum AW SC).

Figure 5



Morphological features distinguishing *Castanea alabamensis* from American chestnut and Ozark chinquapin. (A) American chestnut leaf margin does not have trichomes. (B) *C. alabamensis* leaf margin has simple trichomes. (C) Lower surface of Ozark chinquapin sun leaf is covered with stellate trichomes. (D) Lower surface of *C. alabamensis* sun leaf lacks stellate trichomes. Scale bars = 5 mm.

that occur on the leaf margins of *C. alabamensis*, but not American chestnut (**Figures 5A, B**). When in flower or fruit, *C. alabamensis* can be distinguished from American chestnut by having one pistil per cupule or one nut per bur. *Castanea alabamensis* can be distinguished from Ozark chinquapin (**Figure 5C**), to which it also bears some resemblance, by its lack of stellate trichomes (starfish shaped clusters of hairs) on the lower surfaces of sun leaves (**Figure 5D**).

In summary, our results add a new branch to the *Castanea* evolutionary tree by showing that *C. alabamensis* is genetically divergent from other chinquapins and did not originate from hybridization between American chestnut and Allegheny chinquapin. *Castanea alabamensis* appears to be endemic to the region between Birmingham, AL, in the southwest to Lookout Mountain, GA, in the northeast. It can be easily distinguished from American chestnut in the field by a few leaf and twig traits. However, the morphological similarity of *C. alabamensis* to Ozark chinquapin invites additional questions. Did *C. alabamensis* and Ozark chinquapin independently evolve the tree growth form as an adaptation to similar environments? Or did the shrubby Allegheny chinquapin evolve from an ancestral "proto-chinquapin" that grew as a tree? How has natural selection affected genes involved in growth form? We are currently sampling more plants for whole-genome resequencing, which has the potential to answer these questions. Insights gained from such studies can inform our ideas about adaptation in chestnuts and chinquapins, enhancing our ability to restore these species to the forests of eastern North America.

ACKNOWLEDGMENTS

We acknowledge funding support from the Foundation for the Carolinas, the Southern Appalachian Botanical Society, and the Summerfield Johnston Endowment for the Restoration of the American Chestnut. We are grateful to Dr. Susan North, Dr. Paola Zannini, and Brooke Hadden for review of earlier versions of the paper. The manuscript was improved by following recommendations from Dr. Jared Westbrook. We thank Dr. Rita Lourenço Costa for providing *Castanea crenata* DNA samples. We thank TACF staff and volunteers who assisted with field sampling: David Morris, Ed Schwartzman, Marty Schulman, Dr. Jack Agricola, Bruce and Francine Hutchinson, Will Calhoun, Dr. Martin Cipollini, Dr. Jim Lacefield, Dr. Larry Brasher, Robert Simons, Clint Neel, Dr. Frederick V. Hebard, Dr. Gary Carver, and Kendra Collins.

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Memories that Motivate Restoration

By Brett Andrzejewski, NY-TACF Chapter

Growing up I didn't know the history of the American chestnut. I used to hear the Christmas songs and wonder "where are the roasting chestnuts over an open fire, now?" It wasn't until I lived in Germany for a year and discovered the European chestnut that my eyes were opened. I hiked into the mountains of Germany and would collect a backpack worth of chestnuts to bring home. I would boil them and eat them with a bit of butter. The boiled, buttered chestnuts were often my meal for the week as I was on a poor college student's budget. I would see the chestnuts sold at the city markets, it was a typical weekend activity to see families collecting chestnuts together, and they were being roasted in front of you in the Christmas markets. Crazy to think it was 15 years ago. I've had a passion for restoring the American chestnut ever since that experience in Germany.

Cherokee Chestnut Bread

Recipe and Photo Courtesy of Doug Gillis, Carolinas-TACF Chapter President

This recipe was published 10 years ago in the July/August 2011 *Journal of The American Chestnut Foundation*. We thought it was fitting to share again, one decade later, in celebration of TACF's partnership with the Eastern Band of Cherokee Indians (see article on page 3). Prior to the blight, traditional chestnut bread was made with American chestnuts, which are sweeter than Chinese and European varieties, but the bread is still delicious. Enjoy!

Ingredients

6 corn blades, washed and scalded in boiling water
2 cups hulled, peeled chestnuts, chopped
1 cup coarsely ground cornmeal (such as yellow corn grits)
1 cup of plain, ground cornmeal
1⁄4 tsp salt
1⁄4 tsp soda
1⁄2 cup sugar
1 cup water

Method

Shred one corn blade (leaf) from end to end to create narrow, one-inch wide strips.

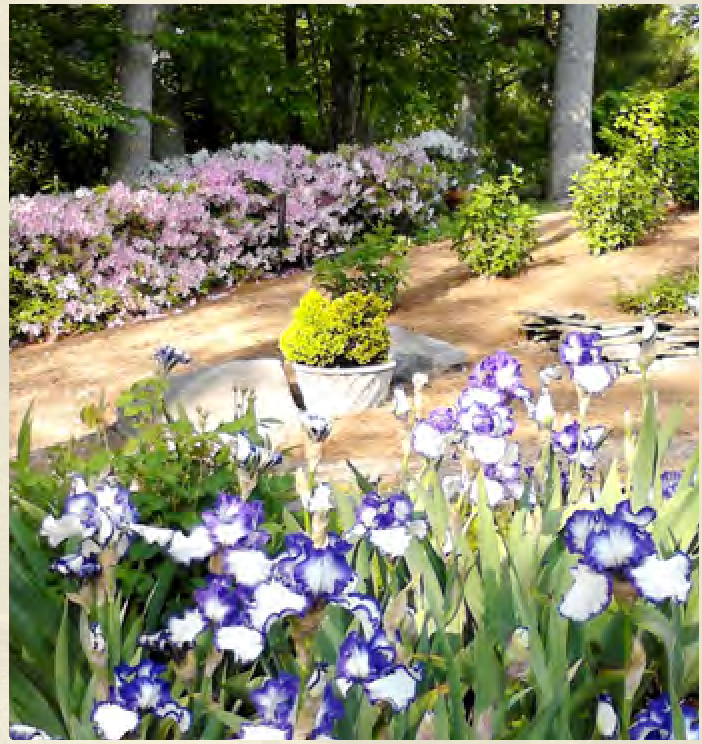
To make the bread dough, mix the cornmeal, salt, soda and sugar. (Use of yellow corn grits will add texture to the cooked bread.) Add the chopped, fresh chestnuts. Add water slowly to make stiff bread dough, a full cup may not be needed. Place a portion of dough on the wide end of a corn blade. Wrap the leaf around the mixture, first the sides and then the large end, molding the dough into a rectangular shape about two inches wide, four inches long, and one inch thick. Be sure the dough is completely covered by the leaf. Leave the narrow end of the corn blade free and unwrapped for the next step.

Split the narrow end of the corn blade into two strands and wrap each strand in opposite directions around the rest of the wrapped bundle. Tie the two strands into a knot to securely bind the chestnut cornmeal mixture within the leaf wrapping. Tie with additional one-inch strips (from first step, above) if necessary. Gently drop the wrapped packets in boiling water and simmer for 60 minutes. After boiling, remove the packets, drain, and allow to cool slightly. Unwrap the packets while still warm and serve with butter. Leftover bread can be reheated in a skillet and served warm with butter.

In Memory Of Buck Blessing

In late December 2020, Lisa Thomson received an email from Joan Blessing, a past supporter of TACF, with the following note:

"My late husband Norbert "Buck" Blessing was an avid supporter of TACF when we lived in North Carolina. I would like to make a gift in his memory before the end of this year. I'm so pleased to honor Buck's memory in this way."

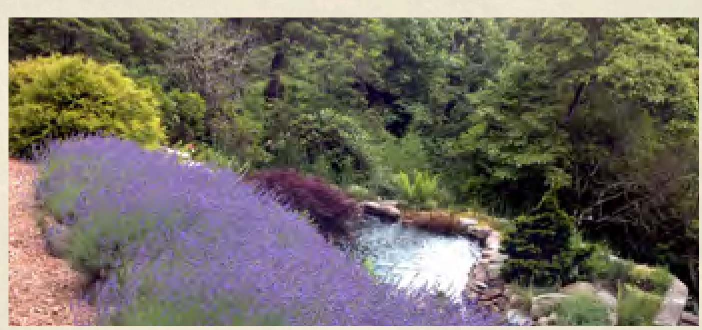


She continued, "After his retirement, I recall that he nurtured a chestnut seedling in his garden in Hendersonville, NC and spread the word among friends and neighbors about the Foundation's work. I also have pleasant memories of a weekend trip to Abingdon, VA, for a meeting.

Sadly, he contracted a lengthy illness and passed away in 2016. Last year a friend, remembering Buck's interest, sent me an article from the *Wall Street Journal* about the environmental and economic impact of reviving the American chestnut and the importance of TACF in that effort. Since then I've intended to make a contribution and am happy to do so now."

Along with his interest in chestnut research, Joan said the acreage around their home in Hendersonville (pictured) featured inviting pathways through flowering shrubs and brilliant annuals. His garden was open to neighbors and other visitors throughout Western North Carolina.

To receive such a meaningful gift, completely unexpected, shows the endurance of TACF's hopeful mission, and the power of positive media coverage. On behalf of all of us at TACF, we will cherish Buck's memory and Joan's generosity to help move our mission forward.



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