THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

CHESTNUT FOUNDATION

Chestnut

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TRIBUTES Dennis Fulbright Ann Leffel William "Bill" Lord

IN HONOR AND IN MEMORY







A BENEFIT



Lisa Thomson President and CEO

DEAR CHESTNUT ENTHUSIASTS,

A new decade is upon us! It is rare in today's fast-paced world that hundreds of citizen scientists, professional researchers, academic leaders and trusted partners are involved in a bold experiment that has taken decades to achieve, with more work ahead of us. That said, we are not daunted by the tasks at hand, because new discoveries and technologies are bringing us closer every year to mission success.

We had much to celebrate at year's end, including a feature story about our work on *CBS This Morning* that aired December 5th! Crews from the network visited our Meadowview Research Farms in June and October and spent hours capturing footage and conducting interviews. An inspirational five-minute story was the result. *CBS This Morning* published it on their YouTube channel. In less than two weeks, the story received more than a quarter million views, and that number continues to rise! If you missed it, enter this link into your web browser: http://bit.ly/CBSchestnutstory

We enjoyed seeing many of you at the fall meeting in Gettysburg, PA. It was a strong turnout of enthusiastic chestnutters and we were treated to excellent presentations, entertainment and field excursions. Mark your calendar for October 3, 2020 for the next gathering right here in Asheville, now renamed the American Chestnut Symposium. We heard your feedback that you would like more scientific content and overall chestnut learning opportunities in the seminars and are gearing it toward that need.

As 2019 draws to a close, and we enter this new decade, it is clear to me and our many dedicated volunteers and partners that restoration of the American chestnut is inching closer to reality. That this effort has been underway for more than a century is nothing short of a miracle. I can't think of any other conservation rescue mission that has endured this long-term tenacity and desire to succeed. We *will* succeed because we have *your* energy, enthusiasm and generosity to ensure it does.

With gratitude,

Lisa Thomson, President and CEO The American Chestnut Foundation



Follow me on Twitter (@MadameChestnut).





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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The Ort Tree

A "Large Surviving American" (LSA) chestnut located near York, PA. This American chestnut tree has demonstrated resistance to chestnut blight both by the appearance of the cankers on the main stem - visible in this photo - as well as in the increased blight-resistance its backcross progeny have above other pedigrees. The Ort tree was one of the first American chestnuts used by the PA/NJ TACF Chapter in its breeding program in 1996, led by Ann and Bob Leffel. We pay tribute to Ann later in this issue, having lost her and two other long-time TACF leaders around the Thanksgiving holiday.

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PART1 of a 4-Part Series

3BUR

USING SCIENCE TO SAVE THE AMERICAN CHESTNUT TREE

The American Chestnut Foundation (TACF) takes a holistic approach toward American chestnut restoration, utilizing a three-pronged research strategy known as the 3BUR method (Breeding, Biocontrol, and Biotechnology United for Restoration). These research tracks are meant to be integrated through collaborations that are mutually beneficial, so we can explore all avenues to reach the common goal of saving and restoring this species as quickly as possible.

In part 1 of this four-part series, we briefly define the three research tracks. Parts 2-4 will detail each track, explaining how employing multiple pathways are essential in order to create a disease-tolerant and genetically diverse population of American chestnut that will adapt to a broad and changing climate.



3BUR: Breeding, Biotechnology and Biocontrol United for Restoration

B1 - Breeding.

Our traditional breeding program is carried out at our research farm in Meadowview, VA, and at more than 500 orchards planted, largely by volunteers and partners, across sixteen TACF chapters throughout the American chestnut's native range. During the past 36 years, offspring from two blight-tolerant hybrids have been bred with American chestnuts from across the species range. Three generations later, our traditional breeding program has produced a genetically diverse population of American chestnut hybrids with improved blight tolerance. Moving forward, our breeding efforts are focused on further improving blight tolerance and incorporating resistance to Phytophthora cinnamomi, which causes a fatal root rot in chestnuts. We are using genomics to increase the speed and accuracy of selecting trees with the greatest tolerance to chestnut blight and root rot.

B2 - Biotechnology.

At the core of our biotechnology program is transgenics, the science of introducing genetic material across species in order to safely create desired traits. Scientists at the State University of New York, College of Environmental Science and Forestry (SUNY-ESF) have discovered a gene from wheat that produces an enzyme that enhances blight tolerance significantly. Incorporating transgenics into TACF's breeding program allows us to stack multiple blight resistance genes and increase the proportion of American chestnut genes in the resulting progeny. The SUNY-ESF research is so promising, that we believe TACF can obtain the governmental approval required from the USDA, FDA, and the EPA to plant transgenic American chestnut trees in the wild in three to five years.

B3 - Biocontrol.

The primary biological control method being explored by TACF and its partners is called hypovirulence. This is the term used for the infection of the chestnut blight fungus by a virus, thereby sickening the fungus and reducing the ability of chestnut blight fungus to cause lethal infections. Using this method, the natural defenses of the chestnut tree may enable the tree to halt canker growth. These methods can keep blight-susceptible American chestnut trees alive and healthy so that they can be used in our transgenic and traditional breeding programs. Other fungi and bacteria are being investigated which may play additional roles in reducing the effect of the chestnut blight fungus on American chestnut trees.

Saving this iconic tree and restoring it to its native range at scale could also help give other endangered tree species a new lease on life and directly offset the effects of climate change and deforestation. While no single intervention can completely eradicate chestnut blight, together the science of breeding, biotechnology, and biocontrol (3BUR) offer our best hope for rescuing the American chestnut tree.

Phytophthora

2019 SCREENING UPDATE

By Ben Jarrett, Southern Regional Science Coordinator

Volunteers with The American Chestnut Foundation (TACF) planted over 3,000 seeds this year at the Resistance Screening Center (RSC) at Bent Creek U.S. Forest Service Facility in Asheville, North Carolina, for Phytophthora Root Rot (PRR) resistance screening. This project is a collaboration between TACF, Clemson University, and the U.S. Forest Service to determine trees and genetic families within TACF's breeding program that have resistance to PRR. This is the fourth year screening seedlings for PRR resistance at Bent Creek.



PRR is caused by a soil pathogen, *Phytophthora cinnamomi* that was introduced to the United States from Southeast Asia in the 1800s. The pathogen is deadly to American chestnuts; however, some trees in TACF's breeding programs contain resistance from their Chinese chestnut ancestors. The goal of this screening program is to identify which trees have PRR resistance and to produce trees that we will eventually breed with trees that have blight resistance to combine blight and PRR resistance.

The seeds this year came from eight state chapters and TACF's Meadowview Research Farms. Planted in the greenhouse in February, the seedlings have grown with the help of U.S. Forest Service employees, who water and monitor the trees until they are large enough for inoculation. By April, the seedlings were growing well and moved into isolation tubs in a complete randomized block design, which helps ensure that there are no influences on our study results

due to where in the greenhouse a given tree is located. In May, Clemson University Professor Steve Jeffers and his colleagues traveled to Asheville to inoculate the seedlings with Phytophthora cinnamomi. This year, for the screening of backcross material, we used isolates of the pathogen from the Mountain Island State Educational Forest in North Carolina. We will plant the most resistant trees from the screening out at the state educational forest in the spring. Utilizing the Phytophthora cinnamomi present in the planting location ensures that we are not transporting isolates of the pathogen to different locations.

New to the program this year, Jeffers and USFS Resistance Screening Center Director, Katie McKeever, also conducted an experiment using TACF seedlings to determine if there are differences in the pathogen virulence from site to site and from plant host to plant host. They utilized seven different strains of *Phytophthora cinnamomi* from different areas of the Southeast United States and inoculated hybrids and Chinese chestnuts with these strains. The results of this experiment will be published after another trial is conducted next year.

On November 1, there were over 650 seedlings still living six months postinoculation. Jeffers and his lab, RSC employees, and TACF volunteers came together to rate the root lesions on every surviving seedling. The process involved removing all the potting mix from each tree and Jeffers, with many years of experience, giving each tree a rating based on the percentage of the seedling's root with lesions caused by PRR.

In 2020, the cycle begins again and seeds will be planted in February. This program would not be possible without our partners at Clemson University, the U.S. Forest Service, and TACF state chapters who send us seeds each year.



The "Avalon" Tree

By Martin Cipollini, GA-TACF Chapter Science Coordinator



ometime in 2005, GA-TACF founding member Tom Pachinger was driving his wife Mary's Toyota Avalon™ along Winding Stair Gap Road in the middle of the Springer Mountain area of the Chattahoochee-Oconee National Forest (not far from Ellijay and Dahlonega, GA). If you know anything about remote U.S. Forest Service (USFS) roads, you'll understand the significance of the fact that he was driving a low-slung sedan that day! Tom was surveying the area for wild American chestnuts, not far from a large survivor we call the "Fowler" tree. He came upon a nice 2-3 inch diameter-at-breast-height (DBH) sapling immediately beside the road, which subsequently became known to us as the "Avalon" tree. Tom noted a number of other fairly large chestnuts in the vicinity during this and subsequent trips with his frequent "chestnutting" partners, Ken McDonald and Mike Hinson (all three having worked together at the Temple-Inland facility in Rome, GA).

I came on as GA-TACF Chapter science coordinator in the summer of 2006, and helped arrange some release work with wild chestnuts that fall. A group including Tom, Ken, James Wentworth (USFS) and several others cleared around and fertilized the Avalon, Fowler, and several other trees in the Springer Mountain area that fall. In May 2008, I returned



The crew's first release work at the Avalon tree in fall 2006. Back row: Milton Bradley, James Wentworth, Martin Cipollini, and Ken McDonald. If anyone knows the name of the volunteer kneeling, please let us know!

Avalon tree in fall 2011, five years after initial release work. GA-TACF volunteer lan Cipollini poses by the tree for size comparison. Photo by Martin Cipollini. to fertilize these trees once more, and have continued to monitor them. Both the Fowler and another Springer Mountain tree have produced pollen that has been used successfully in the GA-TACF breeding program, but the Avalon tree grew so fast that once flowering began, catkins couldn't be reached by pruning poles or ladders!

Despite having the lower branches cut off by a mower in 2009, the Avalon tree was already at 8-inch DBH and 35 feet tall by 2011. In 2013, the tree was in excellent condition with no evidence of blight, although someone (unknown to us) had been mud-packed around the base of the tree (we assume to combat a suspected canker). By 2014, the tree was over 9-inch DBH and 50 feet tall, with near full canopy exposure, no blight, and many catkins near the very top. That year, GA-TACF member Jack Rogers and I were just able to reach a few catkins for a pollination attempt using our 30-foot pruning pole. In 2015, catkins were at the very top of the tree and out of our reach, and the tree measured 11-inch DBH with no apparent blight. I noted that fall that it was "spectacular," its full canopy a glorious blaze of yellow-orange. By this time, it was clear that the only way to do any future breeding work with this tree would be with a bucket truck.

Flash forward to this past summer. After several years of not being able to access the tree (road blockages due to storms and other issues) I suspected, based upon field reports, that blight cankers might begin taking a toll on the tree. Rather, I returned to find the tree in good condition and still growing fast. The tree did have cankers forming at two locations on the trunk but they appeared to be of a hypovirulent type common among the trees on Springer Mountain (swollen, knotty, slow growing). This gives me hope that the Avalon tree will live for many years (the aforementioned Fowler tree is still alive and looks about the same as it did in 2006 despite heavy cankering).

Impressed by its substantial growth since my previous observations, I measured and nominated the tree for the Georgia Forestry Commission's State Champion Tree program. Indeed, at 60 feet tall, 13-inch DBH (40-inch circumferenceat-breast-height), and 34-foot crown spread, the tree was verified this past August as Georgia's state champion American chestnut!

Noticing a number of burs developing, I returned this fall to harvest them with the help of a bucket truck donated by Amicalola Electric Membership Corporation (EMC), and arranged by TACF member Jack Rogers and Amicalola EMC staffer Ray Carpenter. The EMC crew harvested 50 burs yielding three viable seeds. While this might not seem like a significant yield, wild American chestnuts almost



never produce viable seeds in Georgia, so capturing this tree's genetic diversity is very significant for the GA-TACF Chapter. We hope the seedlings will find a home at Berry College, where our main orchard is being converted from a backcross into a germplasm conservation orchard.

I write this to emphasize three important points. First, the impressive growth of this tree in the 14 years since its discovery is testament to the resilience of American chestnuts. Capturing the genetic diversity represented across the natural range is paramount for a successful restoration program. Second, efforts to release relatively small saplings can yield very good results (with appropriate care and patience), particularly if trees are in locations that can be frequently monitored. In situ approaches should not be overlooked in our efforts at germplasm conservation. Three, despite the mud and the bumps, Mary Pachinger's Avalon[™] was an effective way to scout for chestnut trees after all!

UPDATES FROM NE-1833 Meeting 2019

By Tom Saielli, TACF Mid-Atlantic Regional Science Coordinator For 37 years, members and staff from The American Chestnut Foundation (TACF), and colleagues from Universities and public agencies world-wide, converge to discuss the latest chestnut science. This year's meeting took place September 6-7 at Alpine Lake Resort in West Virginia and was hosted by West Virginia University Assistant Professor, Matthew Kasson and Research Assistant Amy Metheny.



The conference began with talks on *Phytophthora cinnamomi*. Steven Jeffers, professor at Clemson University, presented on years of collaborative research on Phytophthora resistance screening, including material from TACF chapters.

Tatyana Zhebentyayeva, research associate professor at Penn State University, found that genes on two chromosomes (linkage groups E and K) were associated with Phytophthora resistance in Chinese x American chestnut hybrid populations. Rita Costa, Instituto Nacional de Investigação Agrária e Veterinária (INIAV), also found resistance in linkage groups E and K, and chose eight candidate genes to evaluate potential roles in Phytophthora resistance. Understanding the mechanisms of resistance and identification of markers can significantly aid in development of Phytophthora-resistant chestnuts.

Presentations during the genomics session included Jared Westbrook, TACF director of science, who presented the latest genomics research, identifying blight resistance linkage groups on at least eight of twelve chromosomes on hybrid chestnut trees. These are significant findings, considering the former assumption that blight resistance was controlled by only three genes. Dana Nelson, director of the Forest Health Research and Education Center at the University of Kentucky, also used QTL mapping to identify multiple genomic regions associated with blight resistance. The conclusion: hybrid chestnuts must balance American traits with resistance from Chinese chestnut. We may need to incorporate more genes from Chinese chestnut to get trees with sufficient blight resistance to survive in the forest.

Researchers at SUNY's College of Environmental Science and Forestry shared an update on mechanisms of resistance in Chinese chestnut, focusing on the enzymes responsible for removing oxalate. Studies of the environmental safety of the OxO gene are ongoing and include comparisons of transgenic and wild type trees with respect to mycorrhizal colonization of root, pollen feeding by bees, and tadpole feeding on leaf litter. No ecological differences were detected between wild-type and transgenic trees with the exception of elevated blight tolerance in transgenic trees. (See full article about the safety tests on page 26.)

Andrew Jarosz, associate professor at Michigan State University, presented on hypovirus studies in commercial nut growing orchards in Michigan and the potential benefits of "hypovirus cocktails" to control chestnut blight in commercial orchards. Angus Dawe, professor at Mississippi State University, followed Andrew's presentation by focusing on the identification genes responsible for differences in pathogenicity among chestnut blight strains. Bradley Hillman, professor at Rutgers, reported that different viruses infect chestnut blight fungus and not all viruses reduce the pathogenicity of the blight.

Meeting host, Amy Metheny, presented on the efficacy of controlling chestnut blight with "super donor" strains of the chestnut blight fungus. The super donor strains of the chestnut blight fungus are capable of transmitting hypoviruses to all or most wild-type strains of the chestnut blight fungus in the laboratory. However, transmission of the virus between chestnut blight strains in natural cankers is lower than expected. This lower than expected transmission could be due to the three dimensional structure of cankers preventing transmission of viruses between hypovirulent and virulent strains of the blight fungus.

Following Amy's talk, NE-1833 attendees visited Savage River State Forest to observe the site where Amy and her colleagues applied hypovirulent super donor strains of the chestnut blight fungus to natural cankers on wild American chestnuts. The group observed significant variation in blight canker expansion and the recovery of treated trees, confirming the inconsistent control of chestnut blight cankers with super donor strains.

Other noteworthy presentations included gall wasp studies conducted by Sandra Anagnostakis, researcher at the Connecticut Agricultural Experiment Station. Sandra identified the Chinese chestnut cultivar 'Eaton' as one genotype that fares well against gall wasps. Fred Hebard, TACF Chief Scientist emeritus, discussed how recurrent selections may aide in the rescue of inferior lines in some VA Chapter seed orchards. Paul Sisco, Carolinas Chapter, discussed the value of planting male sterile F, chestnuts along the borders of seed orchards, because the F, females may cross pollinate with BC x F_2 trees producing F₁x BC x F₂ crosses. John Carlson, professor at Penn State University, provided an update on the Chinese chestnut reference genome. Bill Rittenour, head brewer at Chestnut Brew Works in Morgantown, WV, gave a fascinating presentation on the science of brewing beer. And finally, Mid-Atlantic Regional Science Coordinator and article author, Tom Saielli, presented on how to train citizen scientists to locate wild American chestnut trees so that TACF may increase its collection of wild trees in germplasm conservation orchards.



Pure American Program

BEGINNING THIS FEBRUARY, THE AMERICAN CHESTNUT FOUNDATION WILL ONCE AGAIN BE SELLING 100% PURE AMERICAN BAREROOT SEEDLINGS IN BUNDLES OF 10, 25, AND 50.

TACF members can begin placing online orders Tuesday, February 4. Members will receive an email Sunday, February 2 to give advance notice of the sale, including a link to the order form, which will be live February 4. Those without email may place their order by calling the national office at (828) 281-0047. The sale will then open to the public on Monday, February 17 (while supplies last).

Growing pure American chestnut trees is a wonderful learning experience and helps preserve genetic diversity for future breeding and diversification. Pure Americans will succumb to the blight if exposed but can thrive for many years and produce seed for harvest and consumption.

This is a very popular program and the seedlings sell out quickly. Distribution range is limited to states east of the Mississippi (no exceptions). Orders will be mailed in April.

PRICING FOR PURE AMERICAN SEEDLINGS: Only sold in quantities of 10, 25, 50 – includes shipping

> 10 seedlings - \$65.00 25 seedlings - \$150.00 50 seedlings - \$250.00

Proceeds from this program help fund research to restore the American chestnut.

2019 Photo Contest Winners

The photos in this year's contest presented a variety of angles and settings that made for a distinctive lineup! Once the top three were selected, customers at our neighboring pie shop, Baked, chose their favorites and that's how we determined the 1st, 2nd, and 3rd place winners.

The photographer who came out on top is

MATT NICHOLS

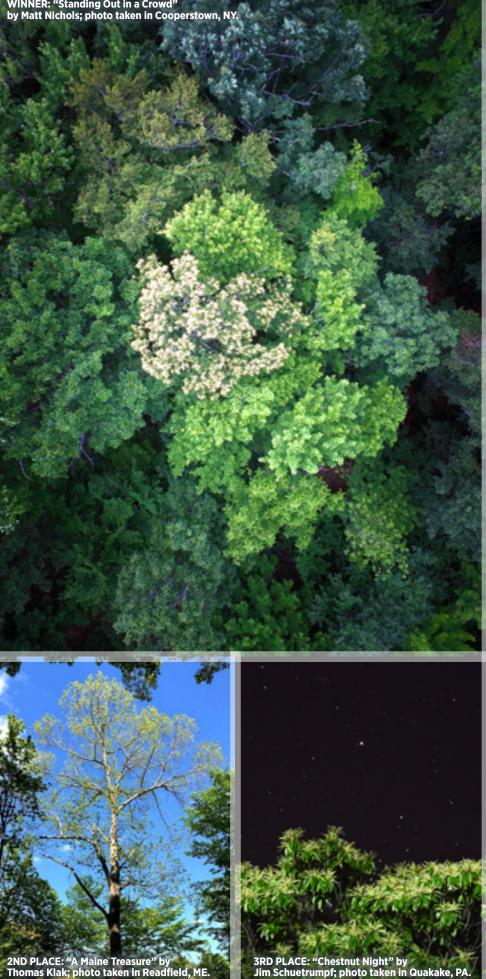
of Toddsville, NY. Matt used a drone to capture this unique angle of a blooming chestnut which easily distinguishes it from the surrounding trees. His photo will appear on the cover of an upcoming issue of *Chestnut* magazine. Congratulations, Matt!

We appreciate all who entered the contest. Even photos that do not win have the potential to be used in a variety of TACF publications, posted on our social media platforms and/or website, or appear in the magazine, so keep them coming! The 2020 contest will begin this spring.

> Second place winner: Thomas Klak, **ME-TACF** Chapter

Third place winner: Jim Schuetrumpf, **PA/NJ** Chapter

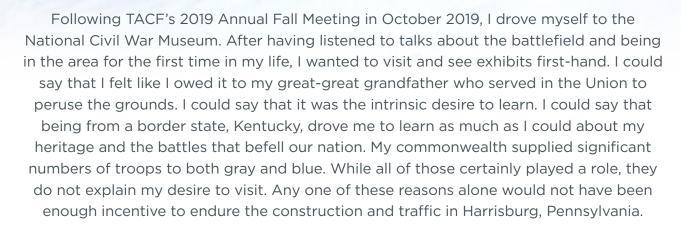
WINNER: "Standing Out in a Crowd"



The Chestnut Address:

A REFLECTION FROM TACF'S 2019 ANNUAL FALL MEETING

By Jacob Pease, KY Chapter



No. Rather, Gettysburg brought forth old thoughts. Thoughts that harken back to the first time I listened to one of my now-favorite songs, "Sticks that Made Thunder," by The Steeldrivers. The song follows the narrative of a tree whose "... roots are deeper than the bones of the others," and whose "branches were higher than anything on the hillside on the day that I watched them all come." Them being the soldiers in a great battle of the Civil War. The tree recounts its awe at the carnage and its interactions with ghosts in the aftermath. The song is a solemn and humbling homage to those killed on the war's battlefields and the horrors therein and thereafter.

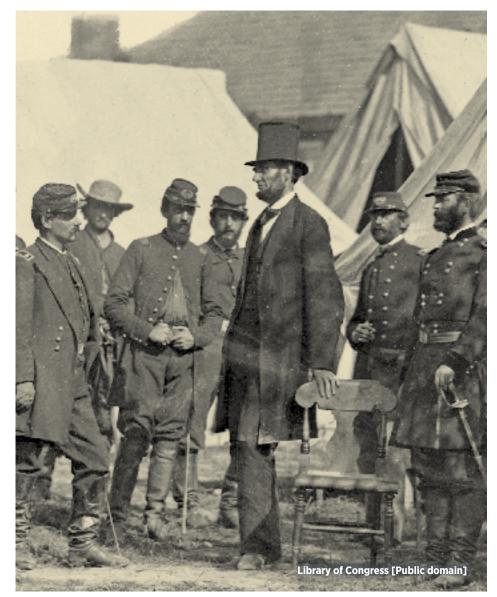
I often understand these lyrics from the perspective of an American chestnut. As members of TACF, we recognize how significant the tree was to our heritage, but this song puts its loss in a different context for me. Gettysburg Battlefield Guide, Britt Isenberg, discussed the dwindling witness trees when prompted during the Q & A portion of his presentation at the fall meeting. Part of me wonders how many would remain today and what our forests would look like if the blight had not been introduced. So, for me, the lyrics and melody bring to the surface myriad emotions - not least among them a great sadness. Sadness for our country, for the ones who sacrificed their lives and well-being, and for our forests that are besieged by so many pests and diseases.

Our forests, like the bellum nation, are engaged in a great war that should never have been brought upon them. However, here we stand, and here we fight. Like Lincoln's speech to that small crowd on November 19, 1863, our mission and our words regarding the chestnut make this war raging in our forests about humanitarianism and heritage. Surely, the chestnut's reintroduction will expand public enjoyment of natural resources and the history we yearn to immerse ourselves in. The more we reach out, research, and recruit, the stronger our message becomes.

Lincoln's words called upon the American people to rise up to the "great task" of preserving our great nation. Truly, bringing back the American chestnut is our great task.

Lincoln's words called upon the American people to rise up to the "great task" of preserving our great nation. Truly, bringing back the American chestnut is our great task. In the two years I have been fortunate enough to attend TACF's annual fall meetings, I can say without hesitation that we have the ability to see our own duty through to the end. Our strength, passion, and science will pave the way for techniques and knowledge ensuring that neither the chestnut nor its contemporaries will perish from the earth. This victory will be our legacy, and I believe Gettysburg epitomizes that.









The American Chestnut Foundation's 2019 Annual Fall Meeting set a record-breaking attendance by bringing together its largest family of donors, members, volunteers, citizen scientists and chestnut lovers to date!

Set in Gettysburg, Pennsylvania, meeting attendees were immersed in the history and majesty of their surroundings, and treated to an abundance of apple delights, as Gettysburg celebrated their annual apple festival during this same glorious weekend.

Partnering this year with The Schatz Center for Tree Molecular Genetics, Gettysburg Nature Alliance, The Gettysburg Heritage Center and The Will Group at Merrill Lynch, the Saturday sessions offered a wellchoreographed celebration of Gettysburg and American chestnut past, present, and future. We hope you enjoy the highlights from this enthusiastic gathering.

2020 promises to be an exciting venture toward the next generation of chestnut restoration. We look forward to welcoming you at TACF's 2020 American Chestnut Symposium, being held in Asheville, North Carolina, October 3, 2020. 2019 Annual Meeting Sponsors

The American Chestnut Foundation would like to

THANK these organizations who helped make this event possible:





Gettysburg Nature Alliance



2019 Awards and Recognition

THE AMERICAN CHESTNUT FOUNDATION HONORS 2019 Volunteer Service Award Recipients

Yurij Bihun, New England Region

Presented by Kendra Collins, New England Regional Science Coordinator

Yurij Bihun has been an active member of TACF for nearly 20 years. As a forester with a broad range of regional,

national, and

international

experience

background

improvement,

he was drawn

to TACF's

challenging

but hopeful

mission of

restoration.

He joined the

forestry

and his

in tree-



Yurij Bihun receives his award from Kendra Collins.

board of the VT/NH Chapter in 2010, became secretary in 2012, and after a very brief term as vice-president in 2013, assumed the presidency later that same year. As chapter president, he worked on all aspects of the chapter's programs and brought his own unique skill set to developing orchard agreements, editing the newsletter, undertaking strategic planning, and salvaging and utilizing the wood of wild American chestnut trees that succumbed to the blight. In addition, he served two terms on TACF's Board of Directors from 2013-2019 and was a member of the Chapters and Restoration Committees.

Robert Sypolt, Mid-Atlantic Region

Presented by Mark Double, WV Chapter

There is no better chestnut advocate in the WV-TACF Chapter than Robert Sypolt. Robert has traveled from one end of the state to the other, working with faculty at West Liberty University in the northern panhandle to monks at the Russian Orthodox Monastery in Robert Sypolt (left) receives his award from Mark Double.



Wyoming County in the southern coal fields. Robert does more than simply plant trees and give presentations; he always has TACF brochures in his truck as he consistently attempts to recruit new members to join the foundation. Robert's trusty truck has carried him on the highways and dirt roads of the great state of West Virginia, all on behalf of the American chestnut tree and the efforts of TACF to restore it.

Blair and Mary Carbaugh, North Central Region

Presented by Ed Saufley, PA/NJ Chapter

Blair and Mary Carbaugh have been members of the PA/NJ-TACF Chapter since 1996 and, during that time, have motivated many people to share their love of nature and chestnuts.

Professor Emeritus at Lock Haven University, Blair is the consummate



Blair and Mary Carbaugh receive their award from Ed Saufley (left).

educator, always teaching and sharing. He has forged Forest Stewardship programs, and worked closely with the Department of Conservation and Natural Resources (DCNR), organizing and planting hundreds of chestnut trees on two acres of land at the Anthracite Outdoor Adventure Area. In 2017, many friends and collaborators gathered at the site, along with Blair, and held a surprise dedication, naming this location the "Carbaugh Conservation Area."

Mary, an honors graduate from Dickinson College, is a Master Gardener and for years has also delighted so many with her chestnut-based dishes.

Together, Blair and Mary have dedicated countless hours to the PA/NJ-TACF Chapter and American chestnut restoration. This award is a wonderful tribute to their love of nature, chestnut, and their enthusiasm to share it with so many.

Kathryn Maley, Southern Region

Presented by Hill Craddock, TN Chapter

As a student, Kathryn Maley has contributed to the restoration of the American chestnut. She grew up knowing the story of chestnut blight and already, as a sophomore at Maryville College, was deeply involved in the school's regional seed



Kathryn Maley receives her award from Hill Craddock.

orchard project. Kathryn was able to plan, plant, water, and carefully tend more than 400 trees in this newest, and largest TN-TACF seed orchard that includes 15 unique B₃F₂ genetic families representing seven Meadowview and Tennessee lines! In May of 2019, Kathryn graduated after defending her Honor's thesis: Success of American Chestnut Hybrid Seedlings in a Walland, Tennessee Seed Orchard.

Special Recognition Award: Volunteer Contributing Author Scott Carlberg, Carolinas Chapter

It didn't take long for Scott Carlberg

to volunteer his services after joining TACF in 2016. Scott has had an extensive career in communications and marketing so when he offered to write articles for Chestnut magazine, Jules Smith, TACF media and communications manager immediately



took him up on the proposal! He began writing the Volunteer Spotlight feature and his first article, written about Carolinas Chapter member Don Surrette, was published in the 2017 fall issue. Since then, Scott has not missed an article, despite a major move from Charlotte to Kansas City and international travels to visit family. He often expresses his enjoyment of getting to know the volunteers he writes about and, in turn, the volunteers have been delighted with the articles Scott has authored about their work.



OUTGOING BOARD MEMBERS

TACF board members Catherine Mayes and Yurij Bihun were recognized during the board meeting on Friday,



October 18 for their long-term commitment and service.

Cathy has been a member of the VA-TACF Chapter since 2001. She was a chapter board member and became president in 2016. Cathy joined TACF's board of directors in 2008 and served on the Executive. Promotion & Outreach, and Chapters Committees.

Yurij has been a member of the VT/NH-TACF Chapter since 2010 and served as secretary, vice-president, and president on the chapter's board. He joined TACF's board of directors in 2013 and was an active member of the

Chapters and Restoration Committees.





The winner of TACF's 2019 American Chestnut Tappan American Chestnut Tappan Rocker Raffle was Luana Maroja from Massachusetts. She bought only one ticket and purchased it on her birthday. Luana considers it one of the best birthday gifts she's ever received. The winning ticket was drawn during the Volunteer Service Awards Dinner on Saturday, October 19. Thanks to Adam Nudd-Homeyer for crafting and donating this exquisite handmade chair.

JENNY ROSE CAREY Keynote Speaker

Jenny Rose Carey treated more than 250 attendees at the Gettysburg fall meeting to an entertaining presentation on the joys and trials of gardening for species enrichment and classic aesthetics. Her long and passionate career has focused on practical gardening, horticultural education, and work with arboreta and formal gardens alike.



Using much of her own stunning photography, we took a visual tour of the many beautiful gardens and formal plantings Jenny Rose has led through the years. Among her skill as a master gardener, she is also an author of Glorious Shade and a co-author of the book, A Century of Cultivation: 1911 to 2011, about the history of the Pennsylvania School of Horticulture for Women.

An alumna of Springside Chestnut Hill Academy (SCHA) in Philadelphia, Jenny Rose is assisting SCHA in establishing a teaching orchard to honor long-time trustee and chestnut enthusiast, Dick Hayne. Jenny Rose resides in Ambler, Pennsylvania at her Victorian home with four and a half acres of gardens designed and lovingly tended by her.





Gettysburg 12.20

The Educational Sessions filled with participants eager to learn about the latest scientific advancements and restoration efforts.



Bruce Wakeland, IN-TACF Chapter, told stories about the American chestnut tree during one of the Educational Sessions.



Winners in order from top to bottom: 1st Place, Hannah Pilkey; 2nd Place, Cindy Ingram; 3rd Place, Taylor Evans.









Meeting participants observe the posters on display in the foyer outside the meeting rooms.

Poster Session Awards

CONGRATULATIONS TO OUR POSTER AWARD WINNERS, AND THANK YOU TO ALL THE STUDENTS WHO PARTICIPATED!

The poster session during this year's annual meeting highlighted thirteen research projects related to the restoration of the American chestnut. In its eighth year, the poster session provides the opportunity for both seasoned professionals and current students to present their work to their peers and TACF membership. This allows the annual meeting to highlight a much broader range of topics than can otherwise be accommodated by the general sessions. Topics presented this year covered a variety of worthy investigations, including chestnut silviculture, habitat suitability, propagation, transgenic chestnuts, and pests and pathogens.

For students in particular, the poster session is a great opportunity to gain experience presenting academic work. In 2017, the poster session program was expanded to include competitive student poster awards. This year ten student presenters participated in the competition. All student posters were judged by members of TACF's research community using a standard rubric, and cash prizes were awarded to those with the three highest scores.

In third place was Taylor Evans, a first-year Masters student at James Madison University. Broadly interested in the intersection of ecological restoration and agroforestry, Taylor's current research focuses on the restoration of the American chestnut by improving seedling quality in the nursery. His poster, "Comparing Four Nursery Production Methods on Chestnut Hybrid Seedling Quality," detailed the research proposal for his Masters' thesis project. He will be comparing propagation methods in order to determine least cost approaches to improving seedling survival and field performance.

Second place was awarded to Cindy Ingram, an undergraduate student at James Madison University. She is pursuing dual Biology and Religious Studies majors, and expects to graduate in 2021. Cindy enjoys field work and crunching numbers, and worked under the guidance of Professor Heather Griscom to study the response of chestnut seedlings to forest management techniques. Her poster, "Response of Restoration **Chestnut Seedlings to Forest** Management Strategies," detailed their work, which consisted of analyzing differences between large canopy gap/small canopy gap and within those gaps; canister/no canister and landscape fabric/no landscape fabric.

The first place winner was Hannah Pilkey, who presented "Genetic Transformation of the Ozark Chinquapin (Castanea ozarkensis)." A Masters student at SUNY's College of Environmental Science and Forestry, Hannah has a passion for conservation biology and was eager to get involved with the American chestnut project. Her primary research involves pollen collection and storage methods for chestnut outcrossing. However, while her pollen samples were in freezer storage, she had time to work with Ozark chinguapin embryos and trees in tissue culture. Following the same protocol that was used to develop the transgenic "Darling" American chestnut tree, the first genetic transformation of Ozark chinquapin was conducted.

Abstracts and posters from the 2019 annual meeting are posted on TACF's website: http://bit.ly/2019postersession

The poster awards would not have been possible without judges who volunteered their time to assess posters and engage with student presenters. A special thank you to John Carlson, Carolyn Keiffer, Dennis Liu, Bill MacDonald, and Jared Westbrook for their conscientious and thoughtful work assessing student posters.



Entirely American Chestnut

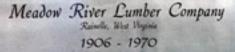
THE RAINELLE, WV UNITED METHODIST CHURCH

By Mark Double, WV Chapter President

In northern Greenbrier County, West Virginia a large white church sits atop a knoll along Route 60. The Rainelle United Methodist Church, dedicated in 1914, is reported to be the largest structure in the world made entirely of American chestnut lumber. Built when American chestnut dominated much of the eastern U.S. forests, the church's ceiling, walls, pews, bookcases, bell tower and bifold doors were all made from pre-blight American chestnut.

The history of the church and the town of Rainelle are fascinating stories. In 1906, Thomas and John Raine, brothers from Ironton, Ohio, purchased 32,000 acres of virgin timber at the base of Sewell Mountain in Greenbrier County, WV. Eventually, they increased their land holdings to 100,000 acres. The tracts of land purchased by Thomas and John were wilderness with no houses and 20 miles to the nearest railroad line. With no access, the Raine brothers began construction of a railroad in 1908, so timber could be accessed via a spur line that connected to the Chesapeake and Ohio Railroad. The Sewell Valley Railroad enabled building materials to be hauled into the wilderness and carried lumber out. The lumber company, built near the Meadow River, was aptly named Meadow River Lumber Company and for decades, it was the largest hardwood lumber operation in the world. Nearly 3,000 acres of hardwood were cut per year, including ash, birch, cherry, hickory, maple (hard and soft), oak, (red and white) and American chestnut. The lumber was turned into flooring, ceiling, siding, molding and trim pieces and 14 large kilns were used to dry the lumber. Construction of the mill began

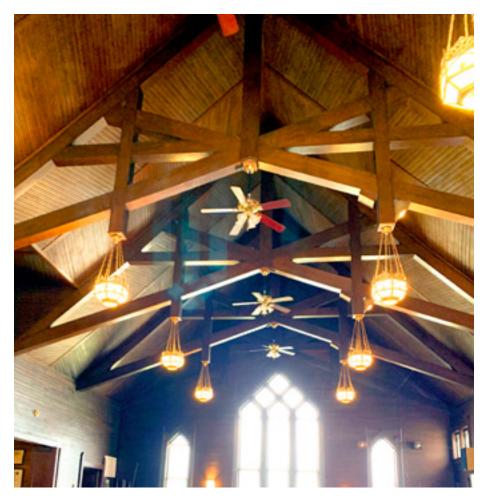






in 1909 and the first logs were sawn in September 1910. The mill had three 9' band saws under one roof and they cut, on average, 110,000 board feet per day. That translates to 20 million board feet per year with a peak of 31.6 million board feet in 1928. Meadow River Lumber Company operated a system of portable lumber camps built on railroad cars that could be moved to new timber supplies. Teams of horses skidded logs to the railroad until 1930 when overhead cable skidders mounted to railroad cars were used. The lumber as the Rainelle Methodist Episcopal Church. It has served as a school and public library, and currently hosts service, civic and scouting organizations. The church has upgraded its facilities and is capable of hosting teams of volunteers who come to southern WV to work flood relief projects. The steam plant at the lumber mill burned sawdust, and heat was provided free-of-charge to the church. As a result, no insulation was needed until 1975 when the steam plant was demolished. The education wing of the facility was

company employed about 500 men and the cut virgin timber was placed in a storage yard that was more than two miles long with logs stacked 40' high. The quality of Meadow River Lumber's oak flooring was famous, and its Sewell Mountain oak adorned the Waldorf Astoria Hotel ballroom in New York City. A ladies' shoe heel plant opened in 1932 and produced four-to-six million shoe heels per year, making it the largest producer of women's shoe heels in the world. Heels that did not meet company standards were provided to workers for cook stove fuel. The company also turned out



completed in 1930 using "wormy" chestnut. The church was forced to add white vinyl siding to the exterior as members could no longer maintain the exposed siding. Because of this, the church is unable to be listed as an historic building.

At the onset of the Great Depression in 1929, ever optimistic John Raine said, *"The time of the temporary lumber camp has passed. The modern town with all advantages is ushered in; a permanent town, built to carry on.*"

The Meadow River Lumber Company did usher in a permanent town, carrying on even now. Though the

chestnut coffins, white ash frames for Packard cars and maple for Ford car bodies.

Construction of houses began in 1908; they were quite advanced for their time, complete with indoor plumbing, bathrooms and electricity. Each house had its own lawn and plot for a garden. The lumber company also provided pasture fields for those employees who had livestock. In 1913, the town officially became known as Rainelle, after the Raine brothers. An 11-room schoolhouse was built in 1913 and the Raine brothers supplemented the salary of the teachers. They also built a bank, company store and a theater. The Raine brothers offered to build one church for the town, allowing the lumber company employees to choose the denomination. The church was dedicated on June 28, 1914 mill was demolished in 1975, the Rainelle United Methodist Church that has suffered through both fire and flood, continues to serve the community and remind folks of the town's legacy – as the home of Meadow River Lumber Company and as home to the world's largest pre-blight American chestnut building.

Information was provided by J.W. Puckett and Andrea Pendleton (former Mayor of Rainelle), both members of the Rainelle United Methodist Church, Joan Browning (Rainelle historian) and Ben Crookshanks of BlueRidgeCountry.com.

RSC COLUMN

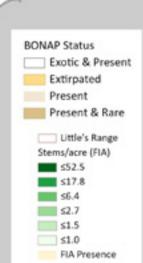
PART 2 OF A 2-PART SERIES

Natural Range

OF THE AMERICAN CHESTNUT

By Sara Fitzsimmons, TACF Director of Restoration

Figure 1: Density of American chestnut stems calculated from Forest Inventory Analysis (FIA) data overlain upon presence/ absence data of American chestnut stems from BONAP (Biota of North America Program) dataset. Map by John Scrivani, December 2019.



The mission of The American Chestnut Foundation (TACF) "is to return the iconic American chestnut to its native range." I've spoken with several members regarding their skepticism at the validity of Little's range map, some issues of which he notes in his own writing regarding those maps (Little 1951). Those issues can have significant repercussions, which brings me to the primary reason of embarking on these articles: to where do we restore the American chestnut?



Looking at **Figure 1** on the previous page, if we use only Little's map to strictly delineate that range, there are several areas where American chestnuts are currently or have historically been found which would be excluded from consideration. For the most part, these eliminated areas are on the outskirts of the range.

The edges and extremes of species' ranges are notoriously difficult to document. One of TACF's members, Roger Willby, splits his time between Maine and Georgia. To the south, Little's map omits potentially important locations for restoration such as the greater Atlanta metropolitan area. Up north, it eliminates central Maine, where several known populations of American chestnut are thriving.

Brian Smith of the VA-TACF Chapter has sleuthed many herbaria of Virginia, West Virginia, and surrounding states looking for historical records of American chestnut in the tidewaters of Virginia. He first found incidences of these trees at the herbarium of his alma mater, Longwood University, which showed instances of American chestnut not covered by Little's range map (Cope 2015).

Looking again at Little's outline in **Figure 1**, there are dozens of disjunct pockets of American chestnut populations through the southeastern U.S. These pockets most likely arise due to the species being largely eliminated from the region by *Phytophthora cinnamomi* (Crandall, Gravatt, and Ryan 1945; Russell 1987), also known as ink rot disease or Phytophthora root rot (PRR). While Little's outline has these pockets, the extrapolated Forest Inventory and Analysis (FIA) densities show low but consistent populations across the area. PRR still exists in large swaths of the southeastern U.S. and will hinder establishment of restoration populations.

Moving westward, there are large regions within the Little boundary, but in which FIA data show no occurrence. For close to 30 years, the IN-TACF Chapter has looked far and wide for American chestnut stems throughout southern Indiana, but few to none have been found. They certainly were there, as documented by Weir (1916). American chestnut reached its prominence in 15 counties of southeastern Indiana, and was present in another seven.

Today in Indiana, most American chestnut populations are found in the north and those southern populations have all but disappeared. Weir (1916) suggests northern incidences of American chestnut were planted, but founding IN-TACF Chapter president Bruce Wakeland suggests otherwise. Wakeland was northern Indiana's first consulting forester, and sees how well American chestnuts grow in northern Indiana (Wakeland 2019). He hypothesizes that the vast Kankakee Marsh kept surveyors from finding pockets of American chestnut further north along the Valparaiso Moraine, home to several known stands of the species today.

Some of the largest trees studied by TACF and other researchers were planted in Michigan and at the West Salem stand (**Figure 2**) in southwestern Wisconsin. The largest American chestnut trees in the United States are in the west coast (Gillis 2017), far from what would likely be considered the native range of this species and known to be planted. What role, if any, would these locations play in restoration?

That brings us to a big, almost philosophical question: What is a native range, anyway? Most literature limits a native range or species to geographic ranges where it was self-established, i.e. not planted. The influence of humans on distribution of American chestnut, however, cannot be denied. Russell (1987) and Ruffner (1999) suggest that trade among Native American populations contributed to the species' spread. Most reviews of American chestnut distribution note the influence of European settlement on the species' expansion. The American chestnut was not always as widely or densely growing as it was just before the chestnut blight was introduced. Faison and Foster (2014) document how chestnut was not extensively distributed in pre-colonial times. From a study of witness tree and other documentation, 19th Century logging is likely the primary reason American chestnut reached its dominance where it did.

This brings us to another limitation of using historic or even current documentation of species to delineate the geography for restoration: the effects of climate change. Many short-lived vegetative species have already increased their northward ranges, and it is likely that American chestnut would find suitable habitat in novel locations such as the Adirondack Mountains or the Upper Peninsula of Michigan as the climate warms.

To account for those changes, a habitat suitability index can help prioritize sites for reintroduction and restoration. Through a review of 26 historical documents covering the site conditions of American chestnut literature, Irwin et al (2010) created an index of site metrics which were used to grade the suitability of

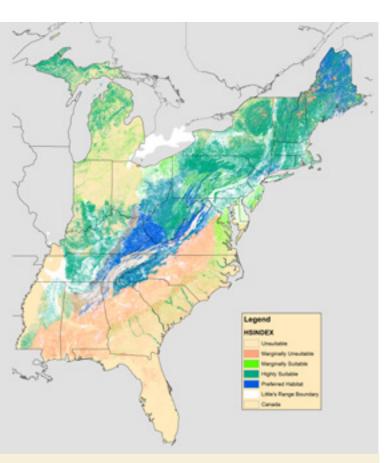


Figure 3: First approximation for suitable habitat of the American chestnut using documented preferred site parameters merged with Geographic Analysis Program (GAP) ecosystems.

Geographic Analysis Program (GAP) ecosystems in the eastern U.S., delineated through extensive geographic parameters as outlined through the U.S. Geological Survey (USGS). Based on that application of an index, there are an estimated 131 million acres of highly suitable habitat available for planting American chestnuts (**Figure 3**).

Range maps like those produced by Elbert Little are a great starting place for analysis. Little's series of maps are available for virtually every endemic species to the U.S. allowing for modelling across them all. For the purposes of American chestnut restoration, however, the Little map should not and will not be used to strictly delineate where restoration will occur.

Priority areas should be those where habitat is suitable, within or near to historically known locations of American chestnuts, and those which have knowledgeable collaborators willing to install and maintain a restoration planting. When it comes to restoration, looking back at these historic maps is the first step. To complete the analysis, we can hybridize our current knowledge, bringing in land-use history, habitat suitability, and estimates of density. As climate shifts, land use and ownership patterns change, and biotic influences transform ecological interactions, the geographic boundaries for American chestnut reintroduction and restoration will shift. For that reason, TACF and its partners should regularly evaluate and update its plans for American chestnut restoration.

ACKNOWLEDGEMENT:

For much of this article, I have referred you to Figure 1, a map created by VA-TACF Chapter member John Scrivani. He produced this map as part of an article he published with Dalgleish et al (2014) documented the current status of American chestnut in the wild. I owe many thanks to him for making his map available for reprinting and in this article.

LITERATURE CITED

Cope B. 2015. Buried Treasure. Longwood Magazine. http://magazine.longwood. edu/article/buried-treasure/

Crandall BS, Gravatt GF, and MM Ryan. 1945. Root disease of Castanea species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. Phytopathology. 35: 162-180.

Dalgleish HJ, Nelson CD, Scrivani JA, and DF Jacobs. 2016. Consequences of Shifts in Abundance and Distribution of American Chestnut for Restoration of a Foundation Forest Tree. Forests 2016, 7(1), 4. https://doi.org/10.3390/f7010004 Faison EK and DR Foster. 2014. Did American chestnut really dominate the eastern forest? Arnoldia 72:18-32.

Gillis D. Winter 2017. American chestnut trees in the Pacific Northwest. Chestnut 31(1): 3-5.

Irwin H, Fitzsimmons S, and CC Pinchot. 2010. The Identification of Priority Forest Sites for Chestnut Restoration – First Approximation. 21st Annual Southern Appalachian Man and the Biosphere (SAMAB) Conference, Gatlinburg, TN, Nov 16 – 18. 2010. Presented by Hugh Irwin.

Iverson L and W Hargrove. February 2014. Tree Habitat Shifts - Species Distribution Models. U.S. Department of Agriculture, Forest Service, Climate Change Resource Center. www.fs.usda.gov/ccrc/topics/species-distribution-models

Little EL. 1951. Mapping Ranges of the Trees of the United States. Rhodora. 53(632): 195-203.

Ruffner, C. M. 1999. Anthropogenic and physiographic influences on the composition and structure of pre-European settlement forests of Pennsylvania. Dissertation. Pennsylvania State University, University Park, Pennsylvania, USA. Russell EWB. 1987. Pre-blight distribution of Castanea dentata (Marsh.) Borkh. Bulletin. Torrey Botanical Club 114: 183–190.

Wakeland B. Fall 2019. Surviving American chestnut trees in Roselawn, Indiana. Chestnut. 33(3): 5-6.

Weir JR. 1916. Pathological observations on the chestnut in southern Indiana. Indiana State Forestry Board Annual Report. (15): 140-163. THE SCIENCE

PART 1 OF A 3-PART SERIES

Safety Tests

ON TRANSGENIC AMERICAN CHESTNUT

By Andy Newhouse, SUNY's College of Environmental Science and Forestry

As part of TACF's 3BUR strategy, transgenic blight-tolerant American chestnuts are being produced and tested for potential use in forest restoration. These trees contain a gene called oxalate oxidase, or OxO, which breaks down toxic oxalic acid produced by the blight fungus. This article (and two more in subsequent issues) will highlight some of the many safety tests we've performed on OxO-containing transgenic American chestnuts.

This first installment describes tests for safety to people, in terms of chestnuts as a food product. While careful safety evaluations are prudent for any restoration efforts, results from these tests will be particularly informative to federal regulatory agencies, who will need to approve transgenic trees before they can be distributed or used for restoration. The primary question (for us as scientists and for the regulators) is whether there are substantial differences between the transgenic chestnut and a similar non-transgenic chestnut produced through traditional breeding.

One way we can evaluate an unfamiliar food product is by looking at the Nutrition Facts label, which essentially describes its composition. This generally tells us how nutritious it is, and lets us compare two food products to see how similar they are.

In order to create a Nutrition Facts label, most food manufacturers send samples to independent commercial testing labs, so that is exactly what we did with chestnuts. We sent samples of both transgenic and non-transgenic



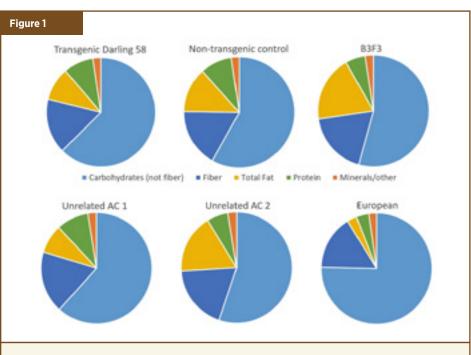
chestnuts, along with various unrelated non-transgenic samples, repeated over three growing seasons. The results (summarized in **Figure 1**) were not surprising to anyone familiar with growing chestnuts: the biggest variances were between different species of chestnuts (e.g. American and European), and then between unrelated American chestnuts harvested from different sites There were small differences between transgenic and non-transgenic nuts, but they were not consistent across multiple seasons, and they were minor compared to the species and site differences.

A more detailed look at one aspect of chestnut nutrition involves a "fatty acid analysis." This means identifying the specific types of fats that make up the total fat in a product, which can affect flavor and texture in addition to nutrition. Some fats are considered healthy (generally unsaturated fats), while others (generally saturated) are less healthy if consumed in large quantities. Our analyses (Figure 2) showed that most of the

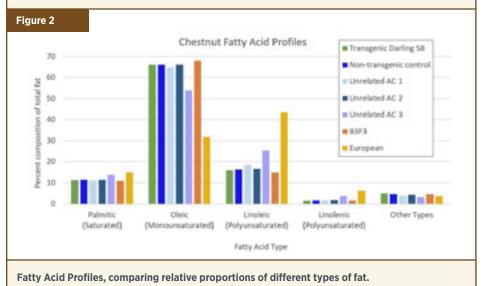
fat in American chestnuts is a monounsaturated type called Oleic acid, followed by the polyunsaturated Linoleic acid. (Interestingly, these relative proportions of Oleic and Linoleic acids are reversed in European chestnuts, which might explain some of the flavor differences between these species.) About 10-15% of fat in chestnuts is saturated Palmitic acid, followed by a few other types totaling less than 10%. Most American chestnut types and a B₃F₃ sample were quite similar to each other, but there were differences between chestnut species (namely the European as mentioned above), and one unrelated American chestnut type (AC 3 in figure 2), whose fatty acid ratios looked more like the European. In contrast to species differences, transgenic and non-transgenic samples were nearly identical, confirming that presence of the transgene does not affect the types of fats found in chestnuts.

Testing for potential allergenicity or toxicity is more complicated than direct nutritional analyses, but one relatively easy way to evaluate a new product or ingredient is to compare it to foods that are already consumed and considered safe. For example, rice is one of the most common foods in the world. Other grains such as amaranth and sorphum are commonly used in health-conscious aluten-free baked goods, while oats and corn are consumed daily by millions of people and not considered dangerous. Spinach, tomatoes, strawberries, and tea leaves are also considered part of a healthy diet. All of these foods contain OxO, the same gene inserted into blight-tolerant transgenic American chestnut trees. Since OxO is not a concern for people who eat these gluten-free, non-allergenic, non-toxic foods, there is no reason to assume it would be a concern when expressed in chestnuts. In order to further verify the safety of OxO, we used the amino acid sequence (the precise order of protein building blocks that make up the OxO molecule) to screen several databases of all known allergens and toxins. Even when we used very conservative thresholds (so any remotely similar matches would be found), there were no matches between OxO and any known allergens or toxins. Results were the same when searching databases of known gluten proteins. It is worth noting that people can be allergic to pollen or nuts from various trees including chestnuts, but the important fact while evaluating transgenic trees is that the presence of transgenes doesn't change or enhance these risks.

In contrast to the lack of matches between OxO and known toxins, oxalic acid (the same tree toxin produced by the blight fungus) is known to be toxic to people, and it showed clear matches in the toxin databases. Oxalic



Percent composition of macronutrients in dried chestnut samples.



acid can be acutely poisonous in high concentrations, and even at low doses it can contribute to problems like kidney stones. Some researchers have actually been testing OxO for use as a medicine to treat oxalic acid poisoning: early tests show promise for successful treatment, with no noted side effects, even when OxO is supplied at very high concentrations. This provides further evidence that OxO will not be harmful to people at the relatively smaller concentrations found in transgenic chestnuts.

Each of these tests confirm that transgenic chestnuts do not present any enhanced safety risks to people. In conjunction with more than 100 years of research on OxO, which also has not shown any risks to people despite being found in many common foods, we are confident that transgenic chestnuts will be just as safe and nutritious as the chestnuts people have been eating and enjoying for centuries.

Seed Stratification

In the previous two issues of *Chestnut* we discussed the storage of chestnut seeds, also called stratification, in terms of temperature, duration, and provenance of the seeds. Another important aspect of seed stratification is the media you use to store the seeds in and surface sterilization of seeds to prevent mold. In this issue, I present the results of a study investigating differences between different stratification media and surface sterilization treatments.

DO STORAGE MEDIA AND SURFACE STERILIZATION PLAY A PART IN MOLD REDUCTION AND SEED VIABILITY?

By Laura Barth, Former TACF Meadowview Horticulture and Pathology Specialist

In order to maintain seed viability, it is crucial to maintain constant moisture conditions. Seeds need to be kept at an adequate humidity in order to germinate. If the storage conditions are too dry, then the seeds will desiccate. If the media is too moist, then the seeds may mold. A variety of potting media are commonly used to maintain adequate moisture for seed stratification (Figure 1). Common media include peat moss, coconut coir (fibers from coconut husks), sphagnum moss, vermiculite (hydrated phyllosilicate mineral expanded by heat treatment), or perlite (volcanic glass expanded by heat treatment). In my work with TACF, I have primarily seen moistened peat moss used as a storage media, as it is thought that the low pH of peat prevents mold during stratification. However, some growers I've worked with have shipped seeds to me in sphagnum moss, or no media at all, with high viability after planting. In my studies at North Carolina State University we usually used moistened perlite as a stratification media. Perlite is often chosen because it is an inorganic material, which is also thought to prohibit the spread of mold during stratification. Another mold-prevention technique is surface sterilizing seeds before they are placed in stratification media by soaking them in bleach, hydrogen peroxide, or several more expensive, commercial treatments.

I conducted a research study at Meadowview Research Farms to investigate differences in stratification media and surface sterilization solutions. My objective was to determine if fewer seeds were lost to mold with different media and sterilization treatments.

METHODS

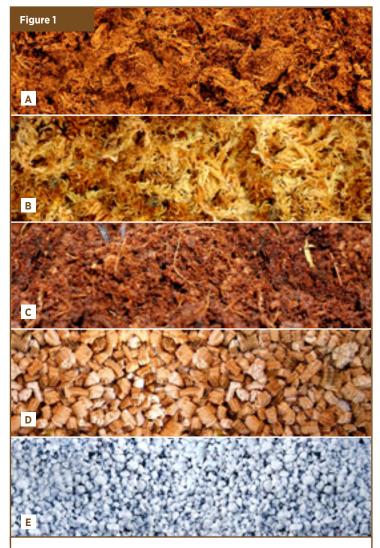
American chestnut BC₃F₃ seeds from Indiana were tested for viability with the "float test" and air dried. The viable seeds were separated into 25 groups of 17 seeds corresponding to the different treatments (5 media x 5 sterilization treatments). Five media were used: peat moss, sphagnum moss, coir, coarse vermiculite, and coarse perlite. All media were moistened until they were saturated but no water drained out when squeezed. The media were placed in plastic resealable bags and laid flat overnight to allow the moisture to evenly distribute. Five different sterilization techniques were used: no sterilization (seeds dipped in water as a control), 20% bleach solution, 1:1 3% hydrogen peroxide/water solution, SaniDate 5.0 (BioSafe Systems; hydrogen peroxide, peroxyacetic acid and proprietary stabilizers and buffers), and ZeroTol 2.0 (BioSafe Systems; hydrogen peroxide, peroxyacetic acid and proprietary stabilizers and buffers). Seeds were soaked for two minutes.

After sterilization, the seeds were placed into one liter of media in a perforated 5"x 7" polyethylene bag and placed in cooler at 40°F for 140 days. The stratified seeds were planted into Oldcastle C/B mix in D40 pots on March 28, 2019. We measured the number of moldy seeds per treatment, percentage of seeds that developed radicles, percent shoot emergence, days to shoot emergence, and tree height after three months.

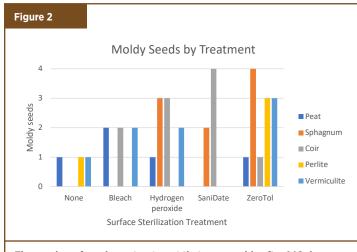
RESULTS AND DISCUSSION

Incidences of mold were low for all treatments with 0 to 4 of 17 total seeds molding per sterilization method and media combination (**Figure 2**). Surface sterilization did not reduce the number of seeds lost to mold when compared with no sterilization. The no sterilization treatment resulted in the lowest number molded seeds as compared to all other sterilization methods. No media/surface sterilization combination was clearly better than the others in terms of number percent emergence after planting (**Figure 3**). Many seeds dried out were and not viable when stored without media due to low numbers (17 seed) within each bag (data not shown).

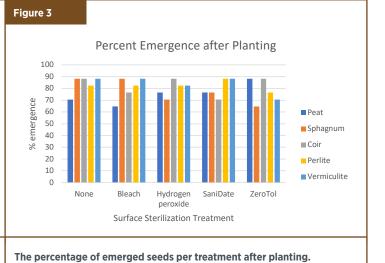
The results presented here show that the choice of stratification media boils down to cost, availability, and preference. Harvest of peat moss can damage peat bog ecosystems, which has led many horticulturists to prefer alternative media, such as coir or wood fiber. Perlite, while easily obtainable and inexpensive, can be an irritant when the dust is breathed in or it gets in the eyes. Sphagnum moss most effectively held moisture, but was by far the most expensive media. When storing seeds without media it is important to fill the bag so that the seeds themselves keep each other moist. However, if the seeds are too moist, seeds can become moldy. While this study did not find that surface sterilization prevented mold, it is a horticultural best practice for stratifying many kinds of seeds, and when shipping seeds to states with quarantine laws, surface sterilization is a requirement.



Different media used for seed stratification: peat moss (A), sphagnum moss (B), coconut coir (C), vermiculite (D), and perlite (E). Images not to scale.



The number of seeds per treatment that were moldy after 140 days stratification at 40 $^{\circ}\text{F}.$



Chestnut Chocolate Torte

from The Fresh Market

Here at TACF, we love to test chestnut recipes. Betsy Gamber, vice-president of operations, made this delicious torte and it was a winner! If you are looking for a simple (no baking involved) and delicious chocolate dessert with a bit of chili spice for that extra kick, give this one a try. We feel sure it will become one of your "go-to" recipes. TACF staff gave this one a thumbs up!



Ingredients

Torte

3½ oz bittersweet chocolate
7 tbsp butter
14 oz bag of shelled roasted chestnuts (Geffen brand recommended)
1 c powdered sugar
1 tsp chili Paste (Zócalo Aji Panca brand recommended)
½ tsp cinnamon
1 tsp vanilla
2 tbsp spiced rum

Topping

3½ oz bittersweet chocolate
2 tbsp butter
½ tsp chili paste
¼ tsp cinnamon
Powdered sugar for serving
Whipped cream for serving

Method

For the torte: In a double boiler on the stove, or in the microwave, melt the chocolate and butter. Meanwhile, in a food processor, blend the chestnuts and powdered sugar together until creamy and smooth. Set aside.

Once the chocolate and butter are melted, remove from the heat and pour into the food processor with the chestnut mixture. Then add chili paste, cinnamon, vanilla and rum. Blend again until smooth.

Line a loaf pan with plastic wrap on all sides, scrape the mixture into the pan and even with a spatula. Cover the pan with more layer of plastic wrap and refrigerate until set, at least 8-12 hours.

For the topping: In a small saucepan, melt the chocolate with the butter until melted. Add the remaining chili paste and cinnamon and stir until blended. Remove from heat.

Turn the torte upside down on a plate or cutting board and remove the plastic wrap. Pour the melted chocolate mixture on top and cool until it is completely set. Dust with powdered sugar, then cut into slices and serve with whipped cream or your choice of topping.

Remembering Dennis Fulbright, Ann Leffel, William "Bill" Lord

The American Chestnut Foundation recently lost three long-time supporters who were instrumental in advancing TACF's mission toward American chestnut restoration. We will greatly miss them and their contributions.



DENNIS FULBRIGHT

The chestnut community recently lost a long-time enthusiast, Dennis W. Fulbright. A California native, he became a Michigan resident when he joined the MSU faculty. His research interest was sparked by the many chestnut groves that dotted the state. Dennis, along with undergraduate, graduate and research assistants, unraveled many mysteries that accounted for the unique recovery of chestnut from blight in Michigan.

His enthusiasm for the species did not stop with science. Dennis was instrumental in organizing the Michigan Chestnut Grower's Cooperative – an initiative that provided leadership among the small and large growers. His enthusiasm was contagious and resulted in a significant chestnut grower community in Michigan.

Dennis will be remembered as a humble, gentle man with a warm and likable personality. Most of all he was a devoted family man. He is survived by his wife Jane and children Kimberly and Scott and their families. He will be sorely missed but long remembered.





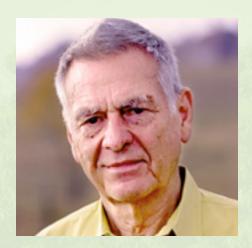
ANN LEFFEL

Ruth Ann Green Leffel was a leader for the creation and growth of the grassroots, chapter network of TACF. Along with her husband, Robert C. "Bob" Leffel, Ann helped form the PA-TACF Chapter, and was the founding president for the chapter.

Born in Dundalk, MD, Ann was a math teacher for many years before she and Bob retired to Brogue, PA. She would continue this role into retirement, mentoring dozens of chestnut enthusiasts across TACF. With slide projector under-arm, Ann and Bob traversed hundreds of miles across the mid-Atlantic, sharing the story of the life, death, and rebirth of the American chestnut, and inspiring people to join the mission to restore this iconic species. Those who had the privilege of working with Ann knew her for her dedication, grace, patience and especially for her sense of humor - all important attributes that made her an effective and charismatic leader.

Ann received Volunteer Service Awards from TACF in both 2003 and 2007. Although Ann effectively retired from her work on American chestnut restoration in 2005, her legacy continues in the Leffel Chestnut Research Center at Penn State, and in the regionalized blight-resistant American chestnut selections found across Pennsylvania.

Ann is survived by her husband, Bob, daughter Meg, son Bob and his family.



WILLIAM "BILL" LORD

William "Bill" Lord was the consummate cheerleader for all things chestnut, often declaring that The American Chestnut Foundation (TACF) was his favorite charity as he fondly remembered chestnuts on the family farm in Ontario. Not only was he a generous donor, he also took on the monumental task of writing the history of TACF from its 1983 founding until 2014 and was given a special recognition award by TACF in 2013 for that work. He served on TACF's board of directors and was a board member emeritus as well.

A proud US Army Veteran, Bill was an Infantry Scout during WWII and in the Battle of the Bulge. He was a graduate of Michigan State University, The University of Georgia College of Veterinary Medicine and the University of Pittsburgh Graduate School of Public Health.

Bill was a naturalist on the Blue Ridge Parkway and the author of the *Blue Ridge Parkway Guide* and he donated royalties from this publication to TACF. He practiced veterinary medicine for over forty years as the public health veterinarian for the Allegheny County Health Department, as the veterinarian for the Animal Rescue League and in his private practice, Plum Animal Clinic.

Bill is survived by his wife Faye (Ferris) Lord, his sons William Scott Lord and James Lord, his daughter Lezlee Holt, and their families.

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We regret any errors or omissions and hope you will bring them to our attention.

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