FALL 2019 ~ ISSUE 3 VOL. 33

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

A BENEFIT TO MEMBERS

THE AMERICAN CHESTNUT FOUNDATION®

Mud Packing ~ Science Meeting ~ American Chestnut Natural Range ~ Breeding Plan ~ Hypovirulence

FALL 2019 ~ ISSUE 3 VOL. 33

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A BENEFIT TO MEMBERS

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Lisa Thomson

President and CEO

DEAR CHESTNUT FRIENDS,

The first half of 2019 was marked with exciting new scientific discoveries, which shed new light on our breeding program, coupled with unique external challenges, making us resolute to stay the course towards mission success. As a result, we are emerging out of this time stronger and more determined than ever to use all of the resources and research at our disposal to ultimately restore the American chestnut species.

In April of this year, after extensive data analyses based on genomic testing and mapping, our director of science Dr. Jared Westbrook, along with his scientific collaborators, learned that the inherited traits for blight resistance are much more complex than originally hypothesized in the Burnham Plan, created decades ago. Since our founding in 1983, the field of genomics has burgeoned in scope and affordability, which we have embraced to unlock the answers to blight resistance at the molecular level. These new discoveries would not be possible without the confidence and generous support from a great number of private foundations, philanthropists, donors and members.

In July, 73 chapter science leaders, board members and staff gathered in Abingdon, VA to help chart a new course in the breeding program. The atmosphere of this meeting was one of renewed energy and resolve (see page 12). We are intentionally embracing new technologies to ensure we are not only using one pathway to our goal of restoring a keystone species. Multiple pathways is the theme behind our **3BUR** science protocol: **B**reeding, **B**iotechnology and **B**iocontrol **U**nited for **R**estoration, now being implemented in real time. It is going to take all the methods in 3BUR to bring back our tree. To learn more, read the article by Dr. Westbrook on page 23 about our collective path forward. We all agree that this is an audacious, long-term conservation rescue mission never before attempted at this scale, which is why we need to use all available knowledge and resources to reach our goals.

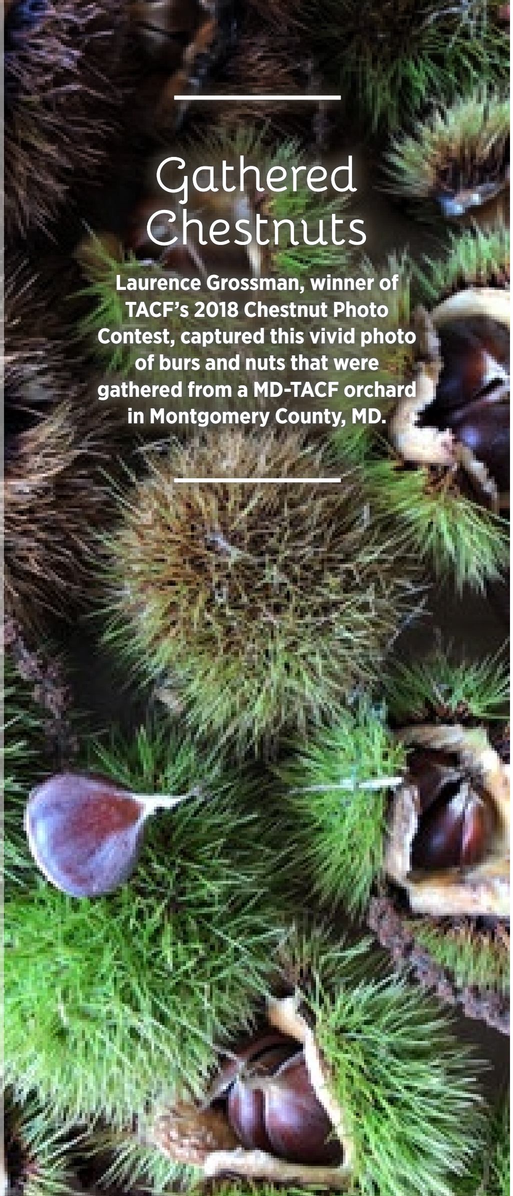
Let us continue to embody our core values: Optimism, Patience, Science-based Decisions, Innovation, Integrity, and Collaboration. Our mission is based on hope and this will carry us forward to overcome any challenges we may face. We will continue keeping you informed of our progress through the e*Sprout* newsletter and updates on social media. Thank you for staying with us throughout this amazing journey; you are the soul of this foundation and we need each one of you to support us as we continue to grow and thrive.

With gratitude,

Lisa Thomson

Lisa Thomson, President and CEO
The American Chestnut Foundation

Follow me on Twitter (@MadameChestnut).



Gathered Chestnuts

Laurence Grossman, winner of TACF's 2018 Chestnut Photo Contest, captured this vivid photo of burs and nuts that were gathered from a MD-TACF orchard in Montgomery County, MD.

DO WE HAVE YOUR EMAIL ADDRESS?

Share it with us to receive additional updates about our work as well as exclusive TACF member benefits. Email **chestnut@acf.org** so you can begin receiving these electronic communications!

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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Wild American chestnut discovered by Erik Carlson in upstate NY. Photo by Erik Carlson.

Mud Packing:

A SIMPLE METHOD FOR THE PRESERVATION OF BLIGHT-SUSCEPTIBLE AMERICAN CHESTNUT

By Erik Carlson, Research Project Assistant and Vernon Coffey, Research Support Specialist, State University of New York College of Environmental Science and Forestry (SUNY-ESF), Syracuse NY

In the effort to restore the American chestnut, three main techniques have taken precedence: biotechnology, breeding, and biocontrol (the 3BUR approach). While biotechnology and breeding focus on the production of new resistant trees, biocontrols are methods of directly treating the effects of blight infection on susceptible trees.

The future of the American chestnut will focus on the production of resistant trees, but in the meantime, non-resistant wild type American trees must be maintained in order to produce pollinated crosses. These trees are susceptible to blight and will eventually become infected and suffer from cankers. In order to keep these trees healthy enough to grow to sufficient size to produce flowers and nuts, the effects of blight need to be minimized as much as possible. At SUNY-ESF, we are employing this technique to help maintain mature wild-type "mother" trees for crossing with the 'Darling' blight-tolerant American chestnut trees. This outcrossing is important to build a genetically diverse and regionally adapted restoration population.

Mud packing, aka soil compress, is a simple biocontrol technique first described by Weidlich in the Proceedings of the International Chestnut Symposium in 1978[1] that takes advantage of a familiar phenomenon: the chestnut's ability to survive underground. Although blight is capable of killing American chestnuts above ground, the tree has the ability to produce new sprouts from the stump. This is possible because the blight is unable to kill the roots and underground portions of the tree. The parts of the tree that are beneath the soil are protected from the pathogen by the teeming diversity of natural soil microbiota. The pathogen is unable to compete with the overwhelming presence of microbes or possibly particular antagonistic microbes. Mud packing takes advantage of this mechanism and raises the soil to places on the tree where blight has taken hold in order to mitigate the infection. More background information on mud packing can be found here: http://bit.ly/2MUgkT1.

How to mud pack

When a blight infection on a stem is discovered, it's better to act sooner rather than later in order to minimize damage to the tree. That said, even large cankers can be treated with this method. A description of mud packing can be found at this link: http://bit.ly/2Th4gwk. Below is the method we use with slight modifications.

First, the surface of the canker should be removed with a sharp tool. A variety of tools can be used to accomplish this such as a utility knife or a draw knife. The tissue beneath the bark that is affected looks very different from healthy

[1]W. Weidlich, Proc. Int. Chestnut Symposium 1978. MUDPACK FOR CHESTNUT BLIGHT DISEASE CONTROL. A Preliminary Report on a Method of Biological Control of the Chestnut Blight Not Involving the Use of a Hypovirulent Strain of *Endothia parasitica*.

Figure 1 A large untreated canker, April 2018 (left); the same canker after carving off blighted tissue (center); healed canker after one growing season, October 2018 (right).

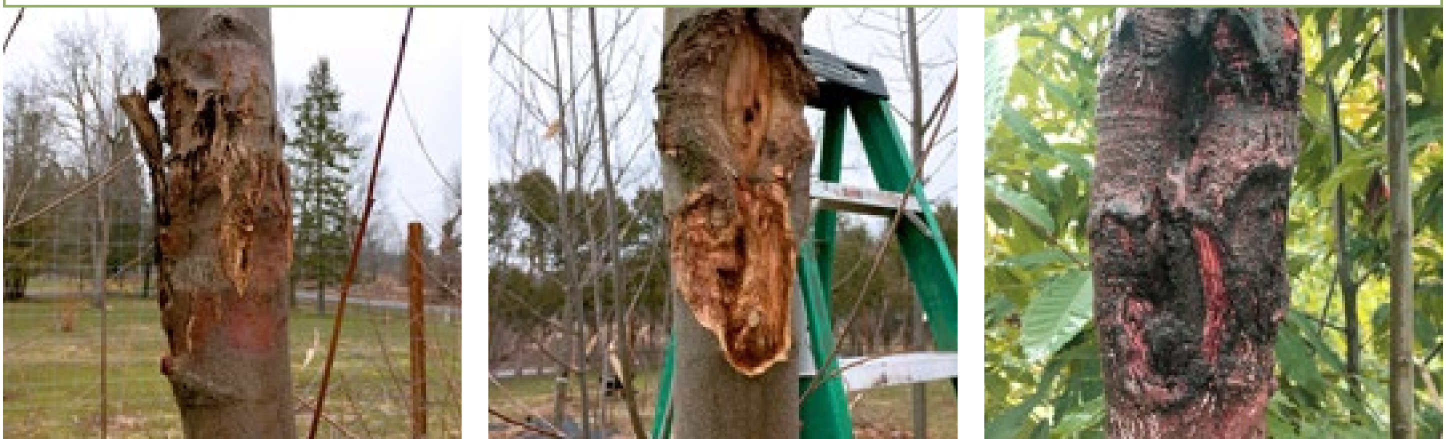


Figure 2 A severe sunken canker originating from branch; Fig 2.2 Branch is removed and surface tissue removed with utility knife; Fig 2.3 Mud is pressed tightly on carved out canker; Fig 2.4 Mud pack covers wound; Fig 2.5 Plastic is wrapped tightly around mud pack compressing soil into wound; Fig 2.6 Plastic is sealed and secured with tape on top and bottom.

tissue; the border between a canker and healthy tissue beneath the bark is a clear demarcation of brown (dead) tissue to green (healthy) tissue. The removal of this outer layer will expose the blight fungi within the stem tissue to the microbes in the soil. If the outer layer of bark is not removed, there will be a chunk of dead rotting tissue stuck in the tree when you remove the mud pack.

Once the outer surface is removed, moistened soil should be pushed into the wound firmly. The soil should be very wet, as its liquidity will help fill every nook and cranny the blight is hiding in. The source of the soil can vary but most sources should contain a large quantity of living microbes.

After the mud is packed into the hollowed canker, it needs to be sealed in. To do this, plastic is wrapped around tightly to compress the mud into the wound. A number of different items can be used for the plastic source, such as plastic bags or plastic shrink wrap. The bottom and top of the plastic-wrapped pack are then sealed with tape. It's important to use tape or something stretchable; plastic zip ties can cut into the stem of the tree as it grows. Mud packs are typically left on the trees over the course of a growing season, with cankers being inspected in the fall.

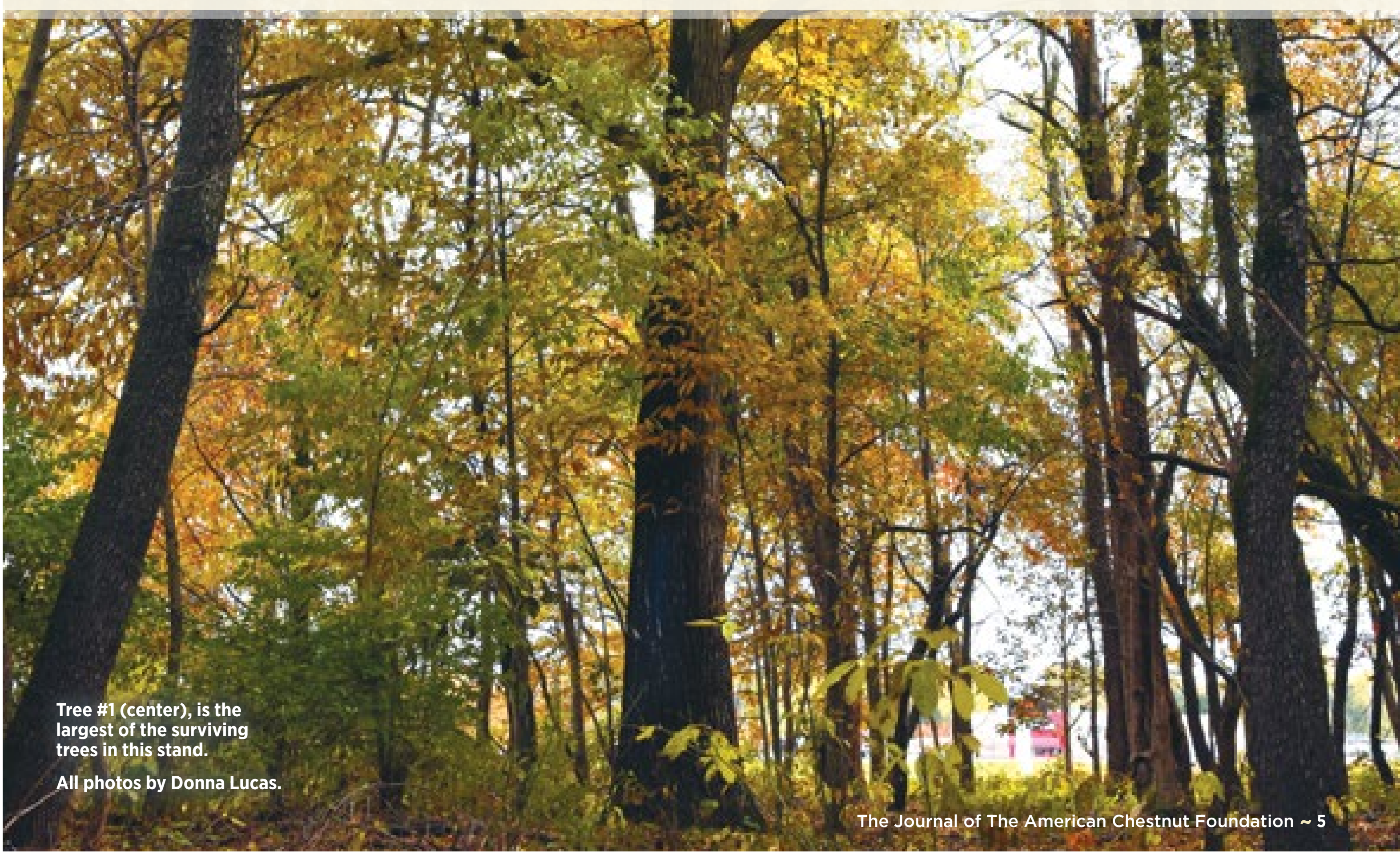
At SUNY-ESF, we have had a high success rate using this technique. In Weidlich's original report in 1978, he accomplished nearly 100% success rate in healing cankers with his mud packs, and in our limited usage we have seen similar results. I hope this article will encourage others to implement this technique in their orchards to help preserve their invaluable American chestnut mother trees.

Surviving American Chestnut Trees

IN ROSELAWN, INDIANA

By Bruce Wakeland, Indiana Chapter

In the summer of 2001, I received a call from a hunter who thought he found some American chestnut trees in an area he planned to hunt. The more questions I asked, the more convinced I became that the gentleman had indeed discovered American chestnut trees. I broke with my normal practice of having callers mail in leaf samples, contacted the landowner, got permission to access the property, changed my schedule for the next day, and drove one-hour to the site. I was stunned at what I saw: four beautiful American chestnut trees. Tree #1 was 26″ diameter at breast height (DBH), Tree #2 was 20″ DBH, Tree #3 was 18″ DBH, and Tree #4, 12″ DBH. I counted only six small chestnut seedlings in the understory during this visit.



Tree #1 (center), is the largest of the surviving trees in this stand.

All photos by Donna Lucas.

The landowner is a developer, realtor, and hardware store owner who planned this area for commercial development. Now that he knows about the treasures growing there, he is trying to protect these trees as he sells development lots. The original four trees were within a one-acre area of a larger wood, and they are well outside of what we believe is the native range of chestnut in Indiana. My guess is that these trees seeded in naturally from trees planted many years earlier.

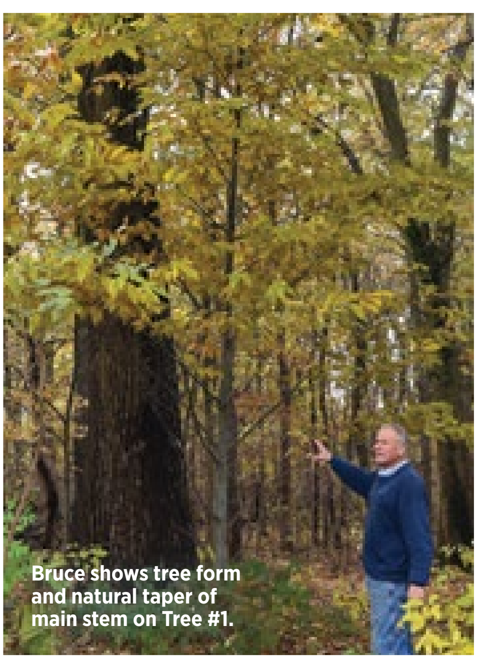
That first summer I began making plans to use them as mother trees for our state breeding program. Because these were woodland-grown trees, they were very tall. We were going to need a bucket truck with a massive reach. Northern Indiana Public Service Company, the local power company, donated the use of two bucket trucks and their crews that could reach a height of 70 feet high. The following spring, we were able to pollinate Trees 1, 2, and 3. It was my first time in a bucket, and I will never forget being 70 feet up on that windy first day. These trees have been conserved as two Clapper lines and as lines in the Indiana Chapter's germplasm conservation orchard (GCO).

Bruce measures Tree #1 at 39" DBH in 2018.

By 2006 Tree #2 was 24˝ DBH and, unfortunately, it was hit by lightning. One side of the main trunk was blown off. I made a deal with the landowner to log and saw what was left into lumber and half it between us. I counted the rings on the stump and learned that the tree was 40 years old. The stump now has several large sprouts. Tree #4 was overtopped by a large leaning maple tree, and during the winter of 2008 the tree fell onto #4 and broke its top off. I again salvaged the butt log for lumber. Tree #4 had grown to 16˝ DBH. Disturbance caused by the loss of these two trees and the construction of an access road and cul-de-sac resulted in a more open canopy near these original four trees. This extra light allowed the growth of over 38 sapling sized American chestnut trees where there had been only four trees and six seedlings in 2001.

In the fall of 2018, I visited the site again, along with photographer Dona Lucas, who took photos to document these beautiful wild American trees. I remeasured Trees #1 and #3. Tree #1, which was 26˝ DBH in 2001, is now 39˝ DBH and 90 feet tall. It has been growing at a rate of .76 inches DBH per year. Based on the growth rate of the tree stump from Tree #2 for its first 40 years, and the known growth rate of Tree #1 over the last 18 years, I estimate the age of Tree #1 to be 60 years old. Tree #3 was 18˝ in 2001 and is now 29˝ DBH. The 38 saplings are now becoming a young forest. During the visit I ventured across the road to the adjoining woods to the east and found 20 more young American chestnut trees there.

Sadly, I also have to report that I found, for the first time, blight on this Roselawn site. It is showing on the stump sprouts of Tree #4 and on one of the newfound trees across the road.



Bruce shows tree form and natural taper of main stem on Tree #1.

Tree #1 is estimated to be 90' tall.

Stump sprouts from Tree #2, hit by lightning in 2006.

TENNESSEE RECOGNIZES THE

Role of the American Chestnut

IN SHAPING THE STATE

By Natalie Bumgarner and Andy Pulte, University of Tennessee Institute of Agriculture Department of Plant Sciences

Have you ever taken the time to ponder what plants have most shaped your state? We often overlook their role, but our lives are really quite intertwined with the plants around us. Plants provide food, fuel and raw materials, while contributing to culture, history, and certainly economics.

In fact, people see more plants than any other organism in their lifetime. We can certainly point to ways that plants impact and shape society. Plants also have the ability to influence us personally in a variety of ways: mentally, physically, spiritually, and emotionally. And yet, plants are still often unseen or unrecognized by many. Bringing an awareness to a sort of plant-blindness is at the heart of this project: The Ten Plants that Shaped Tennessee.

Because it is easy to overlook the role plants have in shaping our environment, communities, and economies, we decided last year to ask residents in Tennessee to nominate plants they viewed as the most significant to the state. And, in just a few weeks, more than 650 Tennesseans joined in! Nominations were invited in a variety of categories including plants known for food, culture, history, landscape, and economics. Respondents came from all over the state and included academics, high school teachers, a few kindergarteners, and members of the plant-loving public. After weighing the nominations, carefully considering the significance of each, and consulting with a range of experts in the field, the 10 plants that most shaped the history of the state were chosen in early 2019. Because the list had to be realistic and balanced, both beneficial and negative aspects of nominated plants were considered. We looked at popular row crops, ornamental plants, forest species, unhealthy and invasive plants, and even some plants that are linked with detrimental aspects of our state's history.

These chestnut burs are the first ever seen by many visitors to the State Botanical Gardens of Tennessee on the campus of The University of Tennessee.

The Ten Plants that Shaped Tennessee:

American chestnut: It's been nearly a century since a fungus eradicated this majestic tree, but it's still remembered for its bountiful nuts and prized wood.

Beans: Several varieties of beans are endemic to Tennessee, and since pioneer days, they have been important to the state's food industry.

Corn: In East Tennessee the plant conjures up images of grits and cornpone, and its association with distillery operations.

Cotton: With more than 300,000 acres devoted to cotton production, it is used in clothing, crushed for oil, and in livestock and human food products.

Dogwood: Among the state's favorite trees, festivals celebrate the dogwood and its spring blooms. Our state ranks first in dogwood nursery production!

Ginseng: This native herbaceous perennial plant has been used for hundreds of years and is entwined in the history of eastern Tennessee and our deciduous forests.

Grasses: Our state has some of the most diverse prairie systems on the planet. Turfgrasses cover our lawns and playgrounds on more than 1 million acres.

Tobacco: One of the earliest crops planted by settlers in Tennessee, tobacco has shaped the state's economy and health since Tennessee joined the Union.

White oak: People in Tennessee have been relying on white oaks to build our houses, grace our hearths, and provide income for centuries.

Kudzu: Easily recognizable by almost anyone in Tennessee, kudzu is among the invasive plant species that damage our natural environment.

For in-depth descriptions of each plant visit: tenplants.tennessee.edu

Several potentially blight-tolerant chestnut trees have been planted at the State Botanical Garden of Tennessee in Knoxville and serve as educational tools for school programs.

The Ten Plants chosen were: beans (several varieties), corn, cotton, dogwood, ginseng, grasses (prairie and turf), tobacco, white oak, kudzu, and of course, the American chestnut.

As we reflect on the plants chosen, we are confident that every plant on this list is important. Some of them we could have guessed would end up on the list due to their role in agriculture or their visibility or even notoriety across the state. However, there were a few pleasant surprises in the nominations that illustrated how much of an impact plants can have – even after they are gone.

Though it's been nearly a century since a fungus essentially eradicated this majestic tree of the eastern forest, those who live in our state were quick to nominate the American chestnut tree. Here in the state of Tennessee, like elsewhere, the chestnut was widespread and valued for timber and wildlife. It was a key member of some of the most ecologically diverse forests in our state. Additionally, chestnuts represent for many the most well-known example of the potentially devastating consequences of accidental introduction of exotic pests and pathogens. Though it is considered functionally extinct, it still stands out in the state's culture and history. Our historical archives display old photographs of long-gone lumberjacks beside enormous felled trees we can only imagine today. And, our family historians share tales of trees that provided lumber, food for foraging farm animals, and even a cash crop that bought new fall school shoes.

This rich history means that at our statewide county UT Extension offices, a multitude of enthusiastic homeowners call in yearly to ask agents where they can get blight-tolerant American chestnuts to participate in the tree's restoration efforts. It is clear that American chestnut still holds a place in our hearts, long after it largely disappeared from our hillsides. It also serves as proof of the widespread hope that it won't be too long before we'll be able to add another happier chapter to the chestnut story in Tennessee.

Andy Pulte and **Natalie Bumgarner** of the Department of Plant Sciences at the University of Tennessee spent much of 2018 developing the Ten Plants That Shaped Tennessee. More than 600 nominations were submitted, and submissions were open to the public. Together with a panel of other experts in a range of fields, all nominations were weighed, and each nomination's significance was carefully considered to develop the final list of 10 plants that most influenced the state.

Wisconsin Lumber Wholesaler

CELEBRATES AMERICAN CHESTNUT

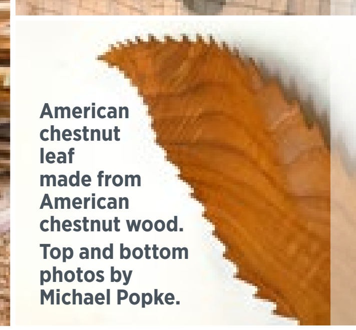
By Michael Popke

Robert "Buz" Holland grew up the son of a Wisconsin lumber wholesaler and eventually entered the industry himself – taking related jobs as a young man in Oregon and Washington in the mid-1950s before joining his dad in the family business. But it wasn't until later in life that he became fascinated with the American chestnut tree.

"I don't think there is another tree whose wood has been used so completely and in so many different ways," said Holland, who is 89 years old and still operates the Robert T. Holland Lumber Co. in rural Green County, Wisconsin. He cites the "nutritious and delicious" nuts that fed both humans and livestock, the bark tannin extracted for tanning leather, and the lumber used for everything from furniture to shingles to fence posts. "It was of enormous economic value to society."

American chestnut lumber. Photo by Michael Kienitz (michaelkienitz.com).

Buz Holland stands by his kitchen cabinets made of American chestnut.



American chestnut leaf made from American chestnut wood.

Top and bottom photos by Michael Popke.

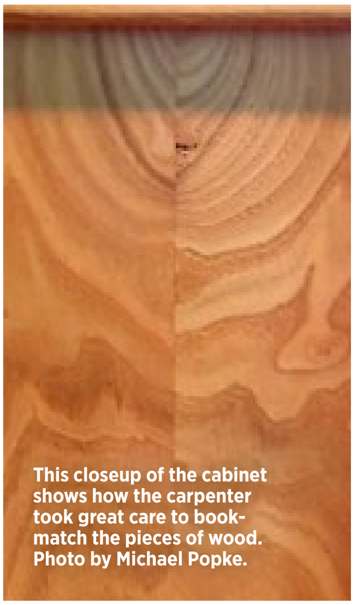
That value also comes alive inside Holland's 22-year-old, 1,500-square-foot home, where American chestnut is extensively showcased in the form of elegant and exquisite kitchen cabinets with book-matched interior-door panels, as well as in the baseboards and window and door casings in a first-floor bathroom. Additionally, replicas of American chestnut leaves carved from American chestnut wood adorn one prominent wall, and Holland also owns a set of wooden bowls made out of American chestnut.

Back in the 1990s, when Holland was calling on a sawmill company in Bangor, Wisconsin, the owner asked Holland if he might be interested in purchasing some American chestnut lumber he had available.

"It just sat around for awhile, because nobody around here knew what it was," Holland said about the wood, seeming still surprised at his good fortune that day. "By the time I came along and purchased it, I think he'd given up hope it would sell. But I knew the history of American chestnut, so I knew it was rare."

Holland purchased several hundred board feet of American chestnut from the sawmill operator at $20 per board foot. He then did some research into the wood's origins and discovered that the lumber was logged on land once considered by many experts to be the world's largest remaining American chestnut stand — established by an early settler from the East Coast in the mid-1890s near West Salem, Wisconsin, located about 150 miles northwest of Green County. That stand has since succumbed to the blight that killed off other American chestnut trees.

Bowls made of American chestnut. Photo by Michael Popke.



This closeup of the cabinet shows how the carpenter took great care to book-match the pieces of wood. Photo by Michael Popke.

American chestnut cabinets are the highlight of Buz Holland's kitchen. Photo by Michael Kienitz (michaelkienitz.com).

Initially, Holland wasn't sure what he would do with the newly acquired lumber. But when he purchased the property next door to his home in the early 1990s and eventually built a new house on it, he contracted with a carpenter to construct the sturdy kitchen cabinets that are now the highlight of Holland's home.

The cabinets' light-brown color brightens the spacious kitchen, and their rich grainlines add character and splendor to the room – perfectly contrasting with the hard maple floors and forest green countertop.

Similarly, the use of American chestnut in the bathroom lends both elegant and rustic touches to a traditional space. Even the tissue dispenser and the linen cabinet are carved from the wood.

Additionally, Holland is in possession of about 1,500 board feet of American chestnut lumber from the now-gone Wisconsin stand, which he said is enough to build another set of cabinets. He'd like to sell the lumber (all or nothing) for $20 per board foot – the same price he paid two decades ago. "That's a fair price for something that's as rare as hen's teeth," Holland said, smiling.

Because he is not online, Holland asked potential buyers to contact him by mail at P.O. Box 546, New Glarus, WI 53574.

Interested in Purchasing the Chestnut Lumber?

Please send a letter to:
Buz Holland
P.O. Box 546
New Glarus, WI 53574

Near the end of *Chestnut*'s visit with Holland, a handyman working on an outdoor project at the property took a break in the kitchen. Holland asked him what kind of wood he thought was used to build the cabinets, and the handyman stepped forward to take a closer look before venturing a guess. "Ash?"

When Holland revealed the cabinets were made of American chestnut, the impressed handyman nodded in acknowledgment and called it an "extinct wood."

Functionally extinct – at least for now. Which just adds to its mystique.

"It's fascinating," Holland said about the rise and fall of American chestnut and his enthusiasm for bringing such a prominent piece of American natural history into his home. "I saw wonderful possibilities."

TACF Gift Membership

Your gift of membership goes beyond the restoration of the American chestnut tree; you're helping to increase biodiversity and repair our fragile environment.

Through the leading edge of conservation science, our new discoveries will ensure other imperiled tree species can be saved from disease, creating more diverse and productive forest ecosystems. Now that's a gift that truly keeps giving. These are just a few of the exciting benefits:

- One-year national membership and state chapter membership
- Subscription to TACF's award-winning magazine, *Chestnut*
- Local breeding and research opportunities
- Access to Regional Science Coordinators and state chapter representatives
- Annual members-only presale for pure American seedlings (February)

Thank you for your generosity and support!

TACF PLANNED GIVING

When was the last time you did something you were extremely proud of? Something that was personally fulfilling? Something you were elated to share with family and friends? Was it last week, last year, last decade, or maybe you are still waiting for that one special moment to stake your personal flag in the ground?

More likely than not, you have been involved with and interested in our work for some time now, so why not leave your permanant mark on our efforts to return the iconic American chestnut to its native range? By becoming a Legacy Tree Sponsor, you join other chestnut enthusiasts who are deeply invested in our mission and take pride in the long-lasting impact they are making. We celebrate these cherished few who have made a $10,000 commitment and whose improved blight-tolerant trees are planted in our prestigious two-acre Legacy Tree Orchard at Meadowview Research Farms. There is a unique joy in honoring or memorializing family and friends in this tranquil sanctuary, where loved ones can visit for generations to come.

If you are interested in leaving this lasting tribute, please give us a call at (828) 281-0047 or email me at david@acf.org.

David Kaufman-Moore,
TACF Donor Relations Manager

PLAN TODAY, GIVE TOMORROW

SUMMER Science Meeting 2019

By Lisa Thomson,
TACF President and CEO

Attendees tour the greenhouse at Meadowview Research Farms on the last day of the meeting.

Stalwart chestnut volunteer science leaders and orchard managers, board of director members, academic and industry partners, and even some dedicated spouses joined TACF staff in Abingdon, VA, for a planning and information sharing meeting July 19-20. Now that the chapter breeding plans may warrant adjustments, and priorities are shifting, discussion and vetting was needed after Dr. Jared Westbrook shared his genomics analyses at the April Board meeting. To learn more about those discoveries, be sure to read Jared's article on page 23.

A call for presentations was made ahead of the meeting, and we were treated to an array of reports, results, and ideas. After opening the meeting, Lisa Thomson turned over the first presentation to Jared, who shared his plans and recommendations for chapters to finish selection in their seed orchards, and present alternative strategies to enhance blight tolerance. Jared was followed by regional science coordinators Ben Jarrett and Tom Saielli, who presented on seed orchard design and regionalization and consolidation of orchards, respectively. Volunteer Bruce Levine, having just finished his master's degree, shared the Maryland Chapter's work on small stem assays to help speed up the selection process. Dr. Greg Miller, Phil Rutter, and John Scrivani shared alternative breeding strategies and other resources.

The rest of the afternoon was a variety of presentations and discussion on transgenics: current status, public review and comment strategies, distribution, diversification and relieving the founder effect. These presentations were given by Dr. Bill Powell, John Dougherty, Dr. Scott Merkle, and Jared, which concluded Day One. The group gathered for a catered dinner where so many long-time friendships were rekindled and even more discussion on restoration next steps went well into the evening.

Day Two's focus was on hypovirulence, *Phytophthora*, germplasm conservation orchards, collection of scion and grafting by presenters Amy Metheny, Dr. Joe James, Sara Fitzsimmons, and Laura Barth. The day concluded with a thought-provoking presentation on discovering and validating candidate genes for blight tolerance and resistance to *Phytophthora* by our collaborator Dr. John Lovell of the HudsonAlpha Institute for Biotechnology. Lisa and board of director chair Dr. Brian McCarthy provided closing remarks. About 20 hearty souls braved the summer heat to tour Meadowview Research Farm's greenhouses and progeny orchards, led by Laura Barth and assisted by Brandon Yañez-Breeding and Lisa.

All presentations were tightly facilitated and all speakers who ended on time were entered into a drawing for *The Overstory* by Richard Powers, a novel where chestnut is featured prominently. The winner was Dr. Joe James!

Ample enthusiasm, curiosity and dedication were demonstrated by all attendees during the entire meeting. The next steps include custom breeding plans for each chapter to be led by respective regional science coordinators. TACF salutes these important partners in our efforts to continue this decades-long effort to restore the American chestnut, as we cannot achieve mission success without the hard work of our volunteers and collaborators.

Participants gathered for a group photo in the Higher Education Center where the meeting took place.

Dr. Jared Westbrook shares his presentation with the group.

TACF's Horticulture and Pathology Specialist, Laura Barth (striped dress), led the tour in Meadowview's greenhouse.

Attendees listen and take notes during a presentation.

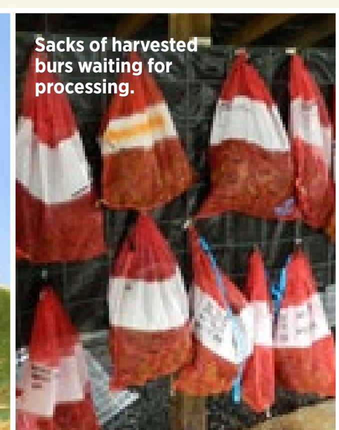
MEADOWVIEW Harvest Season

By Brandon Yañez-Breeding, TACF Meadowview Research Technician

Harvest season begins with the scouting and identification of desired trees with a heavy seed set. Trees previously collected, trees yet to be collected, and control pollinated seeds are identified and slated for collection. Approximate harvest dates are labeled and affirmed daily to ensure seeds are collected at their peak. Typically, a two-person team is sent to a tree with a tractor, tow-behind lift, and a bevy of red onion sacks to collect the harvest. With several trees being over 30′ tall at TACF's Meadowview Research Farms, a self-leveling tow-behind lift is the best means by which to reach the trees. Considering the height of these trees and the surrounding landscape, beautiful views and scenery abound.

It was another chilly autumn morning as the sun shone through the eaves of the barn, bathing the hanging bags in a bright amber glow. In the distance, several song birds could be heard singing through the frosted air. For several days now, the bags of burs have been hanging in the barn seasoning, allowing the burs to slightly break down and become more malleable. Processing of burs is always done by hand, a delicate and tedious task, given the tens of thousands of burs to be processed as well as their rather sharp nature. Standard operating procedure often necessitates wearing two pairs of gloves to reduce the frequency and intensity of the burs' spines that penetrate the gloves. While effective, this by no means guarantees your hands will escape unharmed, as many have experienced.

An open bur during harvest season at Meadowview Research Farms, Meadowview, VA.



Sacks of harvested burs waiting for processing.

Volunteer Jim Warren processing burs.

The steps in processing the burs and seeds doesn't change much from year to year. To begin the process, all of the bags harvested from the mother tree are identified and collected as there are often more than one bag per tree. Once gathered, the bags are emptied onto the table and everyone begins counting burs. After recording the number, everyone begins breaking open the burs to reveal the highly valued chestnuts. Sorting seeds is a critical step in the process as it helps to ensure that records and counts are accurate. When processing, there are several ways to visually identify whether or not a seed is viable: color-comparison to other seeds from the same tree, presence of mold (though not necessarily an eliminating factor), and "fullness" of the chestnut itself. Does it look like someone removed all the chestnut meat from inside the shell? A time consuming yet accurate method of identifying the viability of a seed is to give each seed a squeeze between your fingers; if the seed has any notable give, that is an indicator the seed will likely continue to deteriorate during the stratification period and will not result in a healthy and viable seedling upon sowing. After sorting the viable from the non-viable seeds, a total count of the viable seed as well as the weight of one hundred seeds is measured.

Upon fully processing the entire harvest from a single mother tree, the seeds are sorted into lump sums and placed into perforated plastic storage bags. The bags are labeled with pertinent information: date processed, family codes, numbers of seed bags, and number of seeds in each bag. Throughout the day, as families are completed, the seeds are placed in one of our refrigerated coolers to stratify for several months until they are ready to be sown in the spring. Over a period of weeks, the burs are processed, seeds sorted and stored away, waiting to be shipped to scientists, researchers, volunteers, and donors alike.

Throughout the years, I have gotten to know and connect with the close-knit network of volunteers who offer their time, labor, and care to help process the annual fall chestnut harvest. It has been my pleasure to have spent the last five years working with this small, yet passionate group. The task of processing burs would be immeasurably harder, and require a considerably longer amount of time, if it were not for the dedication and physical contributions of TACF members and volunteers. Given this, I would like to extend my sincere gratitude and appreciation for their efforts, curiosity, and dedication in working towards restoring this once massive species to its former splendor.

Doubling up on gloves to protect from spiny burs.

TENNESSEE CHAPTER

Hill Craddock and Paola Zannini

ARE SPREADING AMERICAN CHESTNUT FEVER

By Scott Carlberg, Carolinas Chapter

Over the past 20 years, Paola and Hill have hand pollinated more than 30 different naturally-occurring American chestnut trees throughout Tennessee, work that contributes to crucial germplasm conservation.

Much of Paola and Hill's work (front kneeling) is interdisciplinary and collaborative such as this nursery experiment - a small stem assay - conducted at UTC in conjunction with volunteers from the TN, GA, and Carolinas TACF chapters.

"I caught the chestnut fever," says YouTube viewer Chestnut Anarchist, upon watching Hill Craddock's lecture. "Hill was my graduate professor. His zest for the chestnut is so infectious."

Hill Craddock spreads chestnut fever along with his wife, Paola Zannini. Chattanooga is home-base for these longtime TACF volunteer leaders.

About Hill: "I teach courses in general biology, economic botany, dendrology and mycology at the University of Tennessee at Chattanooga (UTC). My current research is focused on the restoration of American chestnut (*Castanea dentata*) to the Southern Appalachian hardwood forest. I am also working on establishment of a commercial chestnut industry in Tennessee."

Hill heaps praises when he talks about Paola: "I am the luckiest person in the universe."

It was a long journey to find each other. "I studied 'Agraria,' a discipline-like horticulture in Italy," says Paola. "I just wanted a job out of doors. I liked plants more than animals to work with. I was in a hazelnut growing region, and I came to U.S. to study hazelnuts. Oregon is where you study hazelnuts." Oregon is also where Hill was studying. It was 1985. The rest is history, as they say.

About Paola: "I am a retired greenhouse and nursery manager from Reflection Riding Arboretum and Nature Center in Chattanooga, a 300-acre botanical garden and nature education nonprofit. Before, during, and after that I have been helping Hill at UTC. Harvesting seeds. Planting seeds. Transplanting seedlings. Taking care of the nursery. And especially watering during the hot summer! We grow


Hill (front right) likes to wait for a very hot day to inoculate because it brings out only the most dedicated volunteers, like this group that showed up in July to the Cantrell Orchard in Corryton, TN.

thousands of trees per year. Maybe 4,000 in the nursery," says Paola.

There's enough to do there too. Projects include breeding for blight resistance, root-rot resistance, investigating hypovirulence for biological control of chestnut blight and chestnut cultivar evaluation.

There is the organizational angle, too. Hill convened and hosted TACF's Southern Regional Science Meeting for years, and is currently the president of the TN Chapter. He is also a member of TACF's Board of Directors.

Science is central. Hill says, "The chestnut project is big. We have dozens of growers, some with hundreds of trees on private and public lands. We have experimental forest test plots and public demonstration plantings throughout the eastern half of Tennessee."

"Pedigrees go back many generations," says Hill. "We want to avoid first cousin marriages, of course, so our lines are separate."

Paola manages the database for the trees. "We depend on our chapter volunteers, from finding wild trees and collecting pollen, to making the crosses and planting seeds, to managing the experimental orchards."

Hill's students volunteer. "I work with impressionable young people," says Hill. "The chestnut is a great model system for teaching moments. For instance, Koch's postulates – experimenting with infection of susceptible hosts. Some students are then drawn to research. They learn the scientific method. If I have been able to change anyone it is in this formidable role. They understand the process of science." Because Paola is also a scientist, she is engaged in all aspects of the project, including mentoring students.

"We always seek new information through science," says Hill. "We need to be prepared to see when new evidence challenges long-held ideas. The value of teamwork to solve complex problems lies in the diverse abilities of team members to create opportunities."

Hill and Paola are talking about legacy, really. What you leave the world through those you teach. The viewpoint that you instill in others … whether you spread "the fever."

"We are optimists about the American chestnut," says Hill. "We have seen the disease and its range. It's complex, requiring a multidisciplinary set of solutions. Blight resistance may be more complicated than we thought 20 years ago, but the new information we have is good, based on good science, and provides us with real opportunities to move forward towards our goals."

Optimism in general can be tough. "The world is getting more messed up," he says. "It's hard in the face of reality, the decline of species. The situation is not getting better, but worse. In every course we teach we get to the part of how we are killing the planet: the consequences of global species loss and environmental collapse."

There are chestnuts in our future for generations to come. "Pessimism is not the lesson to teach students," says Hill. Awareness is. Questioning is. Persistence is. You have to catch the fever.

CATCH HILL CRADDOCK ON VIDEO!

He is on YouTube. Look up, "The return of the chestnut – a tree crop archetype. Hill Craddock. TEDxUTChattanooga."

What is a favorite tree memory?

Paola: The fragrance of linden trees in bloom. Horse-chestnuts (*Aesculus hippocastanum*) lining the avenues of Turin, Italy. The smell of pine trees in the mountains.

Hill: A copper-beech tree, well grown, with its lower branches sweeping the lawn, makes the best climbing tree in the world.


Hill Craddock likes chestnuts, a lot!

PART 1
OF A
2-PART SERIES

Natural Range

OF THE AMERICAN CHESTNUT

By Sara Fitzsimmons, TACF Director of Restoration

During a career with the U.S. Forest Service (USFS) that spanned 34 years, Elbert Luther Little, Jr. was the chief dendrologist from 1967 – 1975. During that time, he published a six-volume series which would become the standard for range maps for tree species in the United States. Volume 4 contains the range map for *Castanea dentata* (Little 1977), a map which TACF uses regularly to illustrate the native range of the species (**Figure 1**).

The first published range map for American chestnut appears to be from Sargent in 1884 (**Figure 2**). Sargent was the first director of Harvard University's Arnold Arboretum, and his name can be seen often in historical botanical records in the mid- to late-1800s. In Sargent's map, there are a few things to note. First is that the U.S. species of *Castanea* are combined into a single map, and there is not a separate listing of *C. ozarkensis* in the list of trees and their ranges. Here are some excerpts from the text of that publication, outlining the ranges of the two listed species.

Sargent 1884, Pages 156-157:

Castanea pumila, Miller Lancaster County, Pennsylvania, and the valley of the lower Wabash river, Indiana, south and southwest to northern Florida and the valley of the Neches River, Texas.

Castanea vulgaris, var. Americana, A. De Candolle, Southern Maine to the valley of the Winooski river, Vermont, southern Ontario and southern Michigan, south through the northern states to Delaware and southern Indiana, and along the Alleghany mountains to northern

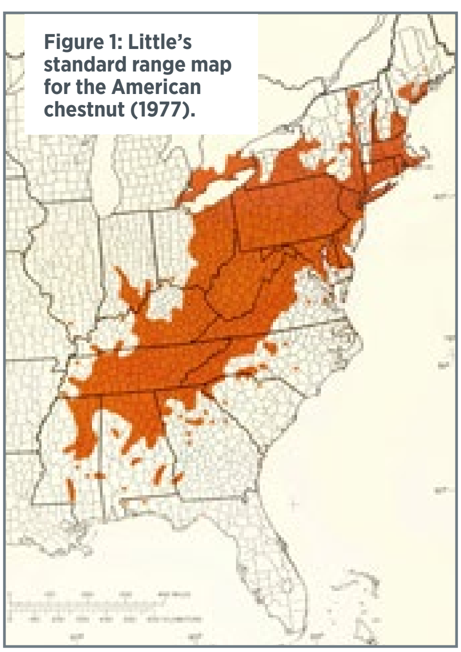


Figure 1: Little's standard range map for the American chestnut (1977).

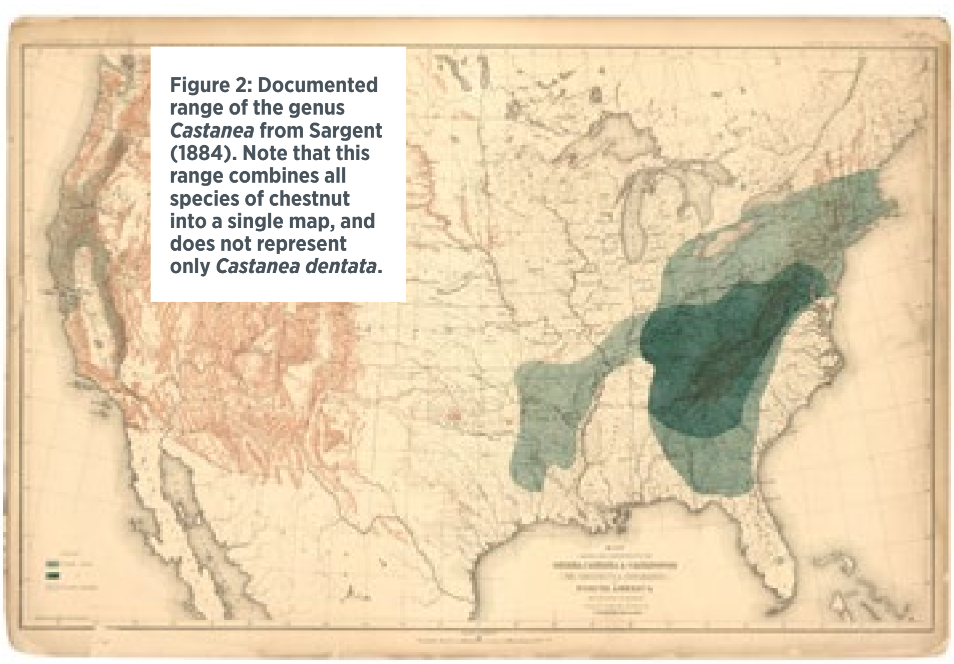


Figure 2: Documented range of the genus *Castanea* from Sargent (1884). Note that this range combines all species of chestnut into a single map, and does not represent only *Castanea dentata*.

Alabama, extending west to middle Kentucky and Tennessee.

Similarly, in **Figure 2**, notice the straight line drawn along the 44th parallel at the northern edge of the range. According to Little (1951), those who worked on these range maps often used geographic limitations to draw boundaries.

Following the publications of maps and a census of forests in the U.S. by Sargent in 1884, then Chief Dendrologist for the USFS, George Bishop Sudworth embarked on updating the maps and range information. Sudworth and his team of W. H. Lamb, Georgia E. Wharton, and Mary C. Gannett, surveyed lands on mule (**Figure 3**), used locations of herbarium specimens, and talked with foresters in every state to mark known locations of tree species on maps in their offices at the USFS Section of Forest Distribution (1951). In a 1916 issue of the *Journal of the New York Botanical Garden*, there is a note stating:

"Miss Georgia Wharton, of the branch of research, Forest Service,

Figure 3: Sudworth and assistant on mules in Hassic Meadow, Middle Tule, Sierra Forest Reserve, 1901. Photo courtesy of Forest History Society, Durham, NC.

Figure 4: Digitization of map created by Sudworth and his team through the early 1900s to document the range of American chestnut. (uwdc.library.wisc.edu/collections/econatres/fplatlas/)

THE AMERICAN CHESTNUT FOUNDATION®

Multiple Path

The American Chestnut Foundation has evolved over the last 36 years in both size and scope but our goal remains unchanged: American chestnut restoration. From utilizing new technologies like genomic sequencing, to assisting our collaborators at SUNY-ESF with transgenic research, to using hypovirulence for enhanced survival, the future is promising for the return of this iconic tree.

While new uses of technology enable us to look at the DNA structure of chestnut families and provide insight into the genes that confer blight tolerance, it

The Richard S. Will Greenhouse at Meadowview Research Farms.

Thank you for suppor

ways – One Goal

comes at a literal cost. Sequencing the entire genome of a single tree is upwards of $100,000. Your 2019 Fall Appeal gift will allow us to use this technology to more accurately select trees with higher genetic traits for blight tolerance in our research and breeding programs.

As noted in TACF's first issue of *The Journal* back in 1985, "…our Foundation is curiously like that of the chestnut itself: great potential, great promise, but in need of some help to achieve that potential and fulfill that promise. Only you can give that help."

ting our bold mission!

Washington, has been at the Garden examining specimens in the herbarium for data to be used in compiling maps of tree species; and in the preparation of a check list of West Indian trees and important shrubs."

Unfortunately, these maps were never formally published and were used only in text publications from the USFS, one published in 1898 and then in 1927. Excerpts below:

Sudworth's Checklist of the Forest Trees of the United States 1898:

Range. *From southern Maine to northwestern Vermont (Winooski River), southern Ontario, and southern shores of Lake Ontario to southeastern Michigan; southward to Delaware and southeastern Indiana, and on the Allegheny Mountains to central Kentucky and Tennessee, central Alabama, and Mississippi.*

Sudworth's Checklist of the Forest Trees of the United States 1927:

Range. *From southern Maine to northwestern Vermont (Winooski River), Southern Ontario, and southern shores of Lake Ontario to southern Michigan; southward to Delaware and Ohio, southern Indiana and Illinois (Pulaski County), and on the Appalachian Mountains to central Kentucky and Tennessee, northern Georgia, western Florida, central Alabama, and Mississippi.*

Recently, those maps were unearthed and digitized by the University of Wisconsin as part of a larger compilation called, "Forest Atlas of the National Forests of the United States" (**Figure 4**).

One of the more interesting details in the maps digitized from the Sudworth expeditions is the deliberate removal of American chestnut from higher elevation areas in West Virginia, Virginia, and western North Carolina. While chestnut is typically not found on these balds or areas dominated primarily by fir and spruce, there are no similar omissions of the range in locations also unlikely to support American chestnut, such as larger swaths throughout the Adirondacks or large wetland or urban areas.

In 1938, Edward Munns, Chief within the USFS Division of Forest Influences used the maps and findings from Sudworth's expeditions in his publications (**Figure 5**). One can see the similar removal of high elevation areas from Munns' maps.

Figure 5: Natural range of the American chestnut from Munns (1938).

Munns' publication was one of "Important Forest Trees." By 1977, the American chestnut had been downgraded, as Little didn't publish its range map until Volume 4 of his maps titled, "Minor Eastern Hardwoods." Little's range maps from the 1970s have largely become the standard when discussing natural ranges for tree species in the United States, but still isn't without its flaws. In his introductions to the volumes of his Atlas, Little noted that there would be mistakes and that these ranges would likely shift over time.

These maps all showcase efforts to document native ranges for the use by natural resource professionals and enthusiasts, but also illustrate the difficulty in embarking on such a mission. This article, the first in a two-part series, serves largely to showcase the beauty of these historical maps and show the groundwork created for subsequent mapping efforts. In Part II, the focus will document the challenges associated with creating these maps and will showcase a more modern look at the range of American chestnut.

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UPDATE:
Breeding Plan

By Jared Westbrook, Ph.D., TACF Director of Science

Recent genomic analysis has revealed that there is a tradeoff between blight tolerance and the proportion of backcross trees genomes inherited from American chestnut (Westbrook et al. 2019a). The most blight-tolerant BC_3F_2 trees from TACF's Meadowview Research Farms inherited an average of 83% of their genome from American chestnut and are less blight-tolerant than 50/50 (F_1) hybrids of American chestnut and Chinese chestnut. We expect similar results for TACF's chapter breeding programs. These results suggest that blight tolerance is controlled by more genes than previously assumed. It is likely not possible to generate hybrids that inherited most of their genome from American chestnut (i.e. > 95%) while also inheriting high levels of blight tolerance from Chinese chestnut. TACF will need to improve blight tolerance beyond the intermediate to low levels of blight tolerance observed for current BC_3F_2 and BC_3F_3 populations. Considering these recent findings, We propose additional strategies for developing populations of American chestnut that are blight-tolerant, genetically diverse, and resistant to *Phytophthora cinnamomi*.

A one-generation backcross breeding strategy to enhance blight tolerance

First backcross (BC_1) trees, which inherited an average of 75% of their genome from American chestnut, have survived for decades at Meadowview Research Farms and at the Connecticut Agricultural Experiment Station, two of the longest-running chestnut breeding programs in North America. Based on this observation, we recommend applying pollen from blight-tolerant American chestnut x Chinese chestnut F_1 hybrids and American chestnut BC_1 trees to advanced generation (i.e., BC_3 and BC_4) hybrids with inferior blight tolerance. This strategy will enable TACF chapter breeding programs to preserve genetic diversity represented by their backcross lines while also enhancing blight tolerance to acceptable levels for restoration. We will inoculate the BC_1 and BC_2 progeny of these crosses with the chestnut blight fungus and we will aim to select a subset of trees that has blight tolerance similar to the average of F_1 tree. To ensure accurate selection, we will monitor selection candidates and F_1 controls for at least five years and perform further culling of trees that develop signs of blight susceptibility. Furthermore, we will screen progeny from selection candidates to accurately select the most blight-tolerant parents. Once we have selected for blight tolerance, then we will use genetic markers to make additional selections to maximize genome inheritance from American chestnut. Large quantities of seed for restoration may be generated with open-pollination among the selected trees.

Although BC_1 trees will likely have improved blight tolerance relative to the currently BC_3F_2 and BC_3F_3 populations, these hybrids may inherit some characteristics from Chinese chestnut that are undesirable for forest restoration. For example, Chinese chestnuts imported into North America generally have lower potential for height growth (Diller & Clapper, 1969; Sclarbaum et al. 1998; Thomas-Van Gundy, 2016), greater stem branching (Clark et al., 2012), lower maximum photosynthetic rates (Knapp et al., 2014), and lower cold tolerance (Gurney et al., 2011; Saielli et al., 2012) as compared with American chestnut. Therefore, as an organization, we will need to grapple with the question, how much American chestnut genome is enough for forest restoration?

In their research to petition the U.S. Federal Government to deregulate transgenic American chestnut, Dr. William Powell's team at State University of New York College of Environmental Science and Forestry (SUNY-ESF) has conducted experiments to compare the ecological interactions of transgenic and wild-type American chestnuts. These studies include caterpillar feeding on leaves (Post et al., 2011), bee feeding on pollen (Goldspiel et al., 2018), tadpole feeding on leaf litter (Newhouse et al, unpublished), germination of other plant species in chestnut leaf litter (Newhouse et al., 2018), mycorrhizal associations (D'Amico et al. 2015; Newhouse et al., 2018), and chestnut leaf litter decomposition rates (Gray, 2015). To determine how much American genome is required to serve those functions, TACF is seeking collaborators to replicate and supplement these studies with hybrids that vary in their genome inheritance from Chinese chestnut. Furthermore, once TACF has generated backcross hybrids with blight tolerance similar to Chinese x American F_1 trees, we will plant these hybrids in forest restoration trials and compare hybrids to wild-type American chestnut with respect to survival, growth rate and form, and insect and fungal associations.

Transgenic outcrossing

Pending U.S. Federal Government deregulation of transgenic American chestnut containing the wheat oxalate oxidase (OxO) gene that enhances blight tolerance, TACF plans to outcross transgenic founder lines with a diverse collection of backcross and wild-type American chestnuts. The aims for this outcrossing are:

1. To increase the genetic diversity and adaptive capacity of transgenic blight-tolerant populations.
2. To minimize deleterious inbreeding effects from developing these outcross populations from few transgenic founders.
3. To combine transgenic blight tolerance with backcross trees' resistance to the pathogen that causes root rot (*Phytophthora cinnamomi*).

Plans for diversifying transgenic populations have been published in previous editions of *Chestnut* (Westbrook, 2018; Jarrett, 2019; Coffey, 2019) and in the peer-reviewed scientific literature (Westbrook et al., 2019b). In summary, the plan is to:

- Increase the number of transgenic founder parents from one (Darling 58) to a minimum of two founders to reduce the founder effect bottleneck on genetic diversity.
- Outcross these transgenic founder trees over five generations to increasing numbers of wild-type American chestnuts (i.e., 2, 25, 50, 150, and 450 wild-type parents in each generation). The purpose of the outcrossing is to increase the representation of genetic diversity from the wild American chestnut population and to dilute out the transgenic founders' genomes.
- Graft-propagate and collect seed from 1,000 unique wild-type American chestnuts from across the *Castanea dentata* range. Plant these wild trees in germplasm conservation orchards where they will eventually be used as parents for transgenic outcrossing.
- Treat blight cankers on susceptible American chestnuts with hypovirulent strains of *Cryphonectria parasitica* to keep them healthy for transgenic pollinations (Stauder et al., 2019).
- Generate 50 -100 progeny per cross between wild-type and transgenic trees. We will use DNA markers to select 1 to 3 progeny per cross that inherited the largest proportion of their genome from wild-type trees as opposed to the transgenic founder tree(s). The DNA markers will be especially useful for reducing the length of the founder genome in the chromosomal region surrounding the oxalate oxidase gene.
- Grow the transgenic outcross progeny under high light treatments in the greenhouse to stimulate the plants to produce pollen in the first growing season (Baier et al. 2012). The high light treatments will accelerate the outcrossing.

Once we have finished the outcrossing, we will intercross the progeny and select approximately ¼ of the progeny that inherited the oxalate oxidase gene from both parents in a homozygous state. We will then plant these homozygous individuals in seed orchards to generate large numbers of seed for restoration from open pollination. The purpose of planting seed orchards composed of OxO-homozygous parents is to ensure that all progeny also inherit the OxO gene.

Combining blight tolerance and Phytophthora root rot resistance

To combine resistance to chestnut blight and Phytophthora root rot, I suggest crossing transgenic trees containing the oxalate oxidase gene with backcross trees that have been selected for resistance to *Phytophthora cinnamomi*. We can inexpensively test for the inheritance of OxO, which is expected in 50% of the progeny, with leaf disk assays of OxO activity. Then we will inoculate those progeny that inherited OxO with *P. cinnamomi* and plant the surviving progeny in orchard locations where *P. cinnamomi* is present in the soil for further selection for root rot resistance. After selections are made for resistance to both chestnut blight (via OxO tests) and root rot (via inoculation with *P. cinnamomi*), then we will intercross these selected trees to make OxO homozygous and to further enhance resistance to *P. cinnamomi*. We will plant selected homozygous progeny in seed orchards where these trees will intercross to generate large quantities of seed for restoration via open pollination (Westbrook et al., 2019c).

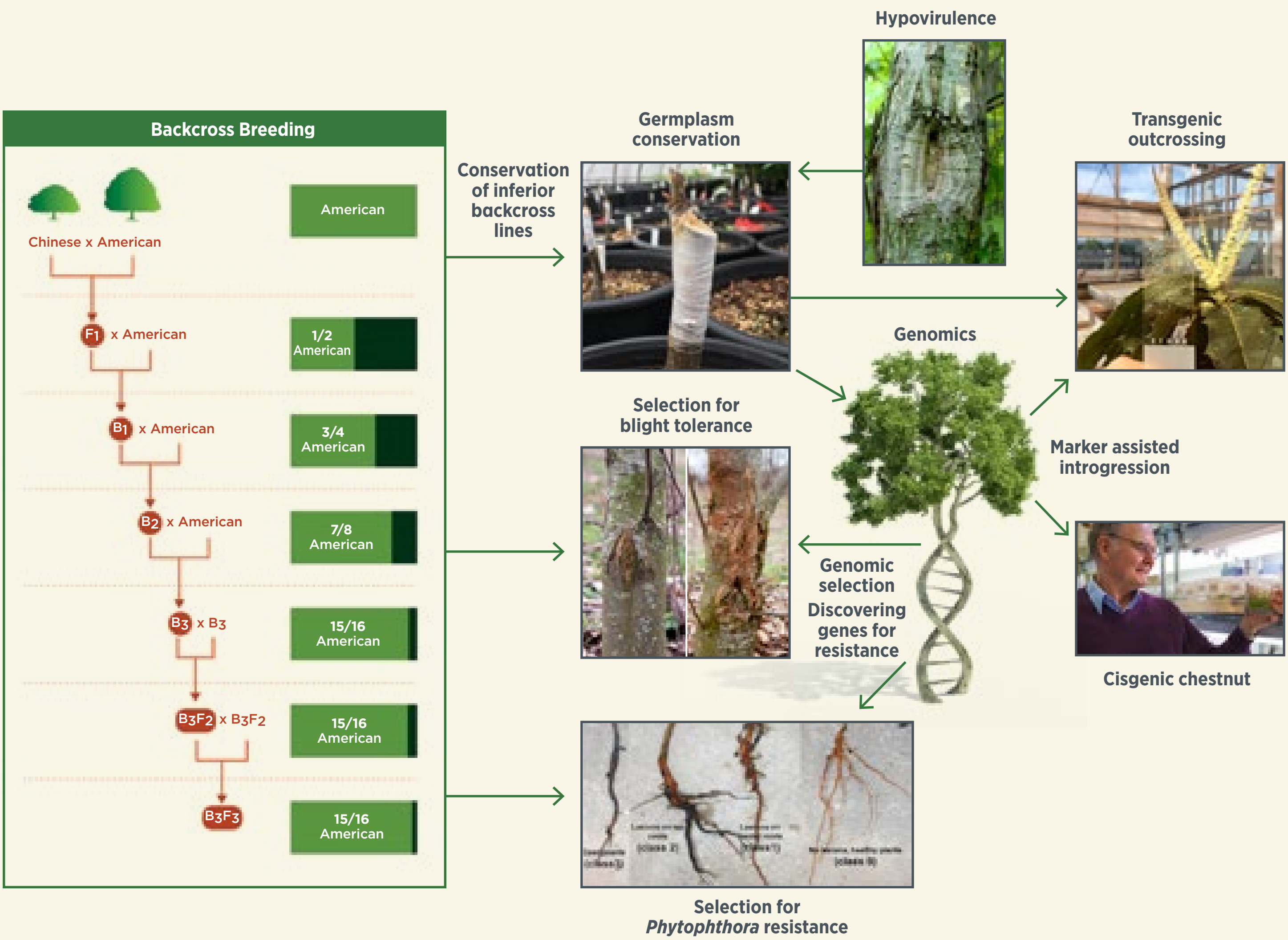
Cisgenic American chestnut

It is not currently known whether transgenic American chestnuts containing the OxO gene will remain blight-tolerant over these trees' lifetimes and whether the OxO gene will be stably expressed over multiple generations of outcrossing. Therefore, TACF and collaborators continue to pursue research to discover candidate genes for resistance to chestnut blight from Chinese chestnut. If we find that blight tolerance conferred by OxO is not sufficient for restoration, we could insert additional candidate genes into American chestnut to potentially enhance blight tolerance. "Cisgenic" is the term for insertion of genes from one closely related species into another.

Previously, Powell's team at SUNY-ESF created cisgenic lines of American chestnut by individually inserting 26 Chinese chestnut candidate genes for chestnut blight tolerance into the genome of American chestnut. These genes were mapped to genome intervals that were associated with

[1]An alternative strategy to rescue backcross genetic diversity is to apply transgenic pollen containing the wheat oxalate oxidase gene to backcross trees with inferior blight tolerance.

Figure 1 **Strategies to develop populations of American chestnut that are blight-tolerant, resistant to *Phytophthora cinnamomi*, and genetically diverse.**



chestnut blight tolerance in hybrid trees and that also had increased expression in Chinese chestnut stems as compared with American chestnut stems after inoculation with the fungus that causes chestnut blight (Barakat et al. 2009; Kubisiak et al., 2013; Barakat et al., 2012; Nelson, 2014). Five candidate genes from Chinese chestnut, when individually inserted into American chestnut, conferred partial blight tolerance. However, none of the candidate genes increased blight tolerance to the level conferred by oxalate oxidase (Powell et al., 2019). Should we need to enhance blight tolerance beyond levels conferred by OxO, we could insert combinations of Chinese chestnut candidate genes for blight tolerance along with oxalate oxidase.

Recently, we began collaborating with Jeremy Schmutz and his team at the HudsonAlpha Institute for Biotechnology and Jason Holliday at Virginia Tech, with the goal of discovering additional candidate genes for blight tolerance and resistance to root rot from Chinese chestnut. Our strategy to discover candidate genes includes:

- Generating high quality reference genomes for an American chestnut and two Chinese chestnut parents in TACF's breeding program.
- Mapping regions of the genome associated with variation with blight and root rot resistance in hybrid populations.
- Comparing genes in Chinese chestnut and American chestnut in genomic regions associated with resistance.
- Comparing the expression of genes in Chinese chestnut and American chestnut after infection of stems with chestnut blight or roots with *P. cinnamomi*.

[2]If we omitted selection for OxO homozygotes and instead started generating large quantities of seed from intercross trees that are heterozygous (i.e. inherited one copy of OxO), then approximately 75% of the progeny from these intercrosses are expected to inherit at least one copy of OxO, and 25% of the progeny would not inherit any copies of OxO. After outcrossing is complete, we may decide to forgo the intercrossing and allow natural selection to cull the population of blight-susceptible trees.

Strong candidate genes for resistance include genes that map to genomic intervals associated with resistance and also:

- Resemble known disease resistance genes in other species.
- Are missing or non-functional in American chestnut and are present and functional in Chinese chestnut.
- Have increased expression in Chinese chestnut as compared with American chestnut after inoculation with the pathogens that cause chestnut blight or root rot.

We have taken the initial steps to discover candidate genes resistance in Chinese chestnut. HudsonAlpha has recently generated a high quality reference genome for American chestnut and we have mapped additional regions for blight tolerance in TACF's backcross populations.

Conclusions

Recent advances in genomics have revealed that genetics of blight tolerance is more complex than previously assumed. TACF is diversifying its strategies to include breeding with earlier backcross generations to find a balance between blight tolerance and American chestnut genome inheritance. Pending regulatory approval, we plan to breed American chestnuts containing the OxO gene with wild American chestnut trees to diversify transgenic populations. We will combine blight and root rot resistance by breeding OxO-transgenic trees with backcross trees that are resistant to root rot. We are pursuing genomics research to discover additional candidate genes for blight and root rot resistance from Chinese chestnut with the goal of creating resistant cisgenic American chestnut lines (**Figure 1**). By pursuing these strategies individually and in combination, we are hopeful that we will increase our odds of success in generating restoration populations with durable blight tolerance, resistance to *Phytophthora cinnamomi*, and sufficient genetic diversity to adapt to a changing climate.

ACKNOWLEDGEMENTS

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In the last issue of *Chestnut*, we presented the results from a seed storage (stratification) study that investigated the relationship between length of warm and cold stratification times and emergence/survival in Chinese chestnut. In this issue, we will present the results from a concurrent stratification study that investigated the relationship between length of cold stratification and emergence in American chestnut seeds from five different provenances across its native range.

Seed stratification

IS IT NECESSARY?

By Laura Barth, TACF Meadowview Horticulture and Pathology Specialist; Bruce Levine, Maryland Chapter; Jared Westbrook, TACF Director of Science; and Annika Socha, Summer Research Intern at Meadowview Research Farms

DO AMERICAN CHESTNUT SEEDS FROM DIFFERENT PROVENANCES REQUIRE DIFFERENT DURATIONS OF COLD STRATIFICATION TO GERMINATE AND GROW VIGOROUSLY?

Provenance refers to the geographic region from which a seed is collected. Seeds of the same species from different provenances do not necessarily germinate or grow the same under similar conditions – trees from Maine may not respond the same when grown in North Carolina or vice versa. Flowering times may be different, as well as growth rate, chilling requirements to break dormancy, frost damage, etc. (Dirr and Heuser, 2006).

Understanding differences in germination and emergence after cold stratification is important for several reasons. In terms of cultivation, understanding the relationship between stratification and seedling emergence may enable greenhouse growers to plant chestnuts earlier (if it is determined that long stratification times are not required) or optimize plantings (if a certain amount of stratification results in higher seedling emergence, survival, earlier germination, and/or uniformity). Understanding stratification requirements based on provenance can help TACF in our restoration efforts, and our increased understanding of the influence of stratification times on emergence and growth can enable us to tailor growing recommendations to members based on their location. It can also provide insight into how trees across the native range may cope with changes due to global warming (or how trees outside of the range could be utilized for breeding in regards to global warming), and how we can combine stratification based growth parameters with future climactic projections for restoration efforts involving germplasm conservation (Glushkova et al., 2012).

Do we even need to stratify chestnut seeds? Does it improve germination and growth?

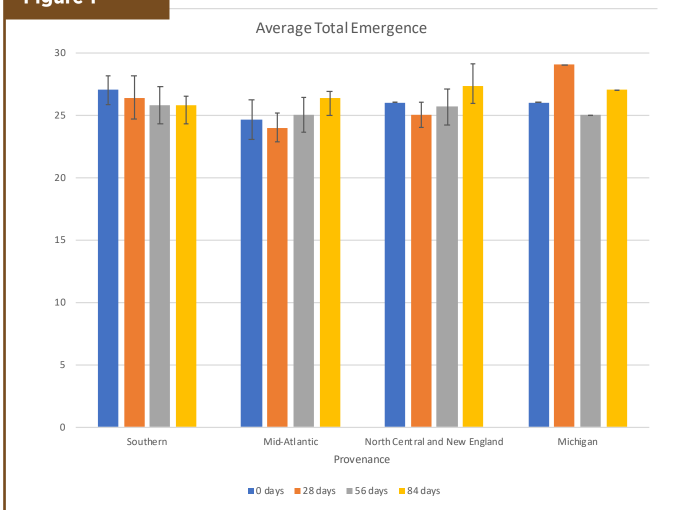
As was mentioned in the last issue, there is very little that has been documented in the literature on stratification times for American chestnut (and *Castanea spp.* in general). Anecdotally, there have been numerous accounts of chestnut seeds not needing any cold stratification to achieve germination, although it is generally recommended and the vast majority of growers do stratify their seeds.

The objectives of the following research were twofold. Firstly, to determine if the length of cold stratification time had an effect on days to emergence and total emergence of American chestnut seeds; and secondly, to investigate any differences in time to emergence and emergence/survival percentage among seeds obtained from different regions of the native range.

METHODS

The study was initiated on October 5, 2018. Open pollinated American chestnut seeds (and one open pollinated B_4 hybrid, CAT-142 X OP; **Table 1**) that originated from mother trees within different provenances in the native range were obtained. All of the TACF designated provenances were included in this study: Southern, Mid-Atlantic, North Central, and New England, as well as one family from a mother tree originating from Michigan (**Table 1**). Seeds were divided into groups of 30 and separated into treatments based on stratification time: 0, 28, 56, or 84 days. Seeds in the 28, 56, and 84-day stratification treatments were placed into moistened peat in perforated gallon plastic bags and stored at 40°F in the cooler at Meadowview Research Farms until they were removed for planting. Seeds in the 0-day stratification treatment were planted immediately after being received. After planting, newly emerged plumules were counted three times a week. The study was terminated on May 1, 2019, after several weeks had passed where no new plumules had emerged from any of the treatments, and it appeared that the majority of the seeds that had been planted had emerged. Heights for all the surviving plants were measured during the first three weeks of July.

Figure 1



Total emergence, out of 30 planted seeds, averaged across provenance for four different cold stratification times. Stratification time is denoted by color, as indicated in the legend on the bottom.

RESULTS AND DISCUSSION

1. Cold stratification is not necessary for germination.

The results from this study confirm data presented in the last issue that show no period of cold treatment is necessary for germination and emergence. No significant differences were observed in total emergence and survival among any of the stratification times or provenances (**Figure 1**).

2. However, longer stratification times resulted in fewer days to emergence.

Our data show that increased stratification times resulted in significantly fewer days to emergence for all of the families among all of the provenances (**Figure 2**). Seeds that underwent 84-day stratification had less variation and more uniformity in time from planting to plumule emergence, around 20-30 days, as opposed to treatments that underwent shorter stratification times. Seeds that were stratified for 0 or 28 days exhibited much greater variation in their emergence, with plumules developing continuously from the time of planting to a few weeks before the study was concluded.

A possible explanation for these results is that individual seeds may display a wide variation in dormancy requirements and maturation periods. This results in a wide variation of emergence times for shorter stratification treatments - the seeds that didn't emerge right away after planting were still undergoing their dormancy requirement, just in the pot as opposed to cold storage, which supports the findings in the last issue's stratification experiment. A longer stratification time may give the appearance of a shorter seed establishment period, but it could just be ensuring that more of the seeds have reached their dormancy requirement at planting before undergoing the seedling establishment phase and emergence.

These results are important for growers and researchers, who are most likely growing on a schedule, and require short and uniform emergence times to optimize the growth and timing of their seedlings for field plantings or use in research projects.

3. Longer stratification times may improve seedling growth.

We observed that 84 days of stratification resulted in taller seedlings for all provenances, except those from the south (**Figure 3**), although the range in height was slightly more variable than that of the shorter stratification treatments. These results could be caused by the fact that the southern provenance seeds were received, and thus planted and stored (depending on treatment), about a month earlier than the seeds from the other provenances. In late March/early April it became necessary to start fertilizing the seedlings, which resulted in the plants from the Mid-Atlantic, North Central, New England, and Michigan provenances being fertilized earlier in their growth than those from the south. They also experienced warmer temperatures and longer days earlier in their growth, although the greenhouse temperature was controlled as much as possible. Another potential explanation could be related to nutrient mobilization; cold storage could make more nutrients available (converting starch to sugar), resulting in faster growing seedlings.

Regardless, these results are of practical use for growers and researchers. Growers may want seedlings of a certain size, depending on their use. Nursery growers may want slightly shorter seedlings for bare root production, as seedlings that are too large are difficult to ship. Commercial or home growers that want to plant their seedlings in the ground right away might want a tree that is a bit bigger and sturdier to better withstand environmental and pest pressure. Researchers who are growing seedlings for use in small stem assays want the largest seedlings possible, since seedlings under 3mm are generally not included in the study (that is the cutoff at Meadowview Research Farms), and

Table 1: Provenance of the mother tree, orchard location, tree code, mother tree location, and elevation of mother tree location for the seed sources used in this study. [a]B_4 hybrid [b]Mixed lot of seeds from various mother trees within provenance.

PROVENANCE OF MOTHER TREE	ORCHARD	TREE ID	MOTHER TREE LOCATION	ELEVATION (ft.)
Southern	Cataloochee Ranch	CAT-142xOP[a]	Avery County, NC	3,600
		CAT-253xOP	Grandfather Mountain, NC	4,534
		CAT-258xOP	Grandfather Mountain, NC	4,077
		CAT-273xOP	Montreat Cove, NC	2,890
		CAT-277xOP	Montreat Cove, NC	2,890
Mid-Atlantic	SCBI, Front Royal, VA	SCBIxOP[b]	Sugarloaf Mountain, MD	1,283
			Wild trees within 50mi of SCBI orchard	Unknown
	Scrivener	Scrivener x OP[b]	Multiple locations throughout VA (possibly other Mid-Atlantic states)	Unknown
	Goshen, VT	VA1-1-1-11	Smyth Co., VA	3,399
		VA1-1-2-9	Smyth Co., VA	3,399
		VA1-1-6-7	Smyth Co., VA	3,399
North Central	Zoar Valley, NY	F-59 Krall	Western NY (location unknown)	Unknown
		H40 Wells	Springville, NY	1,329
New England	Goshen, VT	ME2 2-5-16	Knox Co., ME	223
Michigan	Zoar Valley, NY	H38 MI	Wexford Conservation District, Cadillac, MI	1309

Figure 2a

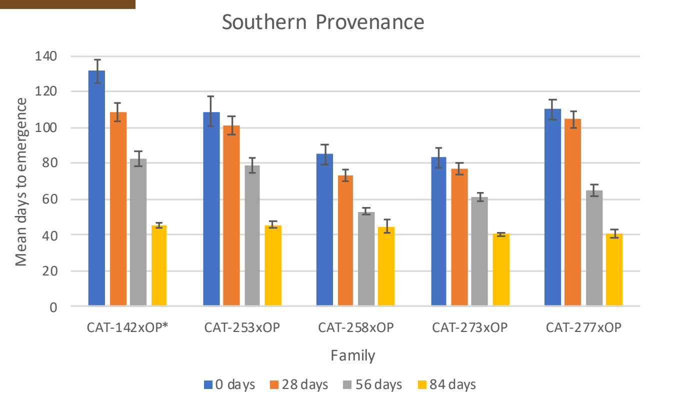


Figure 2b

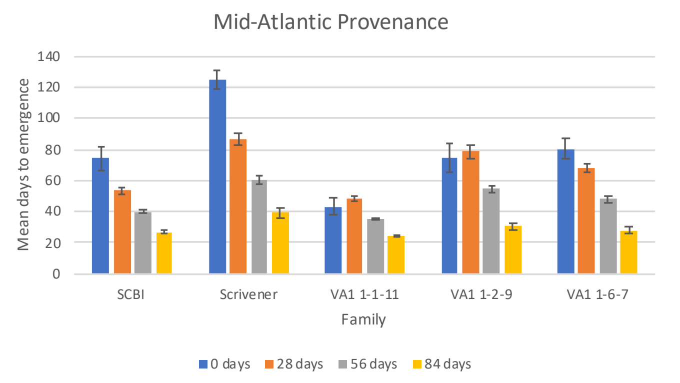


Figure 2c

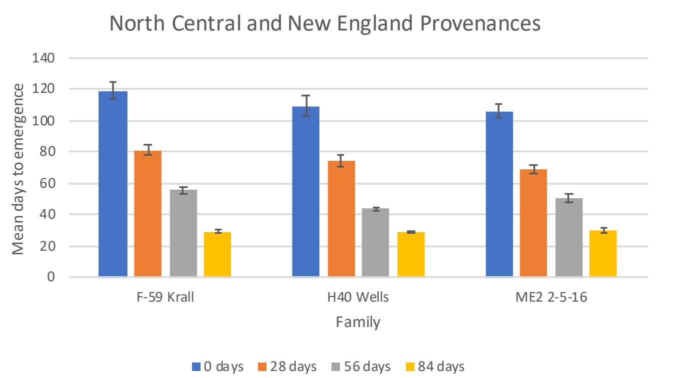
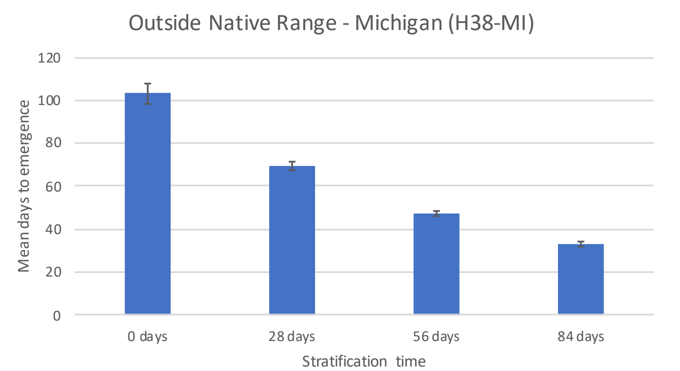


Figure 2d



Mean days to emergence after 0, 28, 56, and 84 days of cold stratification of seeds sourced from Southern, Mid-Atlantic, North Central, and New England Provenances, as well as one family from Michigan. For 2A, 2B, and 2C, stratification time is denoted by color, as indicated in the legend at the bottom. *Denotes B_4 hybrid.

Figure 3a

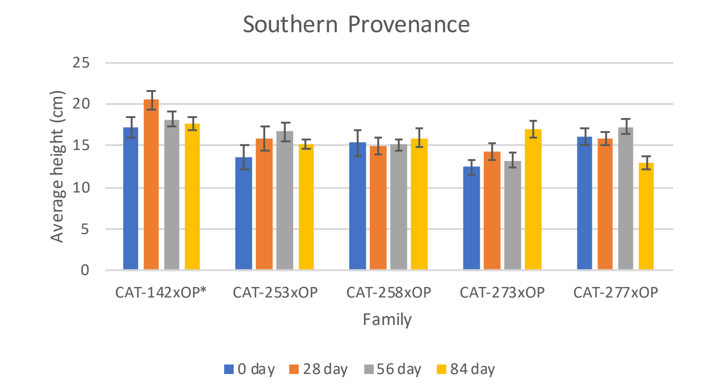


Figure 3b

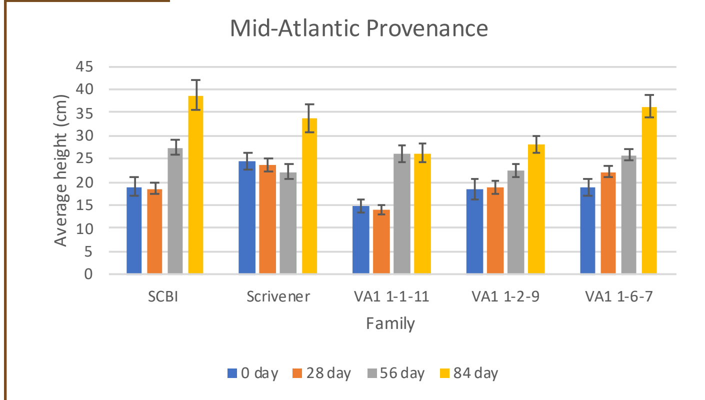


Figure 3c

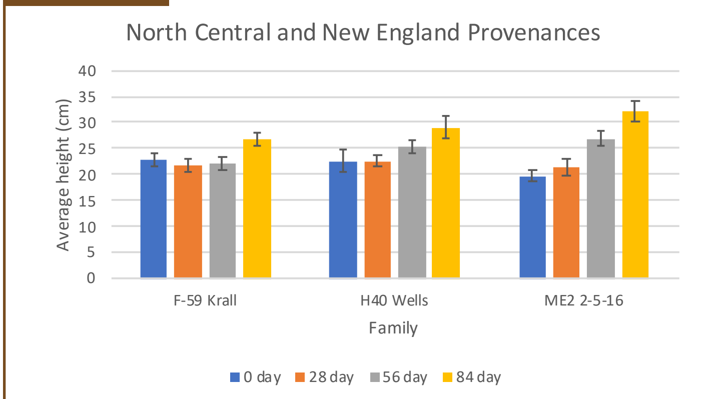
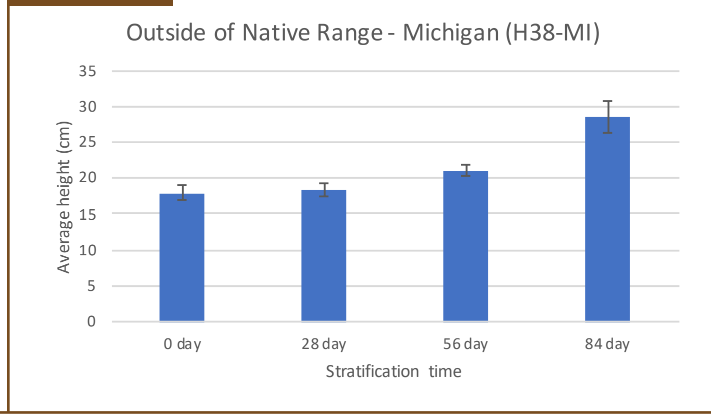


Figure 3d



Average height of plants from Southern, Mid-Atlantic, North Central, and New England Provenances, as well as one family from Michigan, grown after 0, 28, 56, and 84 days of cold stratification. For 3A, 3B, and 3C, stratification time is denoted by color, as indicated in the legend at the bottom.
*Denotes B_4 hybrid.

smaller trees have a tendency to snap at the inoculation/canker site, making them impossible to measure. Larger seedlings are also easier to graft in most cases. These data, combined with the shorter time to emergence, suggest that for the most uniform and rapid emergence and growth, a period of stratification is needed for better results.

4. The provenance of the seeds did not appear to have a strong effect on dormancy requirements.

Instead, our data show greater variation between families within the same provenance. One hypothesis to explain this is genetic variation within provenances. This is something that we hope to investigate further in future studies.

FUTURE RESEARCH

This work provides strong evidence of the relationship between length of stratification and emergence, and, like most research, invites more opportunities for future work. We would like to repeat the study with more sources from within the different provenances (with more representation of North Central and New England families), as well as more sources outside the native range, using both warm and cold storage treatments. This will enable us to better elucidate genetic variation within and between provenances, as well as whether or not the seed maturation period is temperature-dependent. We would also like to reduce some of the cultural variables that were unavoidable during this study, namely fertilization and time of collecting height data – it would be more useful to collect the height data at a set interval after seed sowing to account for differences in planting time.

Storage media and the question of whether to sanitize seeds or not are also important in terms of seed stratification. In the next issue of *Chestnut*, we will present the results from a study that investigated different storage media types and surface sterilization solutions for cold stratification of backcross chestnut seeds.

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Hypovirulence AND Backcross Breeding

A MARRIAGE EXPERIMENT

By Mark Double and William MacDonald, Ph.D.
WV Chapter and West Virginia University

In 1904, the chestnut blight fungus (*Cryphonectria parasitica*) was first identified in New York City, leading to what is considered to be one of the worst ecological disasters to occur in North America. With limited import regulations, chestnut blight was first detected in northern Italy in 1938 and the North American scenario began to repeat itself in Europe on *Castanea sativa*, European chestnut.

In the late 1950s, less than 15 years after the discovery of chestnut blight in Italy, Antonio Biraghi, an Italian plant pathologist, noted that some severely cankered European chestnut trees showed signs of recovery (Biraghi, 1953; MacDonald and Fulbright, 1991). Rather than the typical sunken appearance of killing cankers (**Fig. 1A**), the trees in Italy had odd-looking cankers.

Biraghi noticed a blocky-bark canker that was superficial and not sunken (**Fig. 1B**). Jean Grente, a mycologist from France, accompanied Biraghi in 1964 to examine some of the odd-looking cankers. Grente noticed that fungi isolated from the healing cankers were white-to-lightly orange-pigmented in dramatic contrast to the normal bright orange strains isolated from killing cankers (**Fig. 2**) (MacDonald, 1985). Grente (1981) continued experimenting with these white strains, noting that cankers caused by the white strains were significantly smaller than cankers initiated by the orange strains (**Fig. 3**). Since the white strains produced a much smaller canker, Grente termed them "hypovirulent" or less virulent. Grente (1981) also found that when cubes of both the white and orange fungus were placed side-by-side on an agar petri plate, the orange strain grew normally for about four days but then turned white (**Fig. 4**).

Grente realized that there was a transmissible element in the white fungus. Only decades later was it realized that the transmissible element was a unique double-stranded RNA virus. Since the virus in the chestnut blight fungus caused the phenomenon of hypovirulence, the virus was placed in its own family, *Hypoviridae* or hypovirulent virus.

Figure 1

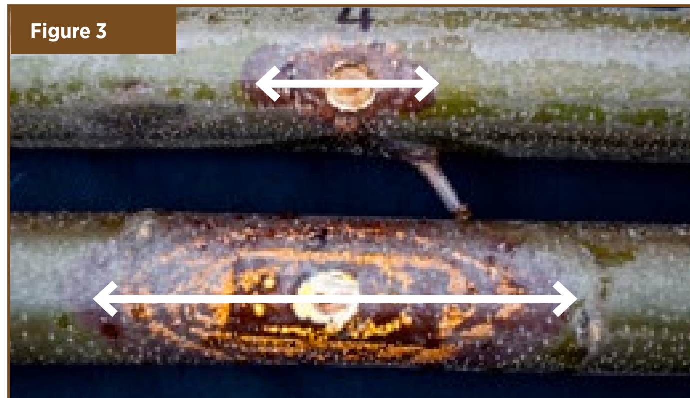
Typical virulent (killing) canker on American chestnut (left, 1A), and a hypovirulent blocky-bark canker on European chestnut (right, 1B).

Figure 2

Isolates of the chestnut blight fungus growing on an agar medium. Left, normal orange-pigmented fungus of killing strain of the fungus. Right, abnormal white-pigmented strain that is hypovirulent (virus-containing).

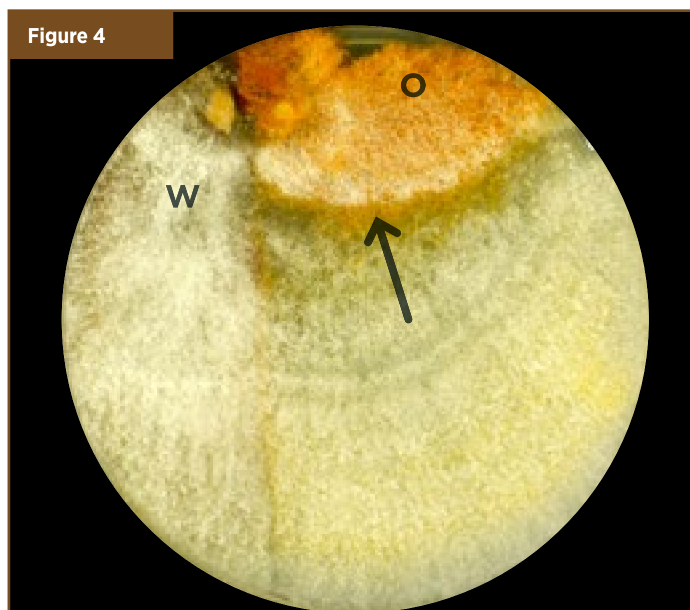
The American Chestnut Foundation (TACF) began its breeding work in 1983 to test the hypothesis that genes from resistant Chinese chestnut could be incorporated into susceptible American chestnut to produce a tree with American form and intermediate levels of blight tolerance. In 2006, an experiment was designed to incorporate hypovirulence with backcross trees to see if the hypovirulent strains could give added protection to trees that had some resistance. Our hypothesis was that hypovirus treatment would lengthen the life of all test trees. Seeds from backcross trees (B_2F_2, B_2F_3 and B_3F_2) along with pure American, Chinese and European chestnut controls were

Figure 3



Excised (cut) American chestnut stems inoculated in the laboratory with a white hypovirulent isolate (top stem) and an orange virulent isolate (bottom stem). White arrows indicate growth of the fungus after 4-weeks.

Figure 4

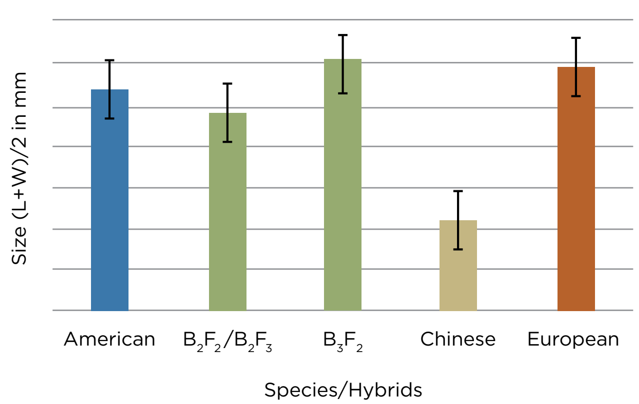


Pairings on agar media of cubes of virulent and hypovirulent isolates. The pairing is an example of transmission of a virus from the white (W) to the orange (O) strain. The orange fungus grew normally for 3-4 days after which the virus was transmitted to the orange strain and all subsequent growth is white (arrow show the time when the orange strain was converted to white).

planted in six blocks at the Agronomy Farm on the campus of West Virginia University. Six blocks of 150 trees each for a total of 900 trees were planted in 2006. Seedlings that died were replaced annually from 2007-2010.

To test the level of resistance of the hybrid trees, all trees over 3" diameter at breast height (DBH) were inoculated in June 2013 with 'Weekly,' a moderately virulent isolate of the chestnut blight fungus. Cankers size ([length+width]/2) was assessed in 2014 (**Fig. 5**). Data from B_2F_2 and B_2F_3s were combined due to low number of stems that were large enough to inoculate. Among all the trees tested, Chinese chestnuts, *Castanea mollissima* (**Fig. 5**, tan bar) had significantly smaller cankers. No significant difference in canker size were observed among the other hybrids and species. These data indicated that all the species and hybrids, except Chinese chestnut, may benefit from the use of hypovirulent strains.

Figure 5



One-year measurement of artificial inoculations with the 'Weekly' isolate.

TACF's 3BUR approach includes biological control as one of the three components for restoration. The use of hypovirulent isolates was integral for the "marriage" of hypovirulence and backcross breeding. Beginning in 2015, cankers in three of the six plots were treated with four hypovirulent strains of the chestnut blight fungus (Grand Haven 2; Euro 7; County Line and Weekly HV). Cankers in the other three plots were untreated to serve as controls. Grand Haven 2 and County Line were isolated from recovering trees in Grand Haven and Manistee County, MI, respectively. Euro 7 was isolated from a recovering European chestnut tree in Florence, Italy. The 'Weekly' HV isolate was created by co-inoculating the 'Weekly' virulent isolate and the Euro 7 HV strain on agar media. All HV isolates were grown on agar media, mixed with sterile water and solidified water agar and mixed in a blender to the consistency of applesauce. The inoculum was added to sterile 500 ml squirt bottles and inserted into holes made around the margin of a canker with a hammer and 1-cm-diameter steel punch (**Fig. 6**). After inoculum was added to the holes, masking tape was applied to retard drying of the inoculum.

Tree survivorship in this study was impacted greatly by hypovirus treatment. As summarized in **Table 1**, all species and hybrids, except Chinese chestnut, benefitted from canker treatment. Comparing hypovirus-treated and non-treated plots, more trees survived (22%, 36% and 20%, respectively for American, European and B_3F_2s) in the hypovirus-treated plots after three years. Survival

	HV-Inoculated Trees					Non-Inoculated Trees				
	# Living	# Planted	% Living	DBH	Rating*	# Living	# Planted	% Living	DBH	Rating*
American	62	90	69%	12.0	1.74	43	92	47%	14.6	1.66
B_2F_2/B_2F_3	97	124	78%	18.6	2.38	78	117	67%	18.5	2.75
B_3F_2	43	62	69%	14.9	2.12	35	72	49%	17.9	2.73
Chinese	82	92	89%	33.0	3.59	89	97	92%	28.2	3.81
European	42	83	51%	9.7	1.74	11	72	15%	11.4	0.63

***Tree Rating:**
4 = No evidence of cankers; healthy crown, no dieback
3 = Cankers; main stem alive; limited crown dieback
2 = Cankers; main stem alive; significant crown dieback
1 = Cankers; main stem dead with epicormics shoots
0 = Cankers; main stem dead and no epicormics shoots

Table 1: Percentage of living trees, diameter at breast height and the 0-4 subjective canker rating of American, backcross, Chinese and European chestnut trees in 2018.

is one measure of success. Tree health is another. After three years of hypovirus treatment, tree health for TACF's hybrids and Chinese chestnut was unaffected, based on the 0-4 subjective whole-tree rating (detailed in **Table 1**). The exceptions were American and European chestnut, as those species had higher tree health ratings in the hypovirus-treated plots. In many instances, the main stem of American and European chestnuts died and resprouted (**Fig. 7**). This was not the case for TACF's hybrids. It appears that TACF's breeding program has provided the hybrid trees sufficient resistance such that hypovirus treatment does not improve overall tree health, despite hypovirus treatment. Thus, our hypothesis that hypovirus treatment could keep blight-susceptible trees alive longer than non-treatment was fulfilled partially, but only for the most susceptible trees (American and European). Trees produced by TACF's breeding program seem to have acquired sufficient resistance to offset aid provided by hypovirus treatment three years following initial treatment. In conclusion, it appears that hypovirulent treatment is most effective with American and European chestnut and treatment should be effective as a biological control agent in TACF germplasm conservation orchards.

ACKNOWLEDGMENTS

The authors thank Dr. Frederick V. Hebard, TACF chief scientist emeritus, who designed the plot layout and provided the nuts for the experiment. Thanks also goes to the 2006 Meadowview farm staff who helped implement the study.

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Figure 6

Treatment of canker using a hammer and steel punch to tap holes around the margin of a canker prior to inoculation with a slurry of hypovirulent isolates.

Figure 7

Resprout from dead main stem in HV-treated plots.

INTERACTIONS BETWEEN American Chestnut ESTABLISHMENT, GROUNDCOVER, AND ECTOMYCORRHIZAL COLONIZATION

By Jenise Baumann, Western Washington University and Jennifer Franklin, University of Tennessee

The American Chestnut Foundation (TACF) has partnered with the Appalachian Regional Reforestation Initiative (ARRI) since 2008 in an effort named "Operation Springboard" to use reclaimed surface mines for the planting of TACF backcross chestnuts as a regional and widespread approach to reintroduce the species throughout its original range.

Figure 1. A chestnut seedling in the first year after planting on a reclaimed mine site.

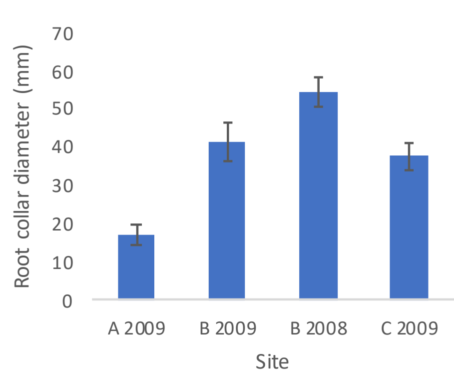
As part of the mine reclamation process, sites are usually seeded with low growing plants to control erosion, but these ground covers can compete with the trees for water and nutrients. They can also be beneficial to trees on these challenging sites by providing organic matter, helping to initiate the nutrient cycling process, and acting as hosts for a wide range of organisms both above, and below ground. Of particular importance to trees are the beneficial ectomycorrhizal (ECM) root colonizing fungi. These fungal symbionts are essential for healthy tree growth, and ECM colonization has been found to result in improved chestnut establishment, and a positive correlation with tree growth. An understanding of how different groundcovers influence the growth and mycorrhizal colonization of young chestnut trees will allow us to select species that can be planted along with the trees to maximize chestnut establishment and growth.

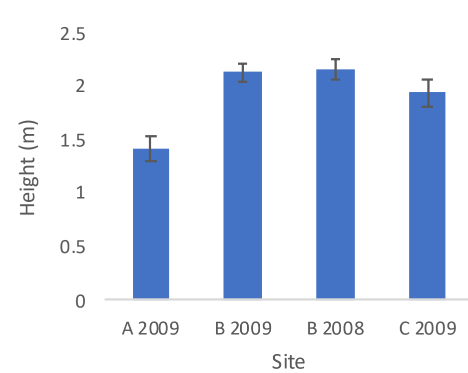
This study evaluated plantings of TACF restoration chestnut that were planted in 2008-2009 on restored surface mines in eastern Tennessee. After mining ended, each site was reclaimed using the Forestry Reclamation Approach as recommended by ARRI in which the material most suitable for tree growth is placed on the surface using minimal compaction. Plots on these sites were originally seeded with different groundcovers to test their influence in the early years after reclamation, but over time, differences in soils and in the vegetation surrounding the site had a larger influence on the development of vegetation. Seventy-eight chestnut trees were sampled in 2016, and 142 chestnut trees were sampled in 2017. Chestnut survival and growth, and the density and species composition of vegetation surrounding each seedling, were measured. ECM colonization of 96 chestnut root systems was assessed; one hundred root tips per seedling were evaluated to determine the percentage of roots colonized by each species of ECM fungi. Fungal species were initially classified based on their color and texture, and other morphological features, then were identified by DNA sequencing.

Figure 2

Chinese chestnut tree planted by the USDA more than 60 years ago in Holmes Educational State Forest, NC. Note that the main trunk divides into several large branches. Photo courtesy of Kent Wilcox.

Figure 3



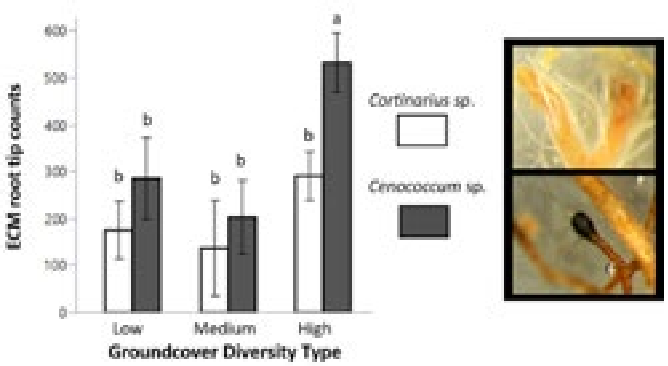


Stem diameter at the base of the stem, and height of seedlings planted in 2008 and 2009, measured in 2017.

Figure 4

Two eight-year-old chestnut seedlings on site B, with a dense cover of lespedeza in the foreground.

Figure 5



Comparing the two most abundant ECM species across the sites. Cortinarius was evenly distributed, however, *Cenococcum sp.* significantly more abundant on site A with higher groundcover species diversity.

Growth was greatest on site B, at an elevation of 2250' on a steep slope with an eastern exposure. Much of this site was quickly colonized from the surrounding mined area by sericea lespedeza (*Lespedeza cuneata*), a non-native, nitrogen fixing legume, which dominates the dense groundcover. Site A is a steep northwestern-facing slope at an elevation of 2950', with slow development of a diverse, native groundcover, and had the poorest growth of chestnut. Seedlings planted on site C, a steep, west-facing slope at an elevation of 1950', are intermediate in growth. The vegetation on this site is also intermediate in diversity, but rich in legumes with patches of lespedeza and native black locust (*Robinia pseudoacacia*) trees that shaded some chestnut seedlings. ECM colonized between 78% and 87% of the chestnut root systems, with the most common species being *Cenococcum sp.* (39%) followed by *Cortinarius sp.* 1 (15%), and *Cortinarius sp.* 3 (8%). Average ECM colonization was similar across sites but the ECM species differed with species richness lowest on the site that also had a low diversity of plant species. ECM diversity was greatest on site A with a moderate plant diversity, higher concentrations of micronutrients in the soil, and intermediate growth of chestnut seedlings. Results show that the groundcover community does influence the species of ECM that colonize chestnut roots, and also suggest that legumes are beneficial to the growth of chestnut seedlings on reclaimed minesites. Although chestnut seedlings in this study had greater growth in a low diversity groundcover, more diverse communities are more resilient to changing environments, and it is important to continue to monitor these sites.

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Bûche aux marrons
Chestnut log cake

In loving memory of my grandparents, Paul and Jeanette Bechetoille

By Florian Carle, CT Chapter

Baking and eating a Bûche de Noel (a cake made to look like a tree log) is a European tradition for the holidays. It comes from an old pagan tradition to cut a large tree trunk and to burn it during Christmas Eve inside the fireplace. The trunk would need to burn very slowly and last up to New Year's Eve. The duration of the burn would be an indication of the harvest for the next year. In the middle of the 19th century, with electricity ubiquity, the tradition evolved and a log-shaped cake is eaten instead.

Well before my birth in 1988, my grandparents on my mother's side would make a chestnut Buche every year for the holidays, which has polarized my extended family for decades. A handful of people (me included) are absolutely in love with this dessert and we look forward to the holidays just to have the occasion of eating the log, while the majority despise it, considering this dessert too rustic. Even those who hate it, though, won't give their slice up to anyone else!

Paul "Bon Papa" Bechetoille (1919-2015) ready to cut the massive bûche for all the family while Jeanette "Bonne Maman" Bechetoile (1921-2005) is holding her butter and sugar-free personal buche! (Valence, France - December 24, 2004)

The tradition continues with this bûche made in our CT home in 2017.

Ingredients

1.5 lb of chestnuts
1 stick of butter
3.5 oz of dark unsweetened chocolate
3/4 cup of granulated white sugar

Method

With a sharp knife, make a small cut into the fresh chestnuts and place them into boiling water for 10 to 15 minutes. Drain and peel chestnuts when they are cool enough to be handled. Cook the peeled chestnuts in boiling water for an additional 15 minutes (chestnuts will start breaking down in smaller bits).

Drain and puree the chestnuts. You can keep a bit of the cooking water to eventually add back if the puree is too dry. If you completely puree the chestnuts, your bûche will be very smooth, but I like to roughly puree them to have some chestnut chunks left in my bûche.

In a saucepan on low heat (or double boiler if you are so inclined), melt the chocolate, butter and sugar and add it to the chestnut puree as soon as all the sugar is dissolved. You can vary the ratio chestnut/chocolate by increasing the quantity of chocolate if you would rather have a more chocolaty bûche.

Mix well and store in a covered bowl overnight in the refrigerator. The mixture will look liquid at first, but it will slowly set overnight.

The next day, the mixture should be malleable and will hold a shape. Transfer mixture onto a large oiled sheet of parchment paper. Rock back and forth lengthwise to form a log. Once you are satisfied with your shape, use the back of a fork to gently mark the log and give a bark texture. Cut the two extremities and use the scraps to form a branch on top of the log. You can use walnuts to decorate your bûche.

Serve with a vanilla custard.

I always eat this dessert thinking of my grandparents, and I never skip a year making this bûche.

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