FALL 2018 ~ ISSUE 3 VOL. 32

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

TACF Historical Timeline ~ Chestnut Experience in Italy ~ Harvesting Chestnuts ~ Ozark Chinquapin Mycorrhizae

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THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

A BENEFIT TO MEMBERS

THE AMERICAN CHESTNUT FOUNDATION®

Lisa Thomson

President and CEO

DEAR CHESTNUT ENTHUSIASTS,

One of the things that continues to amaze me at TACF are the stories and history behind all the scientific effort. Yes, we are a science-based organization, and always will be. But when I ask long-time volunteers what keeps them involved and working so hard, the answer is often, "the people!"

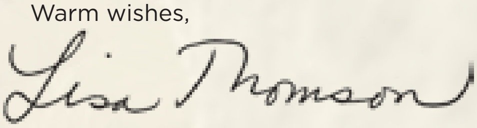
In my nearly four years of having the honor of leading TACF, I have the privilege of meeting the most interesting, committed people. Along with our amazing volunteers, including our highly engaged board of directors, we are honored to work with academic researchers, government officials, educators, and of course, our members and donors. Our constituents know this ambitious experiment is something bigger than they are, and have faith and hope it will come to fruition, even if it is not complete until after their lifetimes. Yet, their confidence and optimism has not wavered. I know we have our skeptics, but they are rare; almost everyone I meet share this confidence in mission success.

At our spring board meeting, we were treated to a story-telling workshop by Scott Mann, a retired LTC Green Beret who travels the country and is in high demand as a storyteller and strategic coach. He is the son of TACF's long-time volunteer leader Rex Mann, so he is a terrific cheerleader for our goals. Rex opened the workshop by sharing his emotional "chestnut story" which was a showstopper. So adept at his storytelling, Rex is now scheduled to be on the national stage at a TEDx event on September 22nd in Youngstown, Ohio which many of us plan to attend. Meanwhile, Scott encouraged us to develop our own personal chestnut story and not be shy about sharing it!

For those of you who may not know him, one of our volunteer leaders is an artist, published author, family business-owner and avid traveler. Michael Doochin has been an outstanding board chair, and has been my sage advisor and mentor as we faced the many changes and growth that have occurred during his three-year tenure. Although he has a full-time business to run, he always made time for TACF and helped institute a higher level of communication and professional rigor to an already strong non-profit, to which I will always be grateful. He will continue as an officer in the Immediate Past Chair position, a newly created seat on the Executive Committee, to ensure seamless transition to our new chair, Dr. Brian McCarthy.

We will also be honoring other invaluable members of our far-reaching constituency, each of whom have their own chestnut story, at Huntsville during our 35th anniversary celebration, October 26-27. Please come and feel the infectious positivity that is our collective TACF family. I look forward to seeing all of you there and hearing *your* chestnut story!

Warm wishes,



Lisa Thomson, President and CEO
The American Chestnut Foundation

Follow me on Twitter (@MadameChestnut).

Still Growing Strong

This GA native American chestnut that was crossed with a tree being tested for *Phytophthora cinnamomi*, was planted in 2008 at Carolyn Hill's demo orchard in Calhoun, GA. Carolyn took this photo in 2014 and says the tree is "still growing strong!"

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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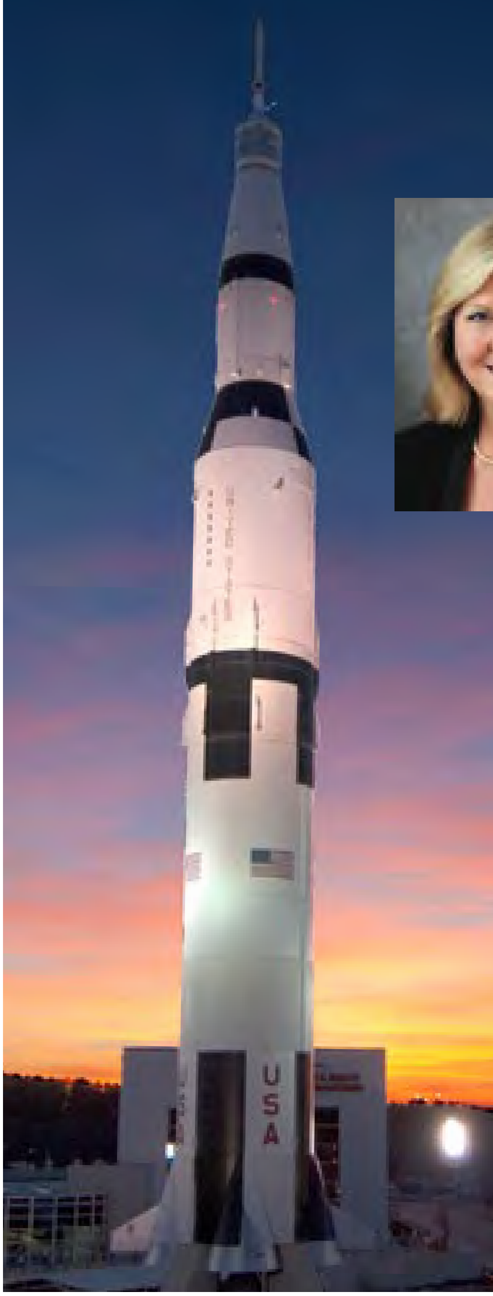
(434) 906-9312

35TH ANNIVERSARY CELEBRATION AND ANNUAL MEETING

Join us in Rocket City

as we celebrate 35 years of unwavering commitment to create a future of forests filled with American chestnut

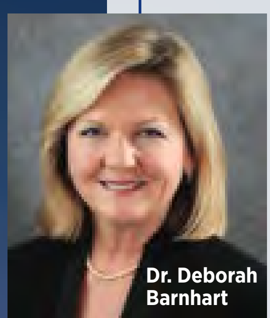
The American Chestnut Foundation® 35 years Restoring the Tree and its Legacy



EMBASSY SUITES HUNTSVILLE HOTEL & SPA
Huntsville, AL
October 26-27, 2018

Hotel Reservation Deadline: Monday, October 1 at 5:00PM
Event Registration Ends: Friday, October 12 at 5:00PM
Registrations after October 12 must come through the National office at (828) 281-0047

TACF's 35th Anniversary Celebration and Annual Meeting will take place in Huntsville, AL, known for its rich aerospace and military technology history.


Dr. Deborah Barnhart

We are very excited and honored to have as our special keynote speaker, **Dr. Deborah Barnhart**, Chief Executive Officer and Executive Director of the U.S. Space and Rocket Center in Huntsville.

Dr. Barnhart will speak about "Inspiring the Mars Generation" at our Friday night Volunteer Service Awards Dinner. Among Dr. Barnhart's many honors, she is a recipient of NASA's Distinguished Public Service Medal, the Honeywell Hometown Heroes Award, and in 2016 was selected as one of the 40 women who are shaping the state of Alabama by AL.com.

Saturday's schedule of events includes speakers who offer a wide range of presentations during the Open General Session and Educational Sessions. Additionally, a lively panel discussion, "What you Always Wanted to Know About... (but were afraid to ask)" will invite participants to ask our panel of scientists those very questions!

On Saturday evening, we will host our 35th Anniversary Gala Dinner at the Von Braun Center. WV Chapter president Mark Double will share his unique perspective of TACF's rich 35-year history. Our first ever Chestnut Conservation Champion Award will also be presented, and the winning raffle ticket for the American chestnut dulcimer will be drawn.

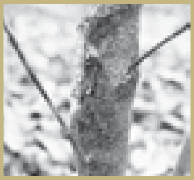
TACF's special guest rate at the Embassy Suites is $119.00 per night (plus tax/fees) for all event guests. This rate includes daily breakfast and the manager's special social hour. Visit the reservation link on our website's event registration page or call 1-800-362-2779 using group code ACF.

For hotel reservations, event details and registration, and to purchase raffle tickets, visit www.acf.org.

Hope to see you in Huntsville!

The American Chestnut Foundation

1904



Chestnut blight first noticed in Bronx Zoological Park in New York City

1905

The blight fungus identified by William Murrill at the New York Botanical Garden in New York City

1911

Pennsylvania Chestnut Tree Blight Commission undertakes efforts to control spread of the blight (efforts discountinued in 1913)

1913

Plant explorer Frank N. Meyer identifies chestnut blight growing on chestnut native to China

1922

USDA breeding program, begun by Walter van Fleet in 1911, reactivated under G. Fillipo Gravat (program abandoned in 1960)

1930

Arthur Graves undertakes a chestnut breeding project under the auspices of the Brooklyn Botanical Gardens

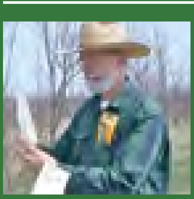
1947

Graves transfers the chestnut breeding project, placing it under the auspices of the Connecticut Agricultural Experiment Station

1980

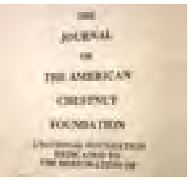
Charles R. Burnham becomes interested in breeding a blight-resistant American chestnut

1983



The American Chestnut Foundation established and Philip Rutter is appointed first President and CEO

1985



Creation of *The Journal of The American Chestnut Foundation*, the first TACF publication

1989

TACF establishes the Wagner Research Farm, a breeding station in Meadowview, VA, and hires Dr. Fred Hebard to manage the breeding program

1990

TACF headquarters established in Bennington, VT

1990

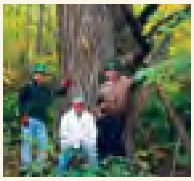
John Herrington hired as President and CEO

1990

ESF

State University of New York, College of Environmental Science and Forestry (SUNY-ESF) begins transgenic research

1991



Researchers begin a biological control study with hypoviruses at the American chestnut stand in West Salem, WI

1995

TACF's Pennsylvania Chapter initiates the first successful chapter breeding program

1997

Marshal Case hired as President and CEO

1998

Dedication of the 93-acre Glenn C. Price Research Farm in Meadowview, VA

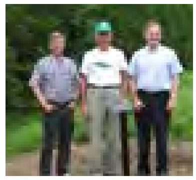
2004

Dr. Joe James leads new breeding efforts launched in Seneca, SC to combat *Phytophthora* root rot

2005

The first potentially blight-resistant chestnuts harvested from Glenn C. Price Research Farm

2006



First transgenic American chestnut tree was planted in Syracuse, NY

2009

Bryan Burhans hired as President and CEO

2009

In partnership with the US Forest Service, US Forest Service Southern Research Station, and the University of Tennessee, the first TACF hybrids are planted in real forest environments

2009

TACF National office moves from Bennington, VT to Asheville, NC

2010

The Journal of The American Chestnut Foundation overhauled to a full-color magazine

2010

Dedication of the Glenn C. Price Laboratory at Meadowview

2011

SUNY-ESF plants potentially blight-resistant transgenic American chestnuts at the New York Botanical Garden

2012

TACF's 16 chapters recorded a total planting of 450+ breeding and test orchards, including the first seed orchards in five states

2013

TACF celebrates 30 years of restoring the American chestnut

2014

TACF National office moves to new location in Asheville, NC

2014

Dr. Jared Westbrook hired as Director of Science

2015

Lisa Thomson hired as President and CEO

2015

TACF's magazine *The Journal* is renamed *Chestnut* and won it's first Printing Industry of the Carolinas (PICA) award for "Best of" category in Environmental Certification

State Chapters Established

1990 The New York Chapter established as TACF's first chapter

1991 Connecticut Chapter established

1994 Pennsylvania Chapter established

1996 Indiana Chapter established

1999 Maine Chapter established

2000 Carolinas Chapter Established

2001 Kentucky, Massachusetts/Rhode Island, and Tennessee Chapters established

2003 Maryland Chapter established

2005 Alabama and Georgia Chapters established

2006 Ohio and Virginia Chapters established

2007 Vermont/New Hampshire Chapter established

2010 West Virginia Chapter established

Board Chairs

1983 - 1992	Philip Rutter
1993 - 1994	Paul Read
1995 - 1998	L.L. Coulter
1999 - 2001	James Ulring
2002 - 2006	Herb Darling
2007 - 2009	Richard S. Will
2010 - 2012	Glen Rea
2013 - 2015	Kim Steiner
2016 - present	Michael Doochin

2016

3BUR Proposal for Integrated Research approved by TACF Science and Oversight Committee

2016

Meadowview Operations Building Established

2017

2017 - 2027 Ten year Strategic Plan adopted by the Board of Directors

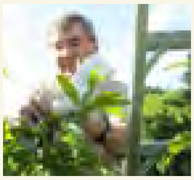
2018

TACF Breeding Plan Revised and Implemented

2018

Meadowview greenhouse and shade houses established and dedicated to Board Emeritus Richard S. Will

2018



Blight-tolerant transgenic tree developed by SUNY-ESF and submitted for regulatory review

2018

TACF's third external Science Review held in Abingdon, VA that focuses on the breeding program

2018

TACF celebrates 35 years of restoring the American chestnut

TACF WELCOMES

Laura Barth

HORTICULTURE AND PATHOLOGY SPECIALIST

HELLO CHESTNUT AFICIONADOS!

I'm very excited to be joining the team as TACF's Horticulture and Pathology Specialist at Meadowview Research Farms in VA. I hail from Eau Claire, WI, where I received a degree in music performance (flute) from the University of Wisconsin Eau Claire. In 2007, I moved to North Carolina and eventually returned to school for horticulture at North Carolina State University (NCSU), earning my BS and MS degrees. During my time at NCSU I worked in several labs: the Gould Entomology Lab, the Plant Disease and Insect Clinic, the Horticultural Substrates Lab, and the Mountain Crop Improvement Lab, where I worked on breeding ornamental nursery crops. Over the past year I've been at Virginia Tech, teaching, taking classes, and working on problems related to nursery production.

I was drawn to this position because it's incredibly exciting to be part of such a large-scale project, that involves so many passionate people who devote their time and resources toward American chestnut restoration. I have already been blown away by the dedicated volunteers who turned out at Meadowview for our spring seedling inoculations in the new greenhouse facilities.

Several of my classes at NCSU discussed TACF's work in detail, and my classmates and I were intrigued and hopeful about the research and its implications. It's thrilling to finally see American chestnut hybrid trees in person, and to now be a part of the research. I love growing plants in greenhouse and nursery settings, designing and conducting scientific studies, working outside, and being in the mountains. I feel this position will offer me the best of both worlds – I get to come in every day and do work that is enjoyable in a beautiful environment, but work that is also incredibly important, impactful, and interesting.

Outside of work and science, my interests and hobbies include music (singing, flute, and guitar), reading, hiking, doing things with my dogs, traveling, and art (drawing, watercolor, and photography). I've had several photographs accepted into juried art exhibitions and competitions and am looking forward to familiarizing myself with the art and music scene in Abingdon and the surrounding areas.

I'm grateful for this new opportunity and look forward to playing a part in the restoration of the American chestnut tree!

From above, catkins in the crown of an American chestnut are very distinct, and could be used to identify unknown survivors in the wild. Photo by ParaTrees

Drones

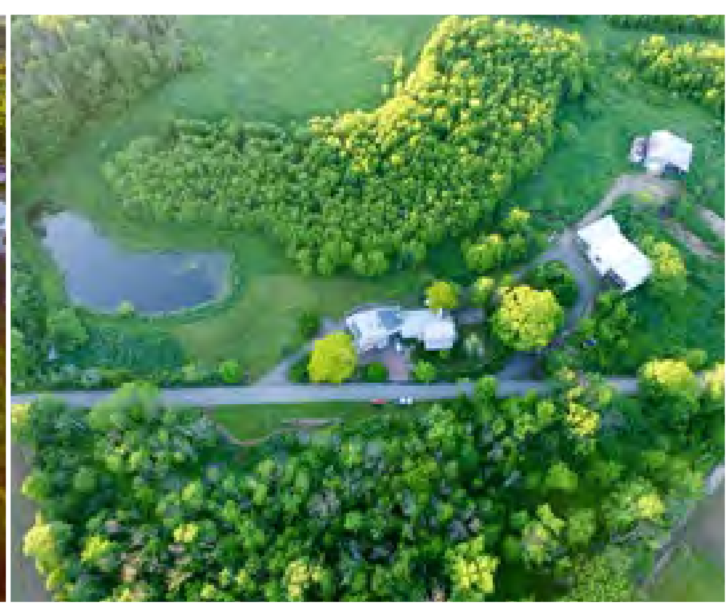
A RESOURCE TOOL FOR AMERICAN CHESTNUTS?

By Clark Beebe, PA/NJ Chapter

We've all seen and heard about drones. Maybe your child or grandchild got one for Christmas or you've seen aerial photos taken by a drone. But the question is, what are their real capabilities and can they help us in our quest to restore the American chestnut?

Drones can be used to hunt for flowering chestnuts in forests. They can fly above the treetops sending back live video, allowing real-time searches for flowering trees. They can also be programmed to automatically create high-resolution photo surveys (one inch per pixel) in a grid pattern. Such surveys can create a baseline for later comparisons.

Drones can be used to inspect individual trees. Once a flowering tree is found, the pilot can move in for a closer look. Built-in stabilization and crash avoidance algorithms allow flying between trees to photograph individual leaves and catkins.



These drone photos of the same property are taken from three different perspectives. Photos by Jeff Street, Medical Drones at Johns Hopkins

It might be possible to develop the capability for drones to help pollinate trees. The down-draft from the rotors could be employed to help distribute the pollen, or a packet of pollen could be lifted to the top of the tree for natural wind pollination. With either approach, its real-time streaming video would help with positioning.

The algorithms mentioned above provide small multi-rotor drones and a high level of automation, making them easy to master. For example, if the operator does not give any input, the aircraft automatically remains stationary in 3D space, resisting wind gusts up to about 20 MPH. When the battery runs low, the drone automatically returns to its launch point. The FAA license required to operate small aircraft (Part 107) is approximately equivalent in complexity and cost to a driver's license. Operational rules require aircraft to remain within the pilot's line of sight (approximately one mile) and avoid airports and flying over people.

Costs vary but a well-equipped drone system with several batteries and multi-battery chargers would run about $3,000. If interested, a good model to purchase would be the DJI Phantom 4.

Now we challenge you to come up with additional uses for drones in forest and orchard applications. Consider other features such as infrared or attachments with servomotors to release payloads. Can drones replace people on ladders for some tasks? Can they be cheaper or faster than current methods? Perspective can change approaches. Consider what the perspective of a drone can do for you and let us know about your ideas!

My Recent Experience with Drones

By John Wenderoth, PA/NJ Chapter

When I met with TACF Board Emeritus, Dick Will several years ago, he spoke about finding surviving American chestnuts by searching from the air. At the time, it seemed too costly to be practical. Since then, I've searched unsuccessfully on foot in some of our local forests for such trees reported by others. In instances when I have found a survivor of decent size and vigor, I haven't been able to confirm how likely it is to flower and potentially produce seeds. These experiences brought Dick's comments back to mind.

He reminded me how chestnuts stand out from the rest of the forest canopy in late spring and early summer. When Clark Beebe said he'd met a new company that was promoting the use of drone technology, I contacted Alec Ratyosyan of ParaTrees to arrange an exploratory flight. PA/NJ Chapter member, Dan O'Keefe, recommended a drone flight over the American chestnut seed orchard at Tyler Arboretum in Media, PA. This would be quite different than looking for a "needle in a haystack."

At the time the drones flew over Tyler's orchard, we had trees that were only beginning to produce male catkins, others just producing anthers, while still others had maturing bisexual catkins. Dan was interested in how this cloud influences open-pollination around the orchard. The images collected help ParaTrees establish a baseline in their software program. As other data is collected, they can analyze it to see whether the signature of a flowering American chestnut tree is present. Automated interpretation of a drone's vision might allow pinpointing the location of surviving American chestnut over forested areas more efficiently in the future.

Photograph from 180 feet over the Tyler Arboretum chestnut orchard. The orchard contains 136 surviving 21-year old trees, grown from American chestnut seeds gathered from various locations in PA. Photo by ParaTrees

PART 4
of a 4-part series
on preserving
American chestnut
genetic diversity in Maine

TACF-Landowner Partnerships:

AN UNFOLDING STORY FROM MAINE

By Thomas Klak, TACF Gene Conservation Committee Chair, Maine Chapter

Located just six miles south of Portland, Maine, in the otherwise suburban community of Cape Elizabeth, Ram Island Farm (RIF) is a 2,100-acre ecological refuge. It is owned and managed by the descendants of Phineas W. Sprague (1860–1943), a Boston businessman and outdoorsman who assembled the estate with a vision of long-term conservation and ecological diversity in an urbanizing region (**Figure 1**).

Figure 1
Signage marking the entrance to the Sprague's 2,100-acre Ram Island Farm, as family and friends refer to it.

Today, most of RIF's nineteenth-century cropland has reverted to forests. These include typical northern New England assemblages of white and red pine, spruce, fir, hemlock, red and sugar maple, birch, and oak. The remaining haying and agricultural fields are leased to farmers following the State of Maine's "Best Management Practices." Sprague's descendants have for decades implemented a managed tree growth and harvesting plan. They continue Phineas' resource conservation aims. These include maintaining a healthy forest ecology, growing high quality sawtimber, removing invasive plant species, maintaining the land's aesthetic qualities, and improving wildlife habitat. Conservation efforts also include protecting the threatened piping plover and rebuilding habitats for the endangered New England cottontail (more on land management at: http://www.ramislandfarm.com/home).

But Ram Island Farm has other features of particular interest to The American Chestnut Foundation (TACF). Fortunately for TACF, RIF's forests include more than a dozen mature American chestnuts (**Figure 2**). And Seth Sprague (Phineas' great grandson) and his wife Laura are committed supporters of our chestnut restoration effort (**Figure 3**). Seth explained their interest: "We have been intrigued by the American chestnut restoration effort ever since Bob BaRoss first brought it to our attention. PW, as my great grandfather is known in our family, created a strong conservation tradition in us, so when we learned that we had a significant collection of a rare species, we wanted to be involved. It's a great thrill to collaborate with Tom Klak and his UNE students to help advance The American Chestnut Foundation's cause."

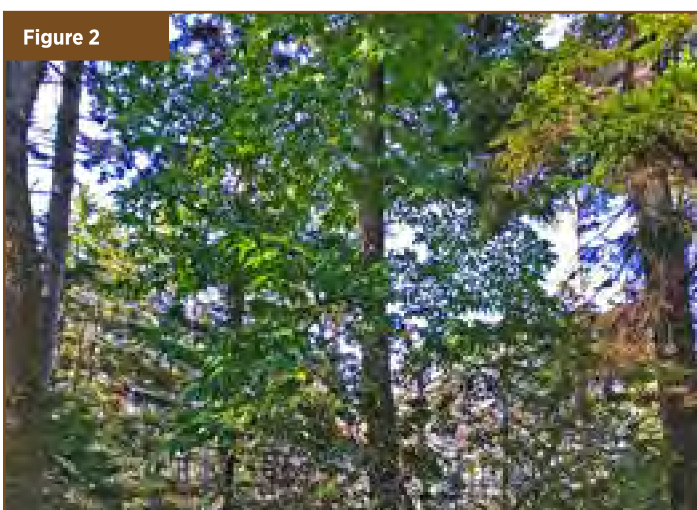
The Sprague-TACF partnership extends back decades. Bob BaRoss, one of the Maine Chapter's earliest board members, hand-pollinated and harvested seeds from one of RIF's big chestnuts in 1998 in an effort to incorporate it in the backcross breeding program. Unfortunately, that line did not survive the breeding efforts and the tree is now no more than stump sprouts. Illustrating the chestnut's diverse value, however, that tree continues to sequester carbon as a beautiful table owned by RIF's land manager, John Greene (**Figure 4**). Another mature chestnut that has also succumbed to blight was recently milled and is now drying, to be fashioned into artisanal table tops in the fall of 2018 (**Figure 5**).

Inventorying Ram Island Farm's chestnut trees follows TACF protocol. We record the tree's latitude and longitude, take and press a twig and leaf sample, complete the Tree Locator Form, and mail it all to TACF's New England regional science coordinator, Kendra Collins. While Kendra has judged most of RIF's chestnut trees to be pure American, a few of them show signs of hybridity, the origins of which remain a mystery. No one, including people who have known the land for decades, seems to know how any hybrid chestnuts came to this land. The plot thickens…

Chestnut seed consumption by wildlife and deer browse has kept Ram Island Farm's mature chestnuts from producing seedling offspring. We therefore assist in the successional process. We collect seeds, grow out seedlings in the University of New England's greenhouse, and plant them in protective Plantra tubes. This has increased the resident chestnut tree population to nearly one hundred. We also experiment by placing RIF seedlings in newly-harvested forest patches (**Figure 6**). In future years, when we have pathogen-resistant seedlings, we can imagine repopulating New England forest patches this way. All of our ongoing chestnut work is a learning experience by trial and error, helping to build a rich knowledge base for future American chestnut forest restoration.

RIF also hosts one of Maine's eight Germplasm Conservation Orchards (GCOs; **Figure 7**). We located it about a half-mile north of RIF's existing cluster of chestnut trees, out of range for natural pollination. We experiment with various

Figure 2



One of the larger American chestnuts at Ram Island Farm.

Figure 3

Laura and Seth Sprague (2nd & 3rd from right) with one of their American chestnuts at an early October nut gathering with University of New England students and land manager John Greene (3rd from left).

Figure 4

John Greene's table, crafted from a Ram Island Farm chestnut that succumbed to blight.

Figure 5

Author mills a Ram Island Farm chestnut that died from fungal blight.

propagation methods in our Maine GCOs, each of which host about 100 seedlings from wild mother trees. Ram Island Farm's GCO will allow us to measure seedling growth and survival as illustrated by this 2-by-2 contingency table:

Number of seedlings	Growth Mat	No Mat
4-foot tube	25	25
6-foot tube	25	25

Each set of 25 seedlings is standardized to contain the progeny of the same mother trees. We then hope to answer questions such as: Do the lower-cost 4-foot Plantra tubes provide sufficient seedling protection, or are 6-foot tubes preferable where deer density is high? Are the growth mats, which warm the soil and reduce weed competition, preferable? Or are those qualities overwhelmed by the fact that the mats can harbor voles which kill seedlings by gnawing at their bark and roots? Look for answers in a future issue of *Chestnut*!

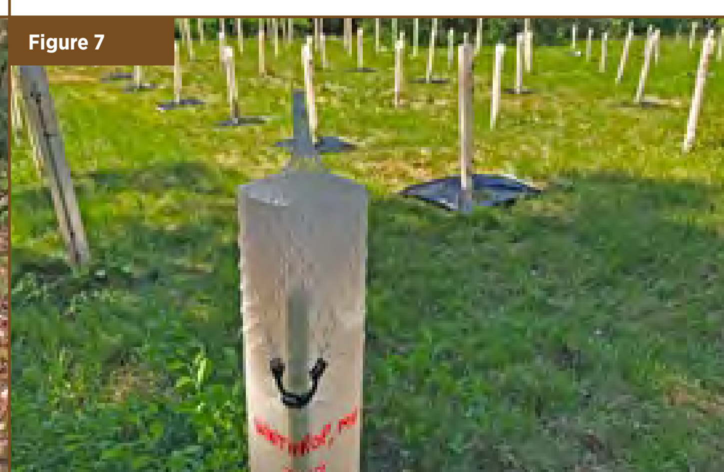
Although the forests of Ram Island Farm have been sustainably managed for decades, ecological threats are intensifying. Many other species besides American chestnuts that have historically been among the forest canopy trees are imperiled by introduced pathogens. Threats include Dutch elm disease, beech bark disease, wooly adelgid on hemlocks, winter moth on red oaks, and most recently for Maine, the emerald ash borer. Mounting impacts on our forest communities create even greater urgency to propagate chestnuts in places like Ram Island Farm, and to continue to learn experientially so we can widely and successfully reintroduce pathogen-resistant chestnuts in the foreseeable future.

If you know of a wild American chestnut tree in Maine, please contact Thomas Klak at tklak@une.edu.

Figure 6

Planting seedlings in a recently-harvested forest opening. Chestnuts should thrive with good sun exposure and growth/shelter tubes (l to r Todd Robbins, Andy Czarnecki and John Greene).

Figure 7



A recently-planted Germplasm Conservation Orchard at Ram Island Farm. Each growth/shelter tube, and an aluminum tag on the seedling inside, records the mother tree's location and harvest year.

This Holiday Season, Give the Gift of a TACF Membership

This year, TACF is celebrating 35 years of unwavering commitment to create a future of forests filled with American chestnut. You can support our continuing progress by giving the gift of a TACF membership today!

Membership to TACF makes a great gift for the people in your life who wish to be a part of a bold movement to bring back a species once thought to be forever lost. Your gift recipient will receive a welcome packet including a personalized letter acknowledging your gift, a membership sticker, and the current issue of our award-winning magazine, *Chestnut*.

Membership to one of TACF's state chapters is also included, along with opportunities to participate in local breeding and research activities. Our monthly electronic newsletter, e*Sprout* and a one-year subscription to *Chestnut* will keep your gift recipient informed of TACF activities, chapter updates, and the latest progress involving our scientific research.

Simply fill out the attached form and send it back with your payment in the enclosed Fall Appeal envelope or visit our website at acf.org and click on the "Join Now" menu tab to purchase your membership gift online.

Thank you for giving us the gift of a new member to The American Chestnut Foundation!

Timothy harvesting chestnut burs. Photos by Kimberly Van Haitsma

How the American Chestnut Tree Helped Me Become an Eagle Scout

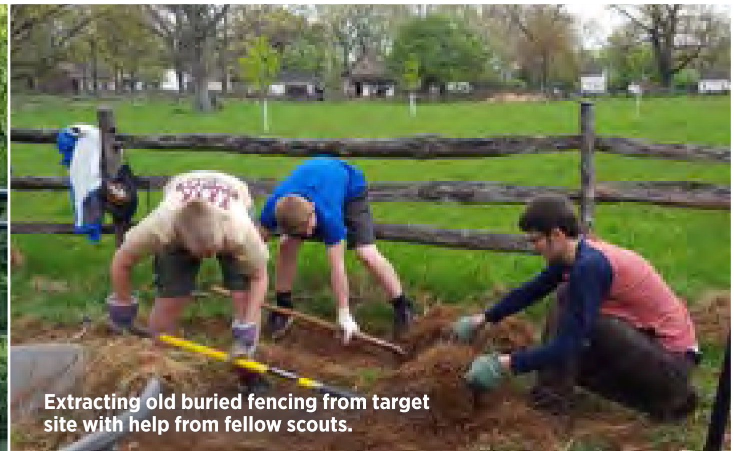
By Timothy Lorandeau, PA/NJ Chapter

My grandfather used to tell stories of the American chestnut tree and how it was lost to the blight. My father kept his story alive by planting native American chestnuts in our yard. So as I pondered options for my Boy Scout Eagle project, one choice contributed to this legacy: I constructed an American chestnut tree orchard at the Pennsbury Manor Historic Site, located in Morrisville, PA.

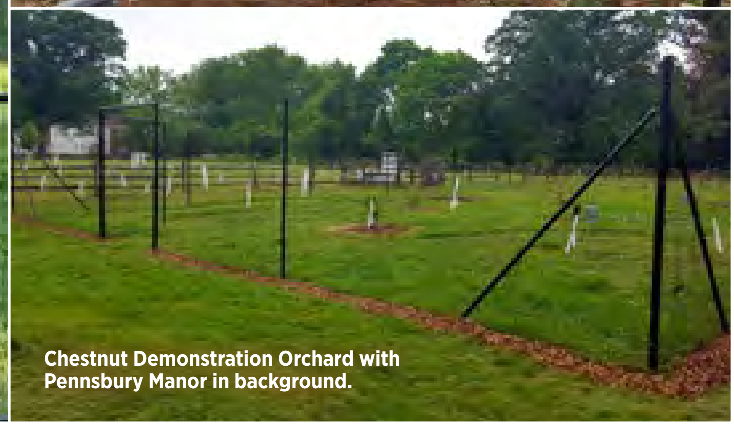
The Manor is a reconstruction of William Penn's home along the Delaware River. Penn founded the Province of Pennsylvania in 1681. The mission of the manor includes maintaining the property as it looked during Penn's time, as well as educating the public about native plants growing there. After discussing project options with Pennsbury Manor staff, an American chestnut orchard seemed like the perfect fit to heighten their mission and fulfill my family's passion for the tree. Three American chestnuts had been planted there seven years prior, but one had died and two were struggling.

In the fall of 2016, I organized a scout service project to collect nuts from the two surviving trees. By early October, they were producing an abundant supply. The burs were quite prickly, and reaching the upper heights of the trees was a challenge. However, following a full day of work, we collected enough burs to fill nine five-gallon pails. By day two, we had five one-gallon bags filled to the brim!

Timothy stands proudly in front of the finished project.



Extracting old buried fencing from target site with help from fellow scouts.



Chestnut Demonstration Orchard with Pennsbury Manor in background.

I delivered the chestnuts to TACF's PA/NJ Chapter office, located at Penn State University's Agricultural College campus. I was delighted to hear that the nuts would be used to help feed endangered wildlife and to develop control trees for research. While there, I also met with several staff members to explore what could be done at Pennsbury Manor, and specifically what I might do as my Eagle Scout project. It was decided that the best path forward would be to establish a Chestnut Demonstration Orchard on a plot of land where the tree line had thinned.

With that idea in mind, I ventured back to Pennsbury Manor to present a proposal. I mapped out the area and outlined a plan for 15 trees. The site would include three native American chestnut, three Chinese, three Japanese, and six hybrid trees. Pennsbury Manor management approved, and the detailed planning began.

The site would require a lot of cleanup. Underbrush, including old fence posts and fencing materials long ensnarled in the undergrowth, had to be removed. In April 2017 the cleanup commenced. Though it was the most challenging and time-consuming part of this project, the site was cleared by mid-May, thanks to the help of my fellow scouts and friends. We were finally ready to build!

Considering there is a substantial herd of deer living on the grounds, the trees would have no chance without a sturdy fence. Our first step was to erect a 7-foot fence to enclose an area 45′ x 165′. By using heavy duty C-Flex fencing, we could repurpose poles from an old Pennsbury Manor project. However, this left us with a need to raise $2,408. Thanks to the generous support from a local land conservation foundation, family and friends, and the magnanimous assistance of deerbusters.com, I was able to raise the money.

To construct the fence, we hammered spiked sleeves into the ground to support side poles and set in concrete at the corner and gate poles. Monofilament wire provided tension to the fencing. The fence was then secured to the tension wire with hog rings.

With the fence completed, and the enclosure now safely secured from deer, we turned to planting. The young chestnut seedlings were planted in early June. We used wire cages to protect them and Pennsbury Manor provided the mulch. I established a routine watering protocol for the following eight weeks.

Since the planting, two of the hybrid trees died. I replaced the trees this past April and am happy to report that they are all thriving.

The folks at Pennsbury Manor were very supportive of my project and appear genuinely pleased with the addition of the new Chestnut Demonstration Orchard. With this project as my final requirement, I became an Eagle Scout in December 2017. I look forward to visiting often and getting to watch the trees grow in the many years to come.

My Chestnut Experience

IN ITALY

By Ana Metaxas, Georgia Chapter

My chestnut experience in Italy was all too brief, and though I wasn't there during the peak of chestnut harvest, I was still able to enjoy some of its sites and flavors. I arrived as burs were starting to drop and was able to harvest a few chestnuts from large trees, some of which were more than 1,000 years old.

Ana Metaxas sits under the trunk of an ancient chestnut tree. Photos by Jonathan S. Starling

What made the experience even more unique was that I collected chestnuts from trees that were alongside an old Roman stone road, and one could see where the trees once lined it. Further evidence of its age was wear marks in some of the stones that had been created by the wheels of carts that rode across them. I later learned that the property had been examined by an archaeologist who indeed confirmed the location to be an old Roman road.

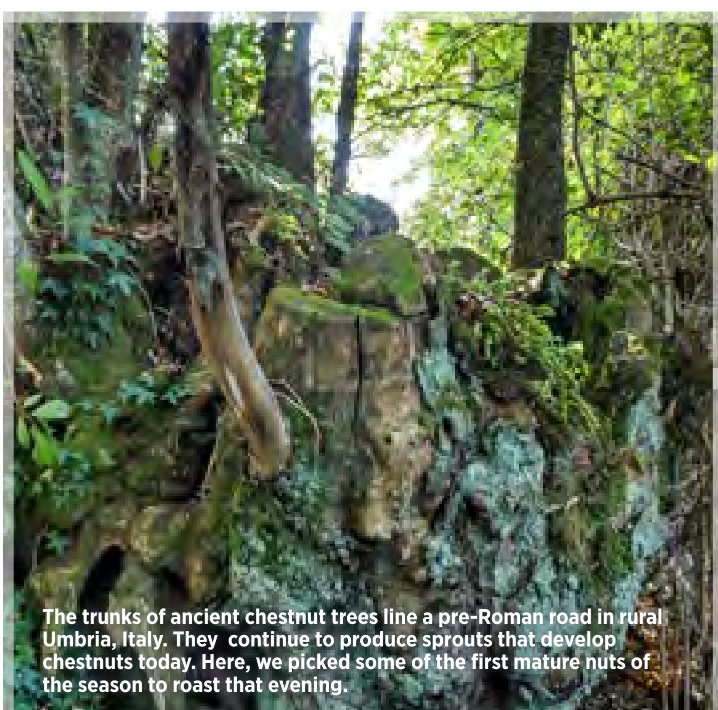
As in that time, to this day, one can see chestnut trees lining the narrow roads in Italy. They are ubiquitous and can be found practically anywhere, so I took great pleasure in harvesting them with the peace of mind that I wouldn't contract poison ivy! I got the sense that the native population views European chestnut trees as commonplace, because when I mentioned the trees, it did not seem to elicit any excitement. Perhaps generations have forgotten how lucky they are to still have such a treasure that, unfortunately, was stripped from North America decades ago with the loss of the American chestnut. However, not all the enthusiasm over chestnuts has faded in Italy. In October, chestnut festivals occur over much of the country, as well as other festivals of which chestnuts are a significant part, such as food festivals or Renaissance celebrations.

When I visited the Trevi fountain, there was a vendor selling roasted chestnuts in the piazza. Can there be anything more romantic than eating fresh roasted chestnuts while enjoying the Trevi fountain in Rome? I asked the roaster what kind they were and he replied, "marrone" (the word for brown in Italian, which can also mean chestnut). When I asked where they were from, he simply said, "Italia." I had to restrain a chuckle as I already assumed as much and was expecting a more regional answer. He finally specified that they were "from North Italia." His chestnuts were big and round, as the highly valued marrone are.

On another occasion I saw chestnuts being roasted and sold in the market at Campo di' Fiori, underneath the statue of Giordano Bruno, who was burned at the stake for heresy at this spot. Enjoying the history and culture of Italy while indulging in roasted chestnuts was a true pleasure. Motivated by that, as well as the smell and taste, I decided to roast chestnuts myself. While doing so, I discovered that their pellicle did not easily separate from the fruit. Therefore, I ate quite a bit of it but wasn't bothered, as it did not have that tannic, astringent taste that Chinese chestnut pellicles do.

While visiting a winery, I got a glimpse of how the European species, *Castanea sativa*, can grow in even dense soils. Typically, we think of chestnut trees thriving on well-drained hillsides and mountains. At Madonna del Latte, the winery located in the hills between Orvieto and Bolsena Lake, one could see the layers of volcanic-exposed soil up-close and it appeared impenetrable. As the owner giving the tour was discussing the soil, I expected him to mention the resilient chestnut tree growing on top of it, but he did not. He explained, however, that this type of soil forces his grape vine roots to spread out looking for water, as opposed to growing strictly downward. He stated that this type of growth improves the quality of the grapes. Chestnut trees on this property seemed to have adapted their root system to this type of soil as well, which I found interesting.

I look forward to visiting Italy again and will plan to go at the height of chestnut harvest. It was refreshing and peaceful to see the abundance of wizened, gnarly European chestnut trees in all their forms, painted across the landscapes there.



The trunks of ancient chestnut trees line a pre-Roman road in rural Umbria, Italy. They continue to produce sprouts that develop chestnuts today. Here, we picked some of the first mature nuts of the season to roast that evening.

A handful of European chestnuts harvested off the floor of an ancient pre-Roman road from Orvieto to Lake Bolsena, in Umbria, Italy.

A street vendor in Rome creates a tempting display of freshly roasted chestnuts served in paper cones to locals and tourist alike, a practice that hasn't changed much in hundreds of years.

Safety Never Takes a Day Off!

by Dan Mckinnon, Director of Farm Operations

We're all guilty of it at some point in our professional and personal lives, far more often than we care to admit - cutting a corner or taking a risk for the possibility of a measurable gain. Sometimes the risk pays off but when it doesn't, the consequences can be serious. Given the dangerous nature of working with trees, TACF has updated our safety protocols in a continued effort to prevent injury before it has a chance to occur.

Most safety guidelines, laws, regulations, suggestions and warning labels are typically created and enforced retroactively in response to an incident, or series of incidents, that were easily preventable had the person(s) known what to expect and how to be proactive in mitigating the often-dire consequences. In other words, the warning labels and precautionary reminders we all see and hear every day have a history behind them and, if heeded, keep us from repeating that history. "Caution: Coffee is HOT!" on a fast food company's coffee cup seems like a "no-brainer," but learning that more than 700 people received burns prior to the company taking action, puts that warning in perspective. There are thousands of examples of warnings and cautionary tales that many of us dismiss as exaggerations or improbabilities until we experience it ourselves.

With that in mind, The American Chestnut Foundation (TACF) recognizes the need to address its most dangerous areas of day-to-day operations, with the initial focus on Meadowview Research Farms. Safety protocols are meant to provide a standard of expectation as well as serve as reminders to those engaged in these types of activities. Much like speed limit signs that cannot stop speeding or protect us from those who do, but are there in an effort to remind us to use caution in a dangerous environment, and that's the goal of TACF's safety protocol reminding you, the person on the ladder, to be safe out there!

Since some of the field work in which we're engaged is inherently dangerous, Meadowview Research Farms compiled a list of the activities that pose the biggest threats to the safety of workers and their tools. In no particular order, the health and safety risk categories include: operating chainsaws or tractors, mixing and spraying pesticides and herbicides, towing trailers and using ladders or aerial lifts. Each topic has three categories:

1. the task/procedure which the worker will perform,
2. the potential hazards they may, or will encounter while performing the task,

3. and the mitigating actions workers can take to greatly reduce the risk to their health and safety and that of their coworkers, and to the equipment and tools they are using.

The information in TACF's Safety Protocols is meant to help everyone and can be used when training to highlight the dangers of the day's tasks to a volunteer work crew during tailgate safety meetings, as well as reminding more experienced personnel that experience does not equal invulnerability. During my 25 years of operating chainsaws and training others in their safe use, I've lost count of the number of times I've heard "I've been doing this around my house for _____ years and I've never worn personal protective equipment (PPE)." My response to that statement has evolved over the years into a biting rebuttal, meant to remind the operator that they're working with an extremely dangerous machine, not an empathetic human: "the chainsaw does not care about your experience or feelings, nor does it discriminate, have either kindness or ill-will. It's a tool that cuts at several thousand rotations per minute until it is stopped." To most, that's a shocking statement and is meant to be. When life, limb and property are at risk, a laissez-faire attitude often leads to a serious problem, and let's face it, injuries from a chainsaw are rarely minor.

In addition to the dangers from the tool itself, our surrounding environment presents an uncountable number of hazards that, without instructions or occasional reminders, we might not remember or recognize until it's too late. For instance, did you notice the 20' long dead limb hanging over your head before you started sawing on the base of the tree? It's commonly referred to as a "widow-maker" for a reason! What about the Yellow Jacket nest or the Timber Rattler between your feet, or the fact that you wore sandals due to the lovely weather that day and left your heavy boots at the office, or you're simply tired and dehydrated from lugging around a chainsaw all morning? On your way to the job site, did you notice the thunderstorm on the horizon and have a contingency plan? Did you know that having tools in the cab of your truck can effectively turn your passenger compartment into what my former supervisor at Mt. Rogers National Recreation Area affectionately referred to as a "meat grinder" or "blender" when your vehicle is rear-ended at a stop sign?

We all know and understand that accidents happen. We all know that there are dangers everywhere, whether in the office or orchard, and we all have a general idea of how to avoid them. TACF's Safety Protocols are presentations of more detailed information for you to learn and use prior to training and carrying out the more dangerous tasks in which we are often engaged. Remember that in addition to our mission of restoring the American chestnut to its native range, we also share the common goal of going home to our families after a hard day's work. We hope you have fun and gain satisfaction from the work you do with TACF, but please take seriously the lessons learned and passed to us by others, and BE SAFE OUT THERE!

PART 1
of a
3-part series

The American Chestnut Tree:
Not Gone, Not to Be Forgotten

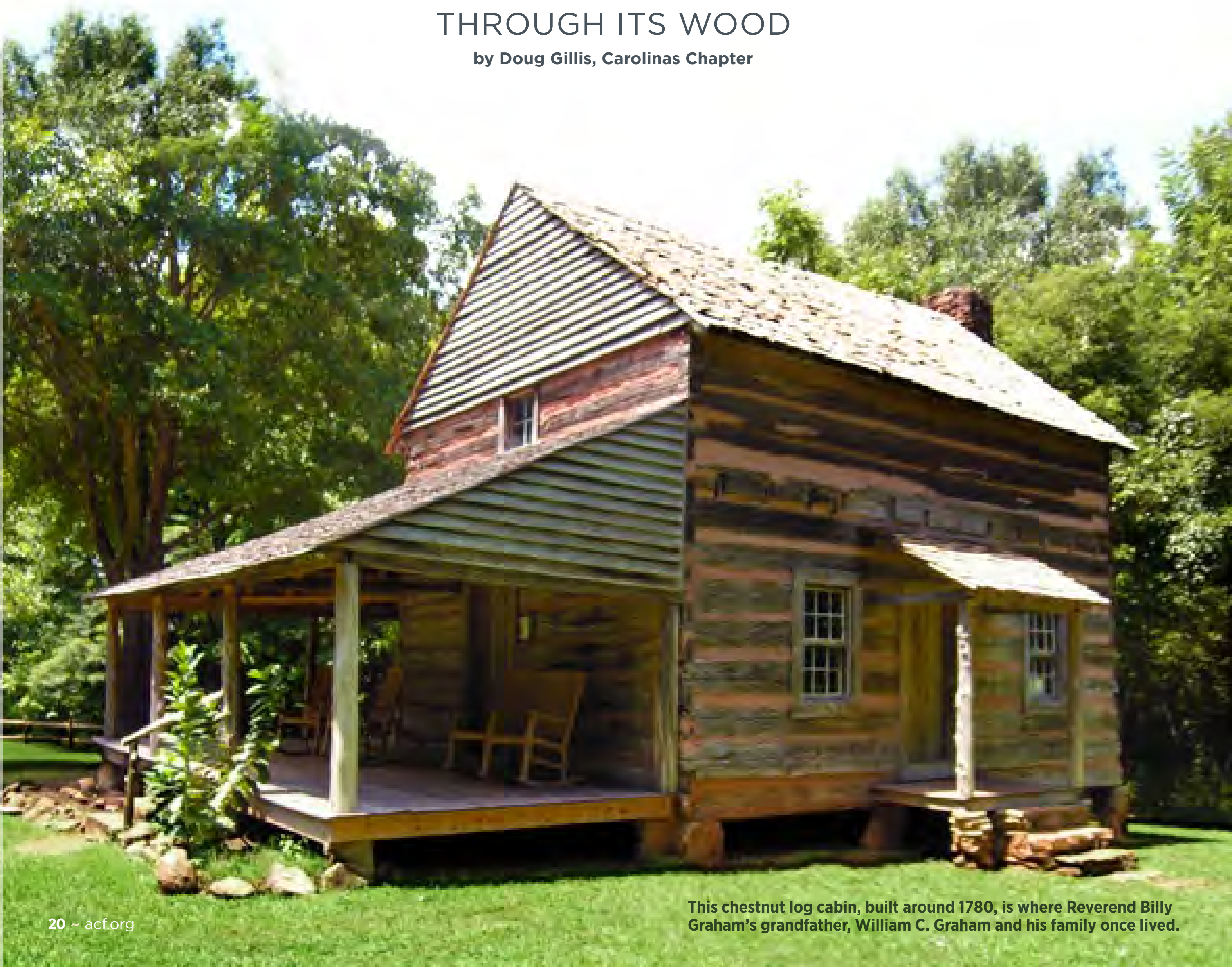
People are introduced to the American chestnut in surprising ways. In this three-part series, Doug Gillis will explore how people learn about the American chestnut through its wood used in structures and in woodcrafts; through illustrations, paintings, quilts, and wall hangings; and through music, poetry and literature. Such arts and crafts can capture one's imagination and reinforce the desire and need to see the tree restored to its eastern forests and woodlands.

The American Chestnut Foundation and its chapters, through promotion and outreach, can help expose people to the history and culture of the tree, to the science and technology that will rescue it, and to the pleasures gained by being involved in its restoration. At the end of this article, we invite you to share your knowledge of uses of American chestnut wood. You can also help with future articles by citing examples of American chestnut themes used in visual arts and in music and literature.

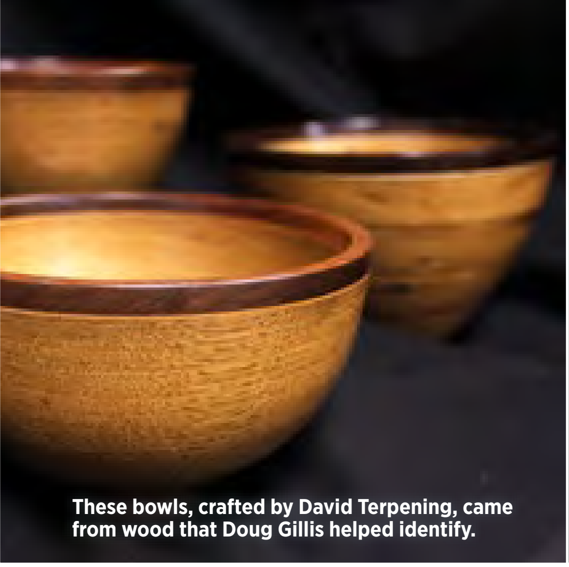
Learning About American Chestnut

THROUGH ITS WOOD

by Doug Gillis, Carolinas Chapter



This chestnut log cabin, built around 1780, is where Reverend Billy Graham's grandfather, William C. Graham and his family once lived.



These bowls, crafted by David Terpening, came from wood that Doug Gillis helped identify.

Maryland Chapter board member, Gary Carver, brings alive a pegged piece of chestnut wood by populating it with nuthatches.

Doug Gillis restored this American chestnut nightstand that used to be owned by his grandparents.

People who know the history and culture of the American chestnut often hold strong feelings for and attach great meaning to conserving and restoring the tree to our eastern forests and woodlands. TACF and its chapters are seeking ways to maintain and cultivate interest in the restoration efforts and reach out to new audiences, especially youth, educating them about and helping them spread the compelling story of the tree and its comeback.

One way to teach children, as well as adults, about the American chestnut is through arts and crafts that use the American chestnut tree as a theme. Experiencing the tree through its wood and its uses, people can renew their interest in rescuing the tree. Parents, grandparents, other relatives, formal and informal educators and TACF members can help educate children about the tree—what it once was, what it meant historically and culturally, and what it can be again through restoration. Action is needed.

Hold a piece of salvaged American chestnut wood in your hands and think of the story it might tell. Think of those who originally harvested the wood, of those who converted it into a useable form, and of those who reclaimed it for a new use. Run your fingers over the grain of the board. Sense how fast the tree grew by looking at the growth rings. Envision how straight and tall the tree was.

Walk around the outside of an American chestnut log cabin and marvel at the size of the logs from which it was constructed. If you live near Charlotte, NC, visit Anne Springs Close Greenway to view the 240-year-old Graham cabin constructed of American chestnut logs. The logs were harvested nearby, a testament to American chestnut trees having grown in the Piedmont before trees were eliminated by root rot introduced into the Colonial South.

Visit Big Meadows Lodge located on the Skyland Parkway in Virginia to experience American chestnut wood used in construction of buildings. The lodge was originally built as a Civil Conservation Corps campground. At the time of the camp's construction, blight was devastating American chestnut trees. The wood of the blight-stricken trees is conserved in buildings at the lodge. Stay at the Big Meadow Lodge and sleep in a room paneled with American chestnut wood.

Experience firsthand Gaither Hall, the Administrative Office Building at Montreat College, NC. It is a former sanctuary and fellowship hall. The vaulted ceilings, paneled walls, and finished carpentry in the building are constructed of American chestnut harvested from the nearby Black Mountains when blight began attacking trees in the early 1930's.

Own a piece of American chestnut wood furniture and pass it on to future generations. I was reworking a nightstand recently which came from my grandparent's Asheville, NC home purchased in 1910. The wood proved to be American chestnut. My wife, Marsha, found a farmer's rake constructed of American chestnut wood at a store in Waynesville, NC. The rake, with a broad stout head and short tines could have been used to rake through leaves and burs, leaving chestnuts behind on the ground.

Check objects for American chestnut wood. It is sometimes found in upright piano cases. Carolinas Chapter member, Don Surrette, reclaimed the wood from a piano case to create a binder that can be used to preserve memories. David Terpening of Charlotte, NC turned two-inch thick pieces of American chestnut wood into bowls that showcase the beauty of the wood. David taught me to build attractive, functional chestnut wood boxes good for safe keeping of mementos. Maryland Chapter board member, Gary Carver, created a sculpture of 11 chestnut nuthatches and affixed them to the end of an old pegged beam from an American chestnut log cabin. Gary says of his carvings, "I often tell people that unlike ceramics, metals, glass and other materials used in artwork, wood was once alive. I believe that when I make a carving from a piece of wood I am bringing part of the tree back to life."

Salvaged American chestnut wood is used by artisans to make guitars, dulcimers, flutes and violins. Tunes played on the instruments can sing our souls further along toward the restoration of the American chestnut tree.

Help TACF catalogue uses of American chestnut wood. Cite locations where its wood used in construction can be viewed. Share information about furniture you know of that is on display and is made of American chestnut. Share pictures and descriptions of wood crafted into functional objects and pieces of art.

If you have pieces made of or know about buildings constructed from American chestnut, please let us know! Contact TACF's *Chestnut* editor, Jules Smith at jules.smith@acf.org.

Past Success

1983: The American Chestnut Foundation was founded

1989: TACF establishes the Wagner Research Farm in Meadowview, VA where the first F1 backcross trees were planted

1995: TACF Honorary Board Director, Mary Belle Price, donates the 93-acre Glenn C. Price Research Farm in Meadowview, VA

2015: *Phytophthora* resistance research begins

Whether you recently became a member of TACF or have been a faithful chestnut enthusiast for the past three decades, you are a part of something special. Over the years, teams of dedicated volunteers, researchers, and our own staff have planted more than 300,000 trees for research, collected over 340,000+ seeds from research orchards, and this year, combed the landscape to identify 815 wild American chestnuts using the TreeSnap app. None of this would be possible without the continued financial support from members and donors like you, and we deeply appreciate your loyalty and generosity.

As many of you know, and have come to expect from TACF, we ensure every dollar raised is used and leveraged to the fullest extent possible, which has allowed us to continue on this journey we began 35 years ago. Of all the donations and memberships we receive, 84%

Continued Progress

Determine "Best of the Best" B_3F_3 backcross tree

Utilizing Small Stem Assays

Regulatory review for transgenic tree

Updated Scientific Review

goes directly to support our scientific research. Your gift, large or small, and how you choose to give it (online, by check, an IRA Charitable Rollover, or through your will), truly matters. Each one helps us move closer to our goal of creating a future of forests filled with American chestnut.

As we gather around the kitchens, dining rooms, and living rooms of our family and friends during the upcoming holiday season, sharing stories and memories of 2018, we hope you'll share your story about why you got involved with TACF. Each chestnut story is unique, so we encourage you to share your passion about and commitment to the American chestnut tree with others.

facebook.com/americanchestnut ~ twitter/chestnut1904

A Chestnut Tree Grows

IN BROOKLYN

By Bart Chezar, New York Chapter

Bart speaks to students following the planting in Prospect Park. Photos courtesy of Barbara Taragan and Ecorama blogspot

In the famous 1943 novel by Betty Smith, *A Tree Grows in Brooklyn*, we learn about Francie Nolan. Francie grows up in an impoverished family in a tenement in Williamsburg, Brooklyn (recommended reading for its timeliness). The story begins in 1900 when the girl's parents, children of immigrants from Ireland and Austria, meet, marry and have two children. Despite poverty, family misfortune and many setbacks, Francie survives and ultimately flourishes. The title of the book is a metaphor for the Tree of Heaven (*Ailanthus altissima*), a hardy street tree that we follow throughout the book that despite being burned, treaded upon and abused, ultimately thrives. This story, the period it covers, and its location could not be more appropriate to the history of the American chestnut in Brooklyn, New York.

At the beginning of the 17th century, when the Dutch arrived in what is now New York City, they experienced a verdant forest covering the land. One of the primary trees making up this forest was the American chestnut. As commerce developed, farming expanded, and intense urbanization occurred over the centuries, the forests disappeared. With the need for recreational space and a place to get away from intense urban life, parks were built, and even large cemeteries were used as a place to feel closer to nature. Two of the best examples of this were Prospect Park and Green-Wood Cemetery in Brooklyn. Concerning the latter, even before Prospect Park was opened, people would travel in their horse and carriage to picnic in Green-Wood. What both these locations had in common was the extensive plantings of American chestnut trees. Prospect Park in particular, was designed and developed by the renowned landscape architect Frederick Law Olmsted. His design included thousands of chestnut trees in forest and meadow areas created in the park. Prospect Park is recognized as one of Olmstead's greatest achievements.

And then it struck. An article in the New York Times on October 2, 1910 headlined, "Mysterious Blight Kills Chestnut Trees by Thousands." The story describes the spread of the disease throughout the city. It states that two years prior (1908) 1,200 infected trees were destroyed in Prospect Park. We know a similar number of American chestnut trees

Second graders from PS146 assist with the planting.

PS 146 student keeping track of plant health.

were lost in Green-Wood Cemetery. These trees were among the first lost after the blight was identified in the Bronx in 1904. For the next many decades, American chestnut trees were not to be seen in Brooklyn.

Beginning in 2004, working with the Prospect Park Alliance and TACF's NY Chapter, a dozen or so native American chestnut tree seedlings were planted in the Park. Though a number of these trees have been lost to the blight over the years, a few are still alive. One of these trees is almost 50' tall and for the last few years has produced fertile seed through cross-pollination with a nearby native American chestnut tree. In 2011 we received a small number of our first potentially blight-resistant seeds from TACF. Seedlings from these seeds were planted in Prospect Park a few years after receiving the seeds.

In parallel, but on a smaller scale, both native and potentially blight-resistant seedlings were planted in Green-Wood. We selected a section of the cemetery named Chestnut Hill. It was initially thought this hill was named for the large horse chestnut tree present there but historical records show the site was named after American chestnut trees growing there in the 1800's. From the Green-Wood planting we collected the first fertile seeds from crosses between native and potentially blight-resistant chestnut trees. A couple of potentially blight-resistant trees were planted in the Native Flora Garden at the Brooklyn Botanic Garden where they are flourishing; one measuring over 20' tall.

In the last couple of years, we have been able to collect more fertile chestnut seeds from the trees in Prospect Park and Green-Wood, and from TACF's NY Chapter. These seeds have enabled us to use them in educating children in the neighborhood about the chestnut story and why it is important. This past year we have worked at three neighborhood schools, PS 321, 146 and 133 in Brooklyn. Speaking to more than 300 students in eleven kindergarten, 1st and 2nd grade classes, we explained why trees, especially chestnut trees are important in our lives; providing numerous environmental benefits, climate change mitigation and a source of food. They were most impressed, though, learning that seeds from the tree can be an important food source for bears (not yet introduced into Prospect Park). We were also able to give each class a chestnut seed to grow in their own classrooms. We charted the growth of these seeds during the school year. This activity matched up with an effort in Prospect Park to reforest a section of the park originally laid out by Olmstead.

On June 6, Amanda Romani's second grade class from PS 146, with their Science Coordinator, Barbara Taragan, met us in Prospect Park with their seedlings. Howard Goldstein, the Prospect Park Alliance forest ecologist, discussed with the students the importance of what they were doing and then we all worked together to plant the American chestnut seedlings as part of a reforestation effort in a new section of the park.

As these and other Brooklyn children grow up, hopefully they can follow the growth of these trees and support the reestablishment of the American chestnut in our nation's eastern forests.

Bart is a retired R&D engineer from the New York Power Authority. Since retiring, Bart has focused on ecological restoration projects in New York City. He was one of the first people to investigate restoring oysters to New York harbor. He also experiments growing long-lost, subtidal plants (eelgrass, Sargassum) along the shore, and restoring the American chestnut in Brooklyn. To receive his annual report on this work or ask questions, contact him at bchezar@nyc.rr.com.


Bart, assisted by Tim McKinney, enlists the help of second grade naturalists to participate in American chestnut restoration.


After the planting, second graders take a break in the shade to eat and talk with Bart about their hard work.

Dave Gill

CHESTNUT SUCCESS ONE STEP AT A TIME

By Scott Carlberg, Carolinas Chapter

Harvesting chestnuts at the 2016 Washington Suburban Sanitary Commission (WSSC) Seed Harvest.

"In every walk with nature, one receives far more than he seeks."
– John Muir, naturalist, environmental philosopher, and father of our National Parks

Muir's quote perfectly describes Dave Gill, TACF MD Chapter member. Dave's TACF journey began when he hiked 1,700 miles on the Appalachian Trail in 2010. He says he felt a calling. "I saw old stumps along the trail with a number of saplings in various stages of growth or decay. It made me inquisitive about what was going on with these trees," he says. The stumps were the remains of the chestnut trees that had once been vital to life along the trail. Dave wanted to bring the trees back to their glory and so began his involvement with TACF.

Trees are in Dave's blood. His inclination to work with wood stems from his childhood. His father built their family's home and was a cabinet maker. Dave and his siblings followed the tradition and each built their own homes. When Dave started to build, many of the trees on his lot were about five feet tall. They are now as tall as 60 feet. "I built the house myself and nature supplied the materials," he says.

"I have white oaks, red oaks, poplars, some ash, some persimmons, cedar, and a stream that runs through my lower pasture. After a bad day at work, I could sit on a bench by the stream and let the troubles of the day drift away in the sounds of nature," says Dave. He's retired now. No more tough days at work but he still revels in the natural habitat that surrounds him.

MD Chapter members may already know Dave as the seed orchard steward of the Central Maryland Research and Education Center (CMREC) near Ellicott City, MD. The orchard was established in 2016 on a two-acre site. "There are now 3,400 trees at the Research Center's site. After all the inoculations and evaluations are complete, we will hopefully be left with 40-45 trees that are significantly blight-resistant seed producers," he says.

The MD Chapter's webpage (acf.org/md/photo-gallery/) shows pictures of the orchard. The aerial shot shows

Dave and wife, Sandy, stand in front of the Chestnut Seed Orchard in its first year at the CMREC.

Dave's third pair of boots from his trek across the Appalachian Trail.

Dave on "weed patrol" at the CMREC.

straight and accurately planted rows of trees. This seems logical when you consider Dave's background as a Certified Public Accountant. "Planting and establishing an orchard requires strong project management skills - organization, attention to detail, effective use of your resources, and the ability to keep to a schedule, which I guess fits in with an accountant's skill set," Dave says.

Dave has new ideas for the trees under his care. "In my seed orchard, I have been testing the use of daikon radishes to de-compact the soil where we will plant future trees and improve the nutrients in the soil. I have two test plots of seedlings under TACF's small stem assay experiment." Dave has even been experimenting with a drone, using its high-definition camera to check the maturity of the female flowers.

There isn't a job in the chapter that Dave hasn't touched, from building new orchards to housing chestnuts in his refrigerator during winter, raising seedlings in his dining room, planting, harvesting, inoculations, evaluations, cullings, and even weed and insect control. Dave was president of the Maryland Chapter in 2016 and has done many presentations to help educate the public. "I keep the story simple and I invite people to volunteer. Best guess would be 100 to 150 volunteers have gotten involved after hearing one of the presentations." Dave has also served as the publisher of the Maryland Chapter's newsletter. Dave estimates he puts in more than 350 hours volunteering in an average year. To him, it's time well invested.

Being the orchard steward would fully occupy most people's time, but not Dave Gill. He tries to give back to his community in many ways. His love of nature is enhanced by being a bike patrol volunteer with the U.S. Park Service on the C&O Canal. In this role he is like a mobile AAA, fixing bikes, giving directions, and being the eyes and ears of the Park. His love of trees also has him serving as a Board member on Montgomery County's Forestry Conservation Advisory Committee. During the winter, Dave volunteers to do taxes for older and low-income members of the community through AARP's Tax Aide program.

He and his wife raise and train service and therapy dogs. They have raised five of them and currently have three therapy dogs in their home. They take the dogs to hospitals, rehab centers, nursing homes, elementary schools and universities to spread a little joy and companionship. He loves working with dogs but jokingly says, "You have to have lost your mind to do it." It's work that requires patience and care. Working with dogs is rewarding, "The smile on the face of the person interacting with one of our dogs means the world to us. There's nothing like it!"

Dave lives and walks among trees. "Hiking has honed my respect and awe for nature's beauty and power. Nothing in life is ever certain, and the chestnut restoration project is no exception to the rule. Just when you think everything is going smoothly, nature throws a curve ball in the form of a storm, a new invasive insect or any of a myriad of obstacles. Patience and resourcefulness are a must to stay in this game. I love the challenge. There is nothing like sitting down in one of our orchards after hours of hard work to enjoy these magnificent trees as the wildlife wanders among them. The joy of watching a fox stalking a mole in the orchard or the swooping swallows gathering their dinner among the trees brings me such peace and pride in our project."

Dave is investing his time for good reason. He sees his work with the American chestnut as the legacy he will leave behind for his children and grandchildren to enjoy. "I have high hopes that chestnuts will be back in the forest. I may never walk among a forest of wild American chestnuts, but with luck, my future offspring will."


This bur is starting to open on its own and is ready to be shucked. Photos by Kendra Collins

Harvesting and Storing Chestnuts

By Kendra Collins, New England Regional Science Coordinator

With the change in seasons comes fall foliage, crisp apples, and of course, chestnut harvest time. Collecting chestnut burs and nuts is an exciting activity. We carefully count the nuts we were able to harvest, package them for storage over the winter, and cross our fingers that we can plant those hard-won nuts in the spring. But many of us have occasionally had the disappointment of checking the bag of nuts at the back of the fridge only to find that they are moldy or perhaps never sprouted. Obviously something went wrong. What's a chestnutter to do?

Harvesting

Planting viable, sprouted, happy chestnuts in the spring relies on proper procedure at harvest time. First, one must collect the nuts before the blue jays, squirrels, bears, etc. do. To ensure a good harvest, we often collect the burs directly from the tree before they are fully ripe. Chestnuts will ripen inside the bur once harvested off the tree. It is best to keep them somewhere cool, in a paper bag or something similar, and away from the risk of predation. The burs will to continue ripening for a week or two post-harvest. The burs should open on their own and you can certainly put on some gloves and pry one or two open to see if the nuts are brown and loose in the bur. If they are still white, or stuck firmly in place, let them go a few more days and check again. As the burs open, or start to brown, the nuts can be shucked and collected for storage.[1] If the burs just won't budge you can try prying them open with a hefty pair of clippers, and if that won't work they are probably a lost cause.

Many of the mold issue we see during storage over the winter can link back to improper handling after harvest. Once the nuts are out of the bur you want to get them into storage as soon as possible. This is particularly important because chestnuts are recalcitrant (see sidebar) and require specific storage conditions to remain viable. A dilute bleach bath and float test can help provide some surface sterilization and also weed out any non-viable nuts (viable nuts sink in water). A small study previously reported on in *The Journal of The American Chestnut Foundation*[2] found that pre-treating seeds with various concentrations of household bleach or over-the-counter hydrogen peroxide were not damaging to germination, though was unable to confirm the efficacy of their treatment

Recalcitrant – obstinately defiant of authority or restraint (Merriam-Webster: https://www.merriam-webster.com/dictionary/recalcitrant). In terms of seeds, or in our case nuts, recalcitrant (or unorthodox) seeds have a high moisture content and do not survive being dried or frozen, as orthodox seeds do. This means they cannot be stored long-term for preservation of their unique genetics.

[1] TACF Fact Sheet – Harvesting, Handling and Storing Chestnuts: Helpful Tips for Success – https://www.acf.org/wp-content/uploads/2016/09/FactSheet_HARVEST_4-23-15.pdf

[2] Gurney, K and Fitzsimmons, S. 2013. How to Not Kill a Chestnut. The Journal of The American Chestnut Foundation. March/April. Pgs 22-23.

of surface molds. Another option is to use StorOx 2.0, a commercial-grade bactericide and fungicide, to treat the nuts for surface molds and other contaminants prior to storage.

Standard Chestnut Storage Method

The standard storage method for chestnuts that are to be planted is to store them in damp, sterile peat moss at about 33 degrees F. Peat should be dampened at roughly a 10:1 peat-to-water ratio, or wet enough to form a ball when squeezed but not so wet water can be squeezed out. Peat is acidic and helps prevent mold growth, but also helps keep the nuts from drying out. Mix the nuts well so all are surrounded by peat – about 200/gallon Ziploc works well. Some swear by poking holes in the bag for ventilation, others do not, but both options seem to work well enough as long as proper moisture levels are maintained. Store the nuts in a cold part of the refrigerator (don't let them freeze) and leave them be, other than checking on their condition once/month or so.

Alternative Storage Options

Of course, there is more than one way to successfully store chestnuts. Some members of the MA/RI Chapter use a slight variation on the peat moss method and carefully layer the nuts in trays full of damp peat moss so that as the radicles emerge they grow down and do not get jostled or re-oriented as the trays are moved around or checked. This method takes up more space and it more time-consuming to set-up initially but does lead to very nice, straight radicles for planting.

There are alternatives to using damp peat moss as well. Members of the Georgia and New York Chapters have successfully used damp sphagnum moss as a storage media and achieved very high germination rates with subsequent planting. Some of our members have also layered nuts in coffee cans full of damp sand with good success. The down side to sand is that it is heavy and not always easy to come by, but may work well for smaller quantities of nuts.

Another method some use is to store larger lots (200+ nuts) in Ziploc bags without any storage media at all. The moisture provided by the volume of nuts keeps them from drying out, but good sanitation prior to storage is key to prevent mold issues. Nuts stored in this manner do not seem to sprout as quickly as nuts stored in damp peat and a float test prior to planting is a good idea, just to be sure nuts are still viable.

Finally, some brave souls allow Mother Nature to handle their chestnuts over the winter. This is what would happen were the nuts to just fall off the tree and makes ecological sense, but the main concern is protecting them from wildlife. Nuts may either be fall-planted within some type of sturdy wildlife protection, or may be stored in bulk (usually buried), also protected from predation with some manner of cage or barrier. Chestnuts are a preferred wildlife food and this method is not generally recommended.

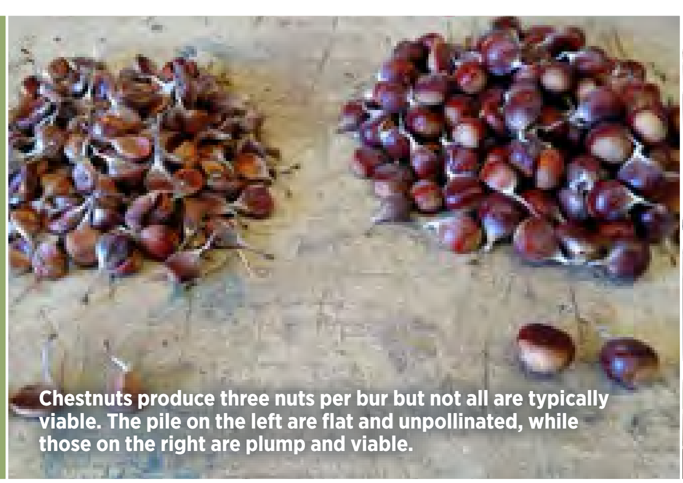
Do chestnuts really need to cold stratify?

The reason we store chestnuts in the cold is that, presumably, they need to stratify, or go through a cold treatment like they would in the wild, before they will sprout. This is true of many plant and tree species. But is it really required of chestnut? Oaks in the white oak group do not need to stratify and their acorns sprout shortly after they ripen in the fall. But oaks in the red and black oak groups do require a cold treatment prior to germination. Chestnut is in the same family as oak, but where does it fall regarding this requirement? Those of you on the TACF Growers Listserv may recall a recent discussion about this subject. The suggestion was made that chestnuts do not really NEED a cold treatment to sprout, but that perhaps storing the nuts in the cold just prevents them from sprouting before we are ready to plant them.

To explore this question, TACF's Meadowview Research Farms plans to run a small experiment looking at stratification times and germination success. Nuts will be subjected to zero cold treatment, as well as varying lengths of cold storage, and then germinated to see if there is a real need for cold treatment, and if so, what works best. Stay tuned!

Interested in chestnut seeds, or tree seeds in general?

Join us for our 35th Annual Meeting in Huntsville, AL! Victor Vankus, Botanist with the USFS National Tree Seed Laboratory will be a featured presenter. See page 3 of this issue of *Chestnut* for more information.



Chestnuts produce three nuts per bur but not all are typically viable. The pile on the left are flat and unpollinated, while those on the right are plump and viable.

A PLAN TO DIVERSIFY A TRANSGENIC BLIGHT-TOLERANT

American chestnut population

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SUMMARY: Incorporating transgenic blight tolerance into The American Chestnut Foundation's breeding program will enable TACF, SUNY-ESF, and other collaborators to efficiently increase the genetic diversity and adaptive capacity of the American chestnut population for large-scale restoration. Simulations of pedigrees were performed to estimate effective population size and average inbreeding coefficients in different scenarios of outcrossing transgenic to wild-type American chestnuts. Scenarios involved 1 or 2 transgenic American chestnut founders outcrossed to up to 1500 wild-type American chestnuts over 1, 2, or 3 generations, using 1 or 3 progeny per cross as parents in subsequent generations. Simulations suggest that an efficient method to increase the effective population size to >500 individuals and reduce the average inbreeding coefficient to <0.01 is to outcross two transgenic founder trees over three generations to 50, 150, and 450 (650 total) wild-type parents using three progeny per cross as parents in subsequent generations. Transgenic progeny of the third generation of outcrossing to wild-type trees may be intercrossed to generate a population of trees that is homozygous for blight tolerance. A diversified population of transgenic blight-tolerant American chestnut is estimated to be available for use in large-scale forest restoration 15 to 35 years after federal approval is granted to distribute the trees. In contrast, trees for personal or horticultural plantings such as yards, parks, arboretums, historic sites, and woodlots would be available almost immediately after federal approval. Methods to accelerate outcrossing, which may cut the diversification time in half, and membership participation are discussed.

American chestnut has been identified as one of 25 trees in the United States that is most vulnerable to climate change (Potter *et al.* 2017). Nearly all of the American chestnut trees in Eastern North America are infected with chestnut blight. The blight-infected stems rarely flower. The inability to reproduce prevents the species from evolving and adapting to the changing climate. Incorporating blight tolerance into the American chestnut population will enable the species to continue to evolve on its own within its native range. The natural range of chestnut is highly climatically diverse, spanning approximately 15 degrees of latitude, from central Mississippi to coastal Maine, which corresponds to mean annual temperature variation of ~12°C. Increasing the genetic diversity of the blight-tolerant restoration population is necessary for efficient local adaptation of trees to variable climates across time and space. Genetic diversity must also be sufficient to minimize the deleterious effects of inbreeding once the species is restored and reproducing on its own within its native range.

Collaborators at the State University of New York College of Environmental Science & Forestry (SUNY-ESF) have developed a transgenic blight-tolerant variety of American chestnut by inserting a gene from wheat into the genome of an American chestnut tree. The enzyme encoded by this gene (oxalate oxidase) detoxifies oxalic acid that the chestnut blight fungus produces to incite cankers on chestnut stems (Zhang *et al.* 2013). When a transgenic American chestnut tree is bred with wild-type American chestnuts that are susceptible to chestnut blight, approximately 50% of the progeny are expected to inherit the oxalate oxidase (OxO) gene. Progeny that inherit the OxO gene have cankers that are as small and superficial as those on Chinese chestnut after artificial inoculation of seedlings with a highly virulent strain of the chestnut blight fungus (Newhouse *et al.* 2014; **Figure 1**). Trees with this gene are readily identifiable using a simple assay: transgenic leaf disk samples change color in a test solution, while non-transgenic leaves remain colorless (**Figure 2**).

In 2018, researchers at ESF plan to submit data on the ecological impacts of transgenic American chestnut trees to the U.S. Department of Agriculture, the Environmental Protection Agency, and the Food and Drug Administration. Research to date indicates no significant differences between transgenic and wild-type American chestnuts in growth, metabolism, colonization of roots by mycorrhizal fungi, chestnut leaf litter decomposition rates, germination of seeds from other plants in chestnut leaf litter, bee feeding on pollen, tadpole feeding on chestnut leaf litter, and nut nutrition (D' Amico *et al.* 2015, Newhouse *et al.* 2018, William Powell & Andrew Newhouse, personal communication). Regulators are expected to decide if these data are sufficient to grant permission to distribute transgenic trees outside confined field trials by 2020 or 2021.

To date, the OxO gene has been inserted into a single American chestnut founder tree, Ellis1, from New York to produce the transgenic 'Darling 58' line being reviewed by the federal regulators. Researchers at ESF and the University of Georgia are collaborating to insert OxO into another tree from Virginia to have two transgenic founder trees to use in future breeding. Each transgenic event

Figure 1

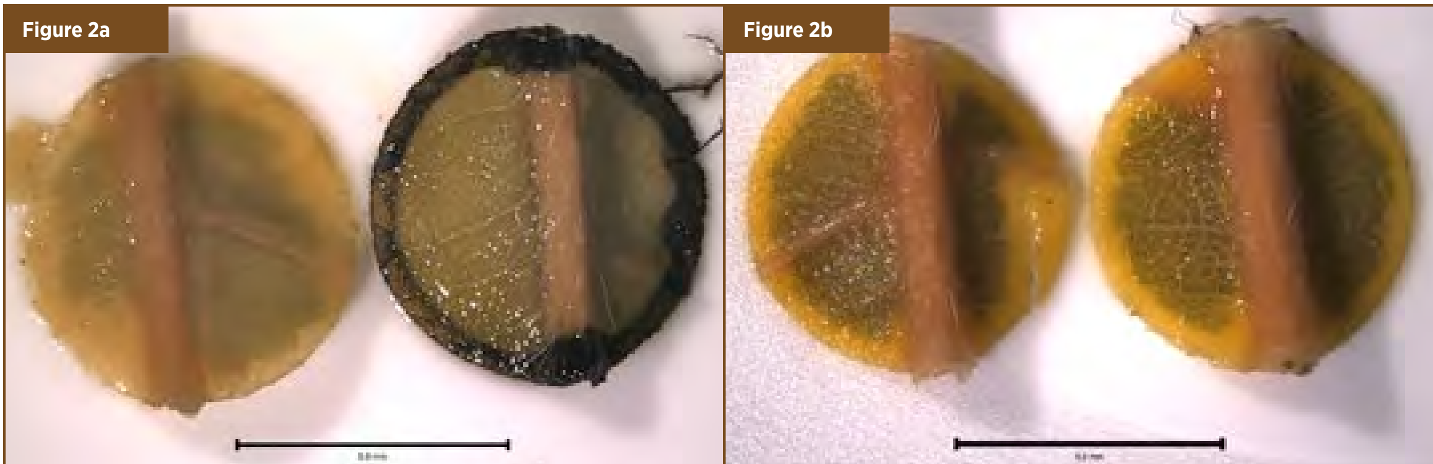
First generation progeny of a cross between a blight-tolerant transgenic American chestnut containing oxalate oxidase and blight-susceptible wild-type American chestnut. Both progeny were inoculated with a highly virulent strain of the fungus that causes chestnut blight (Cryphonectria parasitica). The progeny on the left inherited the oxalate oxidase gene, while the progeny on the right did not inherit the gene. Photo courtesy of William Powell

refers to a line of trees with the OxO gene inserted into a different location of the American chestnut founder trees' genome. The reason for advancing only a few transgenic events (and therefore founder trees) through the regulatory review process is that regulators must evaluate each event separately, making the review process more onerous with each additional event. From a biological standpoint, it is not desirable to have a breeding population composed of progeny from many different OxO insertion events. If these trees were to intercross, their progeny could inherit many copies of the OxO gene at different locations in the genome. It is possible that with many copies of OxO, the American chestnut trees would actually shut down the production of the oxalate oxidase through a process called gene silencing (Rajeevkumar *et al.* 2015).

More than one generation of outcrossing transgenic trees to wild-type American chestnut trees will be required to dilute out the transgenic founder tree's genome and to increase genetic diversity and adaptive capacity represented in the blight-tolerant population. After the first generation of outcrossing transgenic trees to wild-type American chestnuts, the progeny will inherit 50% of their genome from the transgenic founder tree and 50% from wild-type trees. If those first generation progeny were then to intercross, their progeny would be inbred due to the large proportion of their genomes inherited from the transgenic founder parent(s). Inbreeding can lead to mortality and reduced vigor due to the large number of deleterious mutations that outcrossing tree species such as American chestnut carry in their genomes (Wright *et al.*

Figure 2a

Figure 2b



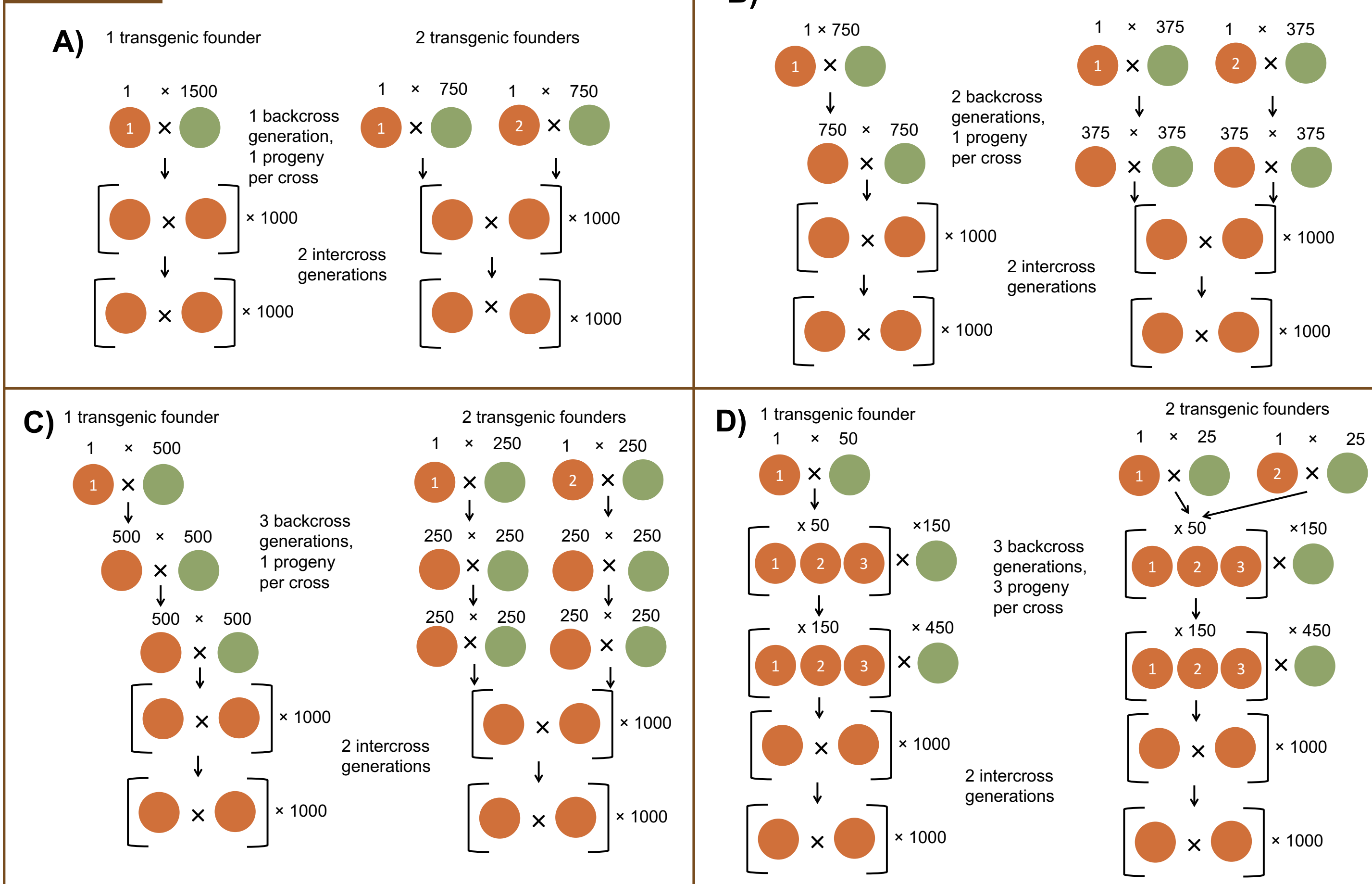
Leaf disk assay for the inheritance of oxalate oxidase among progeny of transgenic American chestnut that were crossed with wild-type American chestnuts. If a tree has inherited the oxalate oxidase (OxO) gene, a dark stain forms on margins of the leaf disk in the presence of oxalic acid in the staining solution (Figure 2a). If the tree did not inherit OxO, then no staining occurs at the leaf margins (Figure 2b). The leaf assay is also performed without oxalic acid in the staining solution to ensure that the staining is a result of oxalate oxidase activities in leaves (Figures 2a-b left). Photo courtesy of William Powell

2008, Charlesworth & Willis 2009). Outcrossing transgenic trees to distantly related American chestnut individuals increases the probability that functional alleles for each gene are inherited from at least one parent. Researchers at ESF have started the process of outcrossing transgenic trees to wild-type American chestnut trees. In permitted field trial plots, four wild-type parents have been used as parents in first generation crosses. Transgenic progeny from two of these wild-type parents have been crossed with 22 additional wild-type trees to generate second-generation transgenic progeny (William Powell & Andrew Newhouse, personal communication). Offspring from early crosses like these will be available for personal and small-scale plantings almost immediately after regulatory permission is granted for distribution. The next step for large-scale forest plantings is to estimate how many outcrosses are needed to produce a diverse and regionally adapted restoration population.

American chestnut pedigrees were simulated to determine how the number of transgenic founders, the number of generations of breeding transgenic with wild-type trees, and the number of progeny per cross used as parents in subsequent generations influences the effective population size and the average inbreeding coefficient after intercrossing the transgenic progeny. Effective population size (Ne) is proportional to genetic diversity and inversely related to the amount of inbreeding in the population. The inbreeding coefficient (F) refers to the probability of inheriting two copies of the same allele from both parents averaged across the entire genome. The American Chestnut Foundation aims to increase the effective population size of the transgenic blight-tolerant population to more than 500. This does not mean that only 500 individual trees will be produced, but rather refers to a hypothetical number of genetically unique tree lines that could be used to establish restoration populations. A target effective population size of 500 is based on the hypothesis that populations of this size have sufficient adaptive variation so as to be at minimal long-term risk of extinction (Jamieson & Allendorf 2012). We also aim to reduce the inbreeding coefficient upon intercrossing to less than 0.05 to minimize mortality and reduction in tree vigor and reproduction due to inbreeding (Hedrick & Kalinowski 2000).

The effective population size and average inbreeding coefficient were estimated starting with 1 or 2 transgenic founder trees, outcrossing these founder trees with wild-type American chestnuts over 1, 2, or 3 generations, using 1 or 3 transgenic progeny per cross as parents in subsequent generations, and intercrossing the transgenic progeny over two generations (**Figure 3**). The maximum number of wild-type American chestnuts available for breeding with transgenic trees was fixed at 1500. An equal number of wild-type parents were used in breeding in each generation such that with one generation of breeding, 1500 wild-type trees were bred with transgenic founder(s), with two generations, 750 wild-type parents were used in each outcross generation, and with three generations, 500 wild-type parents were used in each generation. In scenarios involving two transgenic founders, half of the wild-type parents were bred with each transgenic founder. Only one progeny per cross was used in breeding with the next generation in scenarios in which transgenic trees were outcrossed with equal number of wild-type parents in each generation. A final scenario was simulated in which transgenic founder(s) were outcrossed over three generations with 50, 150, and 450 wild-type trees. Three progeny per cross were used in breeding in subsequent generations. The purpose of this simulation, called "fanning-out" transgenic pedigrees, was to determine if breeding with fewer wild-type parents (650 rather than 1500) and instead using multiple progeny per cross resulted in a similar effective population size as compared with using equal numbers of wild-type parents in each generation. In all scenarios, each transgenic progeny was crossed with a unique wild-type American chestnut that is unrelated with other wild-type parents. After 1, 2, or 3 generations of outcrossing to wild-type trees, the resulting progeny were randomly intercrossed to generate 1000 progeny. Each simulation scenario was repeated 10 times to estimate variation in the effective population size

Figure 3



American chestnut pedigrees were simulated in which one or two transgenic founder trees (red dots) were outcrossed once to 1500 wild-type trees (green dots) (A), outcrossed over two generations to 750 wild-type trees per generation (B), outcrossed over three generations to 500 wild-type trees per generation (C), or outcrossed over three generations to 50, 150, and 450 wild-type trees per generation (D). In scenarios A – C, one progeny per cross was used as a parent in the subsequent generation. In scenario D three progeny per cross were used as parents in the subsequent generation. In all scenarios, effective population size and average inbreeding coefficients were estimated after the second generation of random intercrossing among transgenic progeny.

and inbreeding coefficients due to random intercrossing among the transgenic progeny. Simulations were performed in the R computing environment (R Core Team 2013).

Effective population size was estimated for all individuals and all generations in the pedigree using two methods detailed in Leroy *et al.* (2013): 1) individual inbreeding rate (N_eF_i) and 2) individual co-ancestry rate (N_eC_i). The individual inbreeding rate estimates how trees' inbreeding coefficients change over multiple generations of outcrossing. The individual co-ancestry rate estimates how the the average relatedness between pairs of trees changes over multiple generations of outcrossing. Both inbreeding and co-ancestry are inversely proportional to effective population size. Inbreeding coefficients and pairwise co-ancestry coefficients were calculated with the 'pedigree' package for R (Costar 2013).

Average inbreeding coefficients were estimated among individual trees in the second intercross generation, which is the generation we expect to deploy for restoration. The reason for intercrossing the transgenic progeny after breeding with wild-type parents is that ¼ of progeny of the first intercross that are expected to inherit OxO from both parents. These homozygous blight-tolerant trees will then be planted in a seed orchard so that all seed coming from the second intercross will also be blight-tolerant.

With 1,500 unrelated wild-type American chestnuts available for breeding, a minimum of three generations of outcrossing to wild-type trees was required to reach an effective population size of 500 (**Figure 4a**). Notably, fanning out transgenic pedigrees over three generations (i.e., 50, 150 & 450 wild-type parents, 3 progeny per cross) resulted in a population with a greater effective population size than outcrossing over three generations to an equal number of wild-type parents in each generation (i.e., 500 wild-type parents per generation, one progeny per cross used as a parent in subsequent generations). The estimates of N_e from co-ancestry rates (N_eC_i) were smaller than N_e

Figure 4a

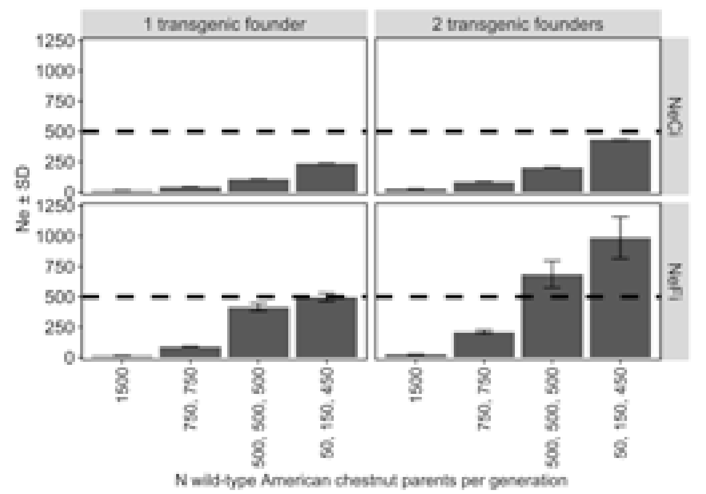
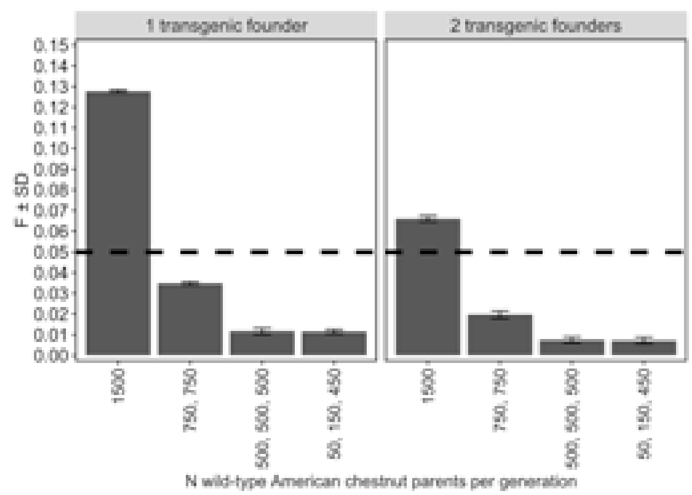


Figure 4b



Estimates of effective population size (Ne) (A) and the average inbreeding coefficient (F) (B) after one or two transgenic founder trees were backcrossed with wild-type American chestnuts over one generation (1500 wild-type parents per generation), two generations (750, 750 wild-type parents per generation) or three generations (500, 500, 500 or 50, 150, 450 wild-type trees per generation). In the first three scenarios involving equal numbers of wild-type parents per generation only one transgenic progeny per cross was used as a parent in the subsequent generation. In the scenario involving increasing numbers of wild-type parents in each generation (50, 150, and 450 parents), 3 progeny per cross were used as parents in the subsequent generation. The backcross progeny were randomly intercrossed over two generations. The average inbreeding coefficient was estimated in the second intercross generation. Simulations were repeated 10 times to obtain the mean and standard deviation (SD) of Ne and F after random intercrossing in each repetition.

estimates from inbreeding rates (N_eF_i) by a ratio of N_eC_i: N_eF_i of 0.55 averaged across simulation scenarios. Increasing the number of transgenic founders from one tree to two trees increased N_eC_i from 236 to 432 and N_eF_i from 495 to 987 in the fanning-out scenario. A minimum of two outcross generations was required to reduce the inbreeding coefficient in the second intercross generation to less than 0.05 regardless of whether the population was derived from one or two transgenic founders (**Figure 4b**). After three generations of outcrossing to wild-type trees the average inbreeding coefficient was reduced to approximately 0.01 regardless of whether one or three progeny per cross were used as parents in subsequent generations.

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 3 outcross generations + 2 intercross generations. To speed up the generation time, TACF and ESF will conduct collaborative experiments to accelerate flowering of transgenic and wild-type American chestnuts with light treatments. American chestnut trees have been demonstrated to produce pollen in their first growing season when the plants are grown in a high light growth chamber (Baier et al. 2012). Using the transgenic trees as the pollen parents and wild-type trees as the seed parents is ideal for outcrossing and diversifying blight-tolerant populations. The pollen of transgenic trees does not contribute to the chloroplast and mitochondrial genomes of the offspring and therefore rescues the cytoplasmic diversity of the wild-type mother trees. Growing 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.

Figure 5

A first year Chinese chestnut seedling grown in high light growth chamber with both male flowers (catkins) and female flowers (burs). The burs did not produce viable seed but the catkins produced viable pollen. Photo courtesy of William Powell

The estimate of two years per generation assumes that the desired number of wild-type American chestnuts parents for each generation all produce female flowers in one growing season, and that all crosses for each generation could be completed in one year. It remains to be demonstrated how long it will take for American chestnut trees to produce female flowers and viable seed when grown under high light conditions that stimulate flowering. Female flowers have

Figure 6

Wild-type American chestnut scion grafted onto a mature American chestnut tree in an orchard to encourage early flowering (Photo courtesy of Andrew Newhouse).

developed on Chinese chestnut within one year in a high light growth chamber, but the plants were too small to develop viable seed (**Figure 5**). Research is underway to determine if female flower production could be accelerated by growing larger plants under high light treatments in a greenhouse. Top-work grafting of scion from wild-type trees onto mature orchard trees is a promising alternative method to accelerate the production of female flowers (**Figure 6**). Chinese chestnut scion top-work grafted onto mature trees produces female flowers and seed within one year (Zhu *et al.* 1998).

If the average number of years for American chestnut trees to develop both male and female flowers could be reduced from 7 years in an orchard to 1 growing season, the total time to complete three generations of outcrossing of transgenic trees to wild-type trees and one generation of intercrossing transgenic trees could be reduced to a minimum of 8 years assuming 2 years for seed-to-seed generation time. Progeny of intercrosses among transgenic trees that are homozygous for OxO will be planted in seed orchards, which will eventually produce seed for large-scale restoration. It will take approximately seven years for progeny of intercrosses to grow large enough to produce large numbers of seed for restoration. Thus, it will take a minimum 15 years from the time of regulatory approval to generate a diversified blight-tolerant population that is homozygous for OxO for use in large-scale restoration. It may take longer than 15 years if more than one year is required to complete the desired number of crosses from each generation.

Pending federal approval, TACF and ESF intend to breed transgenic founder(s) with approximately 50 backcross and wild-type American chestnut trees that are currently reproductively mature in TACF's orchards. Meanwhile TACF will propagate approximately 1000 additional sources of wild-type trees for outcrossing in the second and third generations assuming that some of these wild-type trees will die or will not flower. Methods for propagation of wild trees will include transplanting re-sprouted stems into orchards and greenhouses, grafting wild scion onto root stock, top-work grafting, and collecting seed from rare flowering wild trees. Research is currently underway to characterize the genetic diversity and local adaptation of remnant wild-type American chestnut across the species range. Results will inform TACF's efforts to obtain a sample of wild-type trees that adequately represents the genetic diversity and adaptive capacity remaining in American chestnut populations. ESF is developing methods to store and ship pollen, which could be distributed to citizen scientists interested in increasing genetic diversity using local wild-type mother trees.

In summary, simulations suggest that diversifying blight-tolerant transgenic American chestnut populations will be feasible for TACF volunteers and collaborators at SUNY-ESF. Outcrossing two transgenic founder trees over three generations to 50, 150, and 450 (650 total) wild-type trees appears to be an efficient method to diversify the blight-tolerant population to an effective size of approximately 500 individuals. Seed orchards will be composed of progeny of intercrosses among transgenic trees that inherited OxO from both parents so that all seed coming from these orchards will be blight tolerant.

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Reducing the Risk of Root-rot:

TAKING STEPS TOWARD UNDERSTANDING *PHYTOPHTHORA CINNAMOMI* DISTRIBUTION IN THE EASTERN US

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The American chestnut saga is a long and sad one – ravaged by chestnut blight, billions of trees that once were pillars of the forest community now lay desolate, like skeletons, their bones scattered across the eastern US (Anagnostakis, 2001). Unfortunately, while chestnut blight wrought havoc aboveground, *Phytophthora cinnamomi* crept north unawares, laying waste to the once-hearty roots untouched by blight (Anagnostakis, 2001). *Phytophthora cinnamomi* (Pc) is an oomycete, water mold, which produces zoospores capable of swimming in water (Hardham, 2005). Pc is thought to have originated in Southeast Asia (Arentz, 2017), but has since been introduced throughout the world, and causes dramatic declines in susceptible species in Australia, Europe, and North America (Sena *et al.*, 2018a). Pc zoospores swim toward roots of host plants, where they form cysts that adhere them to the root tissue (Hardham, 2005). The zoospores then form germ tubes that penetrate the host root tissue. In severe infections, Pc hyphae reach the plant's vascular tissue, which is responsible for transporting water and nutrients in between the roots and crown (Ruiz Gómez *et al.*, 2015). Pc rapidly grows within the vascular tissue, causing vascular cells to die and blacken, forming the inky lesions responsible for the disease's common name: ink disease. This rapid destruction of host vascular tissue within the roots reduces the plant's ability to draw up water, and causes the plant to wilt and die (Dawson & Weste, 1982).

FIGURE 2: A blight-resistant backcrossed American chestnut grows on a reclaimed surface mine in Pike County, Kentucky. Surface mined sites have been identified as priority restoration areas for American chestnut, because these sites are "*Phytophthora*-free" at reclamation.

Thanks to decades of intensive breeding efforts, American chestnuts with heightened genetic resistance to chestnut blight are available for planting (Diskin *et al.*, 2006); however, these improved varieties are not necessarily also resistant to Phytophthora root rot (PRR), and some early plantings of blight-resistant varieties experienced significant mortality because of PRR (Brosi, 2001). Additional breeding efforts are underway to improve PRR resistance in chestnut, and preliminary results are promising (Jeffers *et al.*, 2009). However, it is also critical to understand where Pc is found on the landscape, and in what kinds of environmental conditions Pc is likely to cause disease.

To better understand where Pc is likely to be found in eastern US forests, it is important to have access to rapid and sensitive detection assays. Typically, Pc is detected by flooding a soil sample to stimulate zoospore production, baiting with susceptible plant material, and then isolating in culture (Jeffers & Martin, 1986). This process can be time and space-consuming, requiring setting up the baiting process, preparing sterile growth media, transferring baits to media, and making additional transfers to prepare isolates for identification based on morphology. Each of these steps requires lab space for baiting, culture growth, and sterile preparation space. Investigators would benefit from a detection approach that reduces time and space requirements.

To address this need, we hybridized the standard approach – baiting soils with rhododendron leaf discs, with a molecular detection approach using Polymerase Chain Reaction (PCR) to detect *P. cinnamomi* (Kunadiya *et al.*, 2017) from DNA extracted for leaf baits, (**Figure 1**, Sena *et al.*, 2018c). We then compared the traditional culture-based method (baiting with rhododendron leaves followed by culturing on selective medium and morphological identifications) with the hybrid bait/PCR method (baiting with rhododendron leaf discs, followed by DNA extract from leaf discs and Pc-specific PCR), we found that Pc detection rates were similar across these methods for soils from 47 plots in an eastern Kentucky forest. Thus, the hybrid bait/PCR method demonstrated similar sensitivity to the conventional approach, but was more rapid, less costly and more convenient. Also, while Pc is traditionally associated with moist, low-lying soils (Wilson *et al.*, 2003), our screening detected Pc in a total of 21 of 47 plots sampled, representing a range of environmental conditions and landscape positions (e.g., both drier ridge-top sites and moist low-lying sites). Further research will be necessary to clarify the environmental and landscape factors that influence distribution of Pc.

We employed this hybrid detection approach to screen soils from reclaimed mine sites. Surface mining for coal is an incredible force of land-use change in the Appalachian region; an estimated 1 million hectares of Appalachian land have been surface-mined for coal and reclaimed to non-native grassland (Zipper *et al.*, 2011). This vast range of land requires significant restoration effort for reforestation, and may present opportunity for restoration of chestnut (**Figure 2**, previous page)(French, 2017). When initially reclaimed, soils on surface mined sites in Appalachia typically have very little organic matter. While native soils have diverse microbial communities, these reclaimed mine soils are essentially free of microbes—but microbes colonize over time. Recent studies have demonstrated that levels of soil microbial activity only eight years after reclamation are similar to microbial activity levels in forest soils a similar time after clear-cut operations (Littlefield *et al.*, 2013). Several studies have found that chestnuts are capable of rapid growth and high survival on reclaimed surface mined sites (French *et al.*, 2007, McCarthy *et al.*, 2010, Skousen *et al.*, 2013, Bauman *et al.*, 2014); however, it is unknown whether these sites will be conducive for Pc over time.

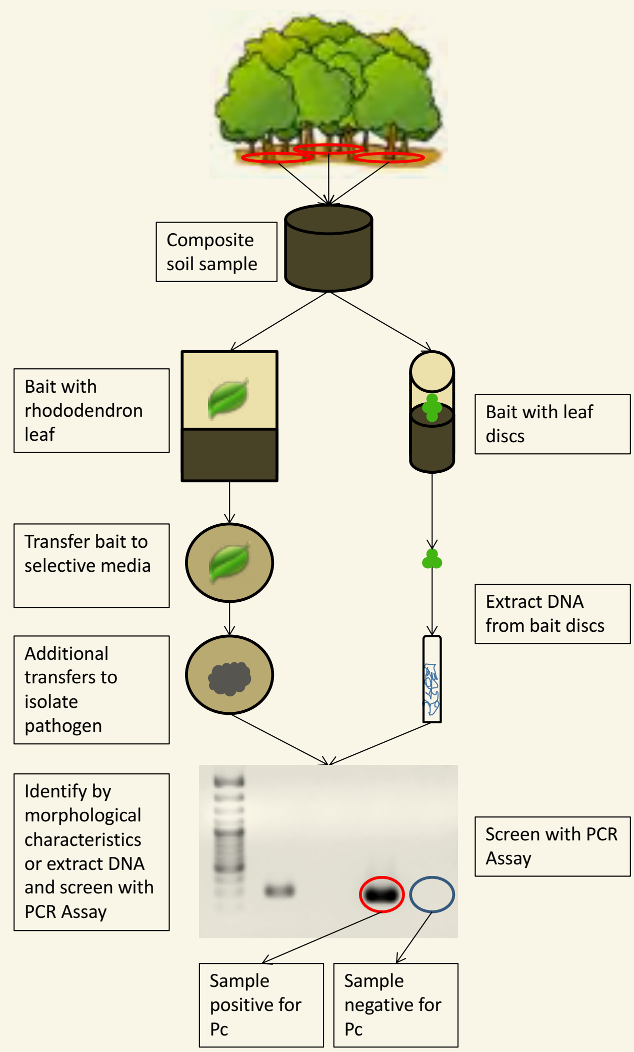

FIGURE 1: Comparison of methods for detection of *Phytophthora cinnamomi* (Pc) from soil samples (Figure modified from Sena et al., 2018c).

In our survey, we screened reclaimed mine sites in eastern Kentucky representing a range of time since reclamation (10, 12, 15, and 18 years). Two sites at each age (20 samples per site, 8 sites total) were screened for Pc incidence, and results were interpreted as percent of samples positive. We detected Pc in mined sites 15 and 18 years after reclamation, but not in sites 10 and 12 years after reclamation (**Figure 3**) (Sena *et al.*, 2018b). It is well-known that soil organic matter accumulates on these sites over time – planted trees continue to grow, contributing organic matter to the soil through roots and leaf litter. As these trees develop a canopy, they produce shade, increasing soil moisture and further improving soil conditions for microbial growth (Sena *et al.*, 2015). Our study demonstrates that Pc is capable of colonizing these sites over time, but it is as yet unknown whether these sites are also suitable for development of ink disease in chestnut and other susceptible species. In some cases, Pc is known to be present in an area, but to not cause disease in susceptible host species (Corcobado *et al.*, 2014, McConnell & Balci, 2014). Soil moisture and microbial community composition are known to influence Pc and disease severity in susceptible hosts (Malajczuk *et al.*, 1983), but these conditions have not been characterized on reclaimed surface mined sites, and further research is necessary.

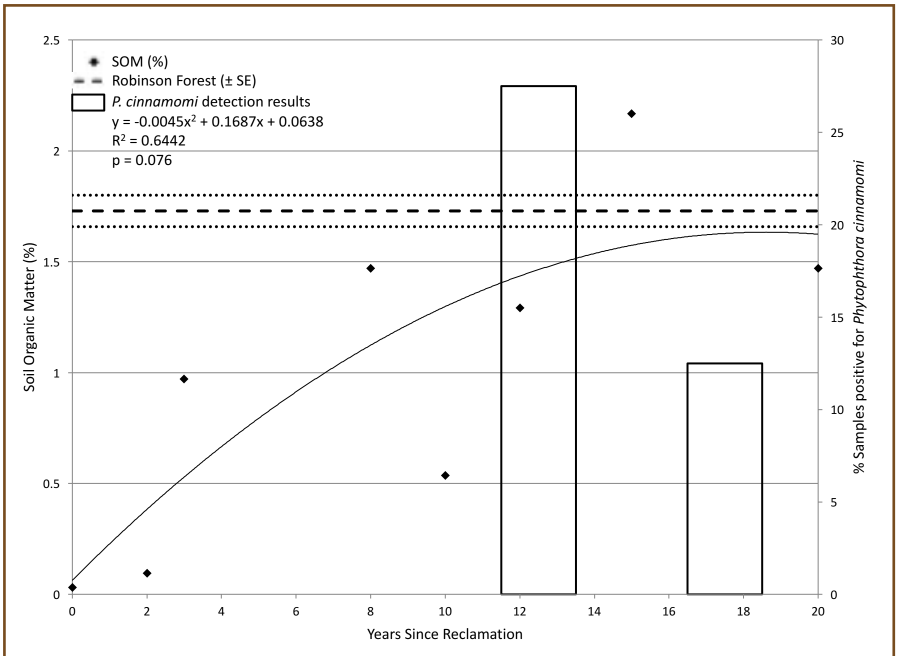

FIGURE 3: Soil organic matter (SOM) accumulation and *Phytophthora cinnamomi* (Pc) incidence on reclaimed surface mined sites representing a range of time since reclamation. (Figure modified from Sena et al. 2018b).

The hybrid Pc detection method will allow better characterization of Pc distribution, by reducing the amount of effort required to process samples. Our initial study of a native forested watershed in eastern Kentucky found Pc at approximately half of the sampling locations and all slope positions, including dry ridge tops. We also found Pc on reclaimed mine lands after 15 years after reclamation. Taken together our preliminary studies indicate that avoiding losses due to ink disease by planting chestnut on dry ridge tops or on mined lands to avoid Pc might not be a successful chestnut restoration strategy if resistance to ink disease is not also deployed.

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Sampling the Mycorrhizae

OF OZARK CHINQUAPIN IN ARKANSAS

By Fred Paillet, University of Arkansas
In memory of Mourad Hassine

After relocating to my current position at the University of Arkansas, it was natural for an old American chestnut enthusiast to develop an interest in an endemic relative of chestnut growing in that area – the Ozark chinquapin (Bost and Paillet, 2010). It came as a bit of a surprise to find that our native chinquapin was a substantial tree in contrast to the shrubby Allegheny chinquapin, with which the species is regularly confused (Paillet and Cerny, 2012). The remains of massive old Ozark chinquapins killed when the bight arrived here in 1957 provide ample evidence in that regard (**Figure 1**). Documenting the growth form and distribution of these old remains has provided many hours of pleasant outdoor experience for myself and some of my students.

Leaves and maturing burs of the Ozark chinquapin in northwest Arkansas; each bur will contain a single acorn-sized nut in contrast with the larger American chestnut burs that typically contain three nuts.

An unusual opportunity to further investigate local chinquapin ecology arose when a colleague introduced a visiting postdoctoral fellow, Mourad Ben Hassine (**Figure 2**), with a special interest in the mycorrhizae that are such an important factor in the health of our forests. Mycorrhizae are associations between a fungus and the root system that almost all forest trees require for their roots to properly provide the nutrients and water that they need to grow (**Figure 3**). Chestnut researchers involved in planting chestnut as part of the reclamation of mine spoil understand that the inoculation of purely mineral-based soils with mycorrhizal species of fungi is critical to the establishment of healthy trees. After talking with Mourad and his advisor, Dr. Steve Stevenson, I thought that helping Mourad to study Ozark chinquapin mycorrhizae and perhaps comparing his results with results from American chestnut might be an interesting project. I was able to provide Mourad with transportation and tools (trowel and shovel) as I learned from him about collection of mycorrhizal samples in the field. All we would need was a small grant, which TACF generously provided through its External Grants Program, to cover the miscellaneous expenses of chemicals and lab work required to identify the various mycorrhizal species that were collected.

Samples were obtained in the field over the summer of 2016 (**Figure 4**). This sounds easy, but the devil was in the details. Forest soil is full of root tips. In order to insure that chinquapin roots were collected, we had to follow roots from the base of a sprout clone out to where root tips and their mycorrhizal bulblets could be sampled along with small parcels of the soil surrounding the root tips. Each such sample was placed in a small bottle with a stabilizing fluid and then processed in the laboratory to yield DNA samples with which to determine the particular fungal species (at least to genus) present in

FIGURE 1: Stump of a large Ozark chinquapin killed by the original chestnut blight pandemic that arrived in northwest Arkansas in 1957.

FIGURE 2: Fred Paillet (right) and postdoctoral fellow Mourad B Hassine whose untimely death from an undiagnosed coronary ailment curtailed the chinquapin mycorrhizal study before the work could be extended to compare results with samples of mycorrhizae from American and European chestnut.

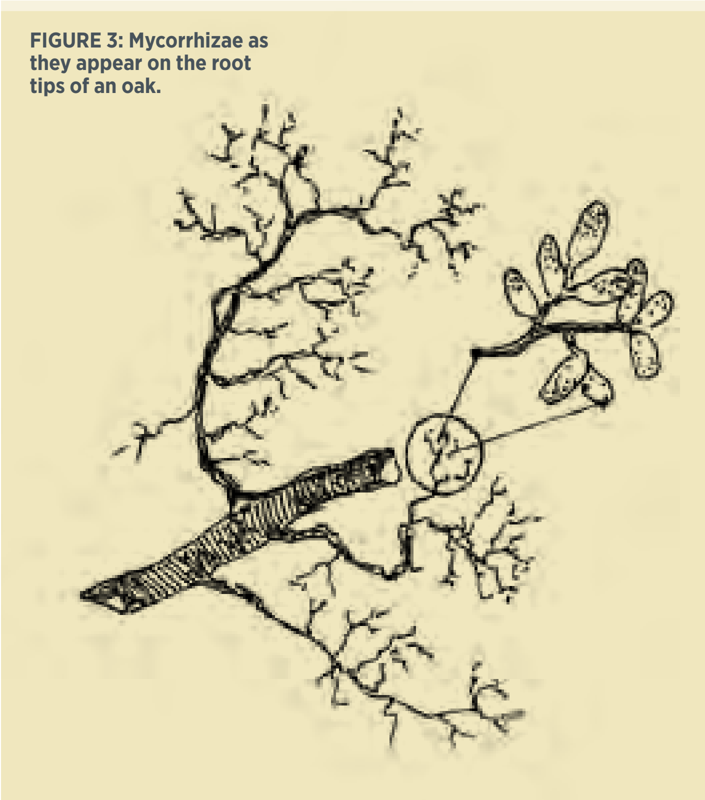

FIGURE 3: Mycorrhizae as they appear on the root tips of an oak.

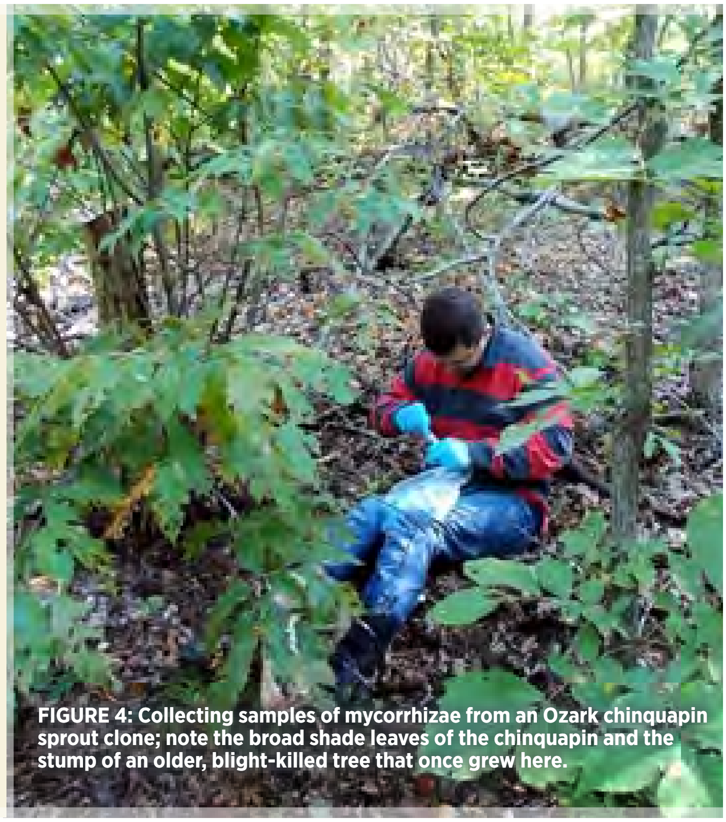

FIGURE 4: Collecting samples of mycorrhizae from an Ozark chinquapin sprout clone; note the broad shade leaves of the chinquapin and the stump of an older, blight-killed tree that once grew here.

the sample. Root tip samples were collected from 18 chinquapin clones at three different study sites, yielding 42 species of fungi, 40 of which were known or suspected to form mycorrhizae. Some of the species of fungi were new records for the Ozarks. The diversity of the mycorrhizae associated with Ozark chinquapin was indicated by the fact that none of the taxa identified in this study were found at more than one of the three sites. Root tip samples from four chestnut clones in Tennessee were also obtained as part of this study (Stephenson *et al*, 2017). The main conclusion from the analysis was that the list of species of mycorrhizal fungi associated with Ozark chinquapin was much more diverse than that for chestnut, even after allowing for the smaller sample size of the latter.

These results were expected to be just the beginning of a continuing study. However, we were saddened to hear that Mourad had unexpectedly passed away during a visit to his home in Tunisia last year. We had hoped that he would be able to do much more with the results on hand and analysis of additional chestnut root samples in the future. As it is, we have had an interesting peek into the subsurface world of the Ozark chinquapin, expecting more surprises in the future from a species shown to have deep roots in time as determined by genetic analysis of the origins of *Castanea* in the ancient past.

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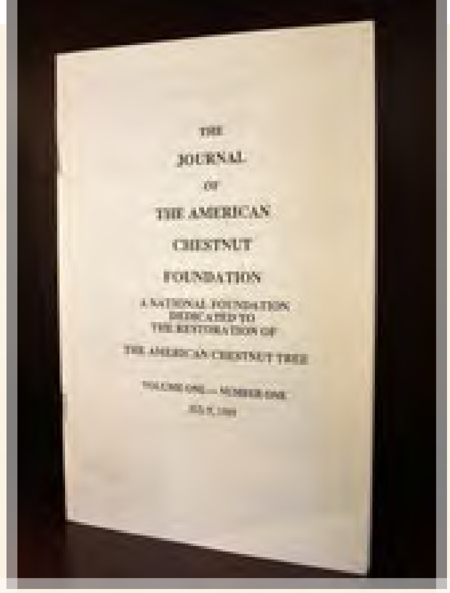

A Third of a Century of

The Journal of The American Chestnut Foundation

By Donald C. Willeke, First "Non-scientist" Member, former Vice Chair and Secretary, and a Director and General Counsel of the Foundation (and First Editor of *The Journal*)

It is hard to believe that *The Journal of The American Chestnut Foundation*, now known as *Chestnut*, has been published for a third of a century. But that's the truth! I was the first " non-scientist" member of The American Chestnut Foundation (TACF) when it was formally established in Minnesota (yes, Minnesota!) in 1983, although its founders and I had met for several years before creating the Foundation. All other founders were scientists with "Dr." in front of the names of most. I'm a lawyer, but I was Chair of Minnesota's Urban Forest Council, and knew most of them from working on urban forestry issues. We formed a foundation because we knew we needed an institution that would last as long as necessary to bring back the American chestnut, and not be dependent on budgets of governments or universities, since we had just observed losses of a number of chestnut research projects.

Several years of our Board's meetings convinced us that we could create a self-sustaining research effort. So, we began to talk about promoting our work. Establishing a formal publication for TACF was suggested by some directors, including me, as I had been an editor of *The Iowa Law Review* when I was in law school. Having "opened my big mouth," the others suggested that I attempt to put together a prototype "first issue." I had just acquired a new type of computer, a "Lisa," built by a company called "Apple." The Lisa was the predecessor of the Macintosh and cost the then-princely sum of $7,800. It was a marvel that could handle many typefaces and produce work that looked like it had been done by a printer. So, I set to work and in July 1985, produced "Volume One, Number One" of *The Journal*. I typed every word in it! Although modest compared to *Chestnut*, it contained several scientific articles, one by Drs. Charles Burnham, Mary Hosier and Paul Read on TACF's breeding plan, and another by Dr. William MacDonald on hypovirulence. TACF's President, Philip Rutter, wrote the first President's Message, explaining our goals and appealing for help. We printed several thousand copies of the 18-page issue and sent them out.

From that modest beginning, a fine publication has developed and lasted 33 years! I'm proud to have been "present at the creation," and to still be participating in what we know is the effort of a lifetime, or more.

Wild Rice Pilaf with Mushrooms, Chestnuts and Cranberries

From *The Sioux Chef's Indigenous Kitchen* by Sean Sherman with Beth Dooley
(University of Minnesota Press, 2017) Copyright 2017 Ghost Dancer, LLC.


Photograph by Mette Nielsen.

Ingredients

1 cup roasted, peeled, chopped chestnuts
2 tablespoons sunflower or walnut oil
1 pound assorted mushrooms, cleaned
1 tablespoon chopped sage
1/2 cup chopped wild onions or shallots
1/2 cup corn stock or vegetable stock
2 cups cooked wild rice
1/2 cup dried cranberries
1 tablespoon maple syrup to taste
1/2 to 1 teaspoon smoked salt to taste

Method

Roast and peel the chestnuts: pre-heat the oven to 350°F. Use the sharp point of a small knife to score an X on the flat side of the chestnuts and place them on a baking sheet. Roast in the oven until the skins begin to peel back. The length of roasting time will depend on the freshness and size of the chestnuts and ranges from about 10 to 25 minutes. Remove the chestnuts, and when they're cool enough to handle, peel.

Make the pilaf: in a large skillet, heat the oil over medium-high heat and add the mushrooms, sage, and onions. Cook, stirring, until the mushrooms are nicely browned and the onions are soft, about 5 minutes. Stir in the stock, wild rice, and cranberries and cook until the liquid is nearly evaporated. Stir in the roasted chestnuts. Season with maple syrup and smoked salt to taste.

Yield

4 to 6 servings

IN MEMORY AND IN HONOR OF OUR TACF MEMBERS

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Other special speakers, educational sessions, a lively panel discussion, and Saturday's 35th Anniversary Gala Dinner will all be part of this celebratory event.

For hotel reservations, event details, registration, and to purchase raffle tickets, visit acf.org.

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