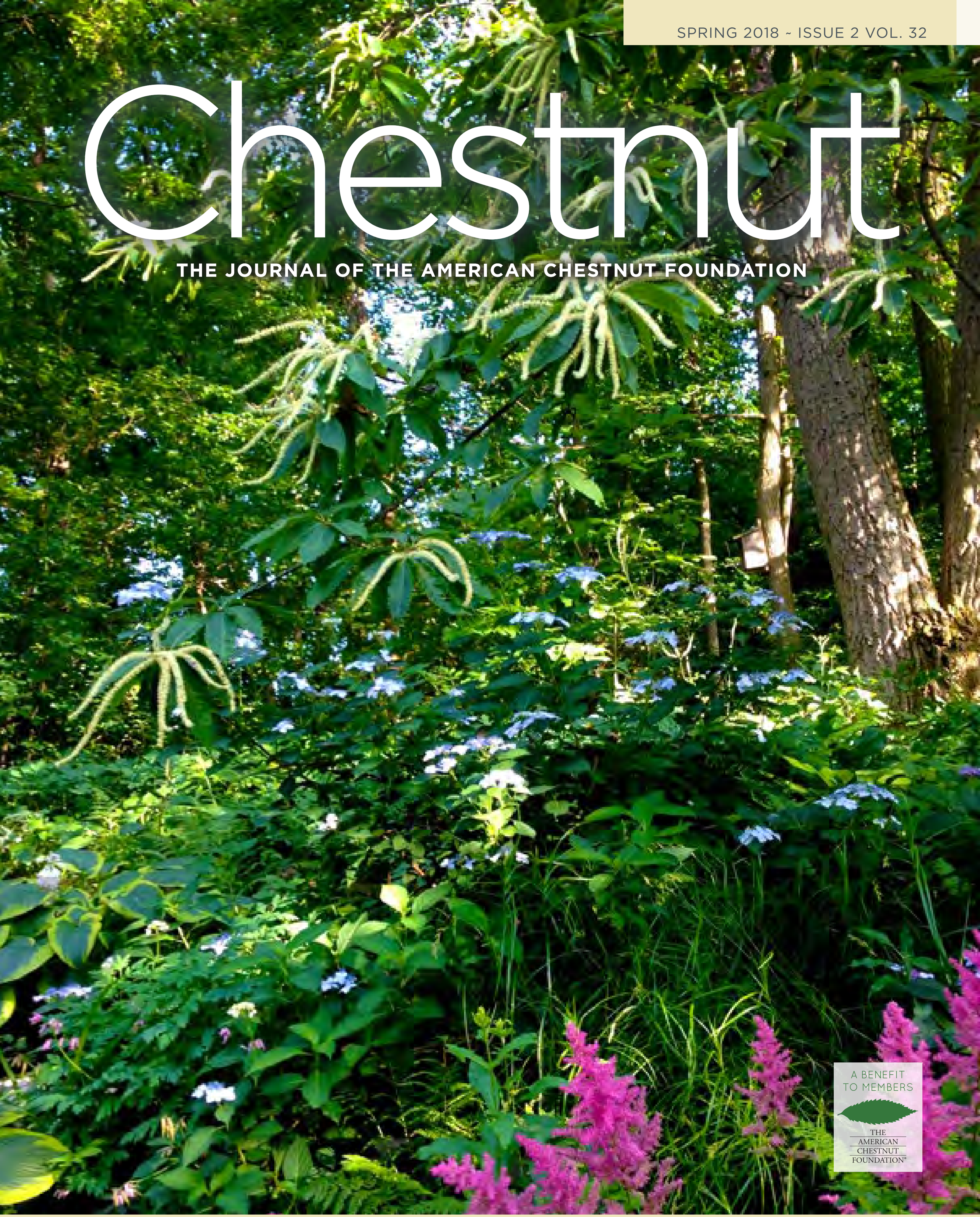
SPRING 2018 ~ ISSUE 2 VOL. 32

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION



TreeSnap App ~ Biodiversity in Maine ~ Direct-Seeding Chestnuts ~ Fire's Implications for Restoration

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A BENEFIT TO MEMBERS

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Lisa Thomson

President and CEO

DEAR CHESTNUT ENTHUSIASTS,

Seed catalogs are arriving in mailboxes and jonquils and daffodils are popping up in yards and fields. Spring is the most exciting time at TACF, because it embodies the hope of another year of growth and promise. After all, we are not only researchers, but also farmers at heart!

For those of you who may have grown up working the land, or are even new to plantings, you know what the potential of another growing season means. At TACF, our growing season has been given a jumpstart, thanks to those generous individuals who gave to last year's spring appeal. The goal was to build a greenhouse and two shade houses at Meadowview Research Farms by this growing season, to double our capacity of seedlings produced and ensure we have optimum growing conditions. Those ambitious goals were met, and at the spring board meeting in March, we dedicated the finished greenhouse to our chairman emeritus Richard S. Will (see the photos and read more on page 3).

Dick Will had audacious goals in the beginning of this organization. He had faith that in spite of tremendous odds, we would be successful if we just stuck with it. He is still involved through his generosity and encouragement, and participated at the spring board meeting. We are excited about these new facilities, and the greenhouse that honors one of our founding leaders who never gave up on the goal to restore the American chestnut to its native range.

Undoubtedly, many of you share Dick's optimism and longevity of purpose. This is a long-term mission in a very fast-paced world, so thank you for your dedication, patience, and loyalty. Our grandchildren will thank us when they walk under a canopy of mature chestnut trees in the future.

With gratitude,

Lisa Thomson, President and CEO
The American Chestnut Foundation

Follow me on Twitter (@MadameChestnut).

American Chestnut in Bloom

by Carolyn Puckett

This blooming American chestnut is the winner of our 2017 TACF Photo Contest. Carolyn took the picture in Hampstead, MD.

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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YOU Built It!

THANKS TO YOUR DONATIONS DURING LAST YEAR'S 2017 SPRING APPEAL, a new greenhouse facility stands complete at TACF's Glenn C. Price Farm in Meadowview, VA. The greenhouse will increase our capacity to grow seedlings, which in turn helps us increase our ability to conduct research, and the work has already begun! Thank you for your generous support and for making our new greenhouses a reality.

A SPECIAL DEDICATION:

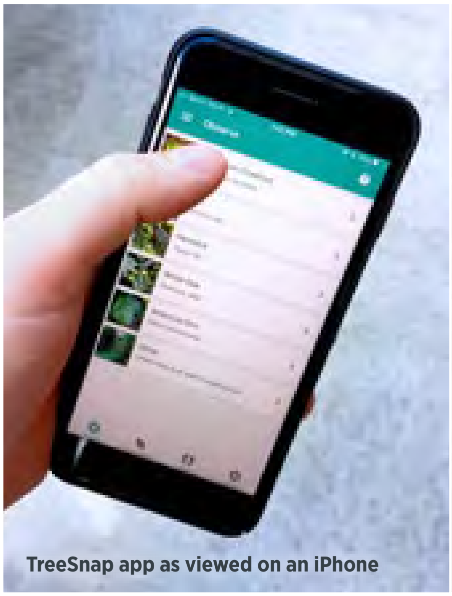
As part of TACF's spring board meeting in Abingdon, VA in mid-March, the board of directors recognized chairman emeritus Dick Will for his years of unwavering support, advocacy, and leadership by naming the newly constructed greenhouse in his honor. As a member of TACF for more than 20 years, Dick assisted the foundation in areas of organization and structure, he helped raise money for his state chapter, and was elected to serve a number of positions for TACF. The Richard S. Will Greenhouse is a testament to how our volunteers, members, and donors truly leave a lasting impact in the restoration efforts of the American chestnut.

TreeSnap:

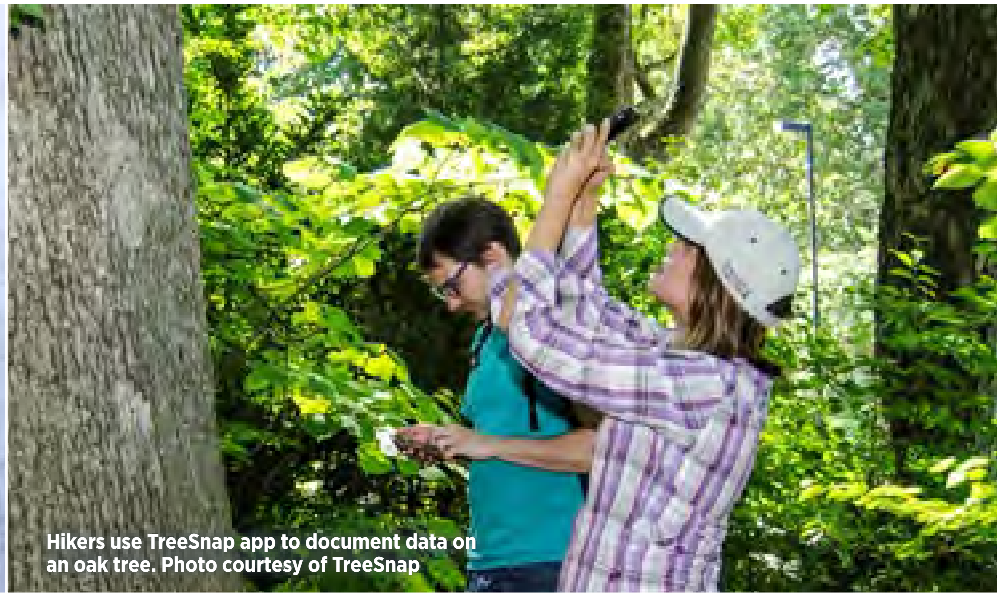
A CITIZEN SCIENCE APPLICATION FOR SMART PHONES

Ben Jarrett, TACF Southern Regional Science Coordinator and
Ellen Crocker, Department of Forestry and Natural Resources, University of Kentucky

Have you found any American chestnut trees recently?



TreeSnap app as viewed on an iPhone



Hikers use TreeSnap app to document data on an oak tree. Photo courtesy of TreeSnap

Each year, hundreds of interested people contact TACF via phone, email, mail, and social media to tell us about American chestnuts they saw hiking, on their property, or even while driving down the road. There's a new way to report these trees to us called TreeSnap, a citizen science application for smart phones.

The goal of TreeSnap is to make it easier for citizen scientists to contribute to forest-related research projects, in particular to help restoration tree breeding programs aimed at finding trees that can resist invasive threats. Using this app, anyone with an iPhone or Android phone can easily send information about chestnut trees to TACF scientists. TreeSnap was created in 2017 through a collaboration between researchers at the University of Tennessee and University of Kentucky as part of a National Science Foundation-funded Plant Genome Research Program grant. American chestnut is one of the species highlighted in the app, but other tree species of interest, such as hemlock, ash, elm, and white oak are also included. Lastly, there is an option to collect data on any tree species you'd like, so users can track any species of interest to them.

Previously, reporting American chestnut trees required using TACF's paper submission form and your own GPS device. TreeSnap streamlines this process and trees you observe on your phone will be immediately visible to scientists. In addition, your digital observation will be tagged with an ID number that can be included when you send in a physical sample of the tree to ensure it is the correct species. TACF's Tree Locator Form has been updated to allow for a TreeSnap submission ID. By using the TreeSnap application, we hope to empower any interested individual or group to help us scout for undiscovered, surviving American chestnuts. Instead of having to use a stand-alone GPS or trying to remember exactly where you were, you can use TreeSnap to geotag your sample, and by providing the TreeSnap submission ID on the Tree Locator Form, you don't have to write down all the details about the tree.

The data on American chestnuts will be used in a number of ways that benefit our mission to restore the species. Finding new sources of American chestnuts, particularly chestnuts in areas underrepresented in our breeding program, allows for us to increase the genetic diversity of our hybrid material. The American chestnuts found can also be potentially moved or progeny can be planted into our germplasm conservation orchards (GCOs). Trees in GCOs are useful for preserving trees with unique genetics, as well as serving as a mother tree orchard for diversifying the SUNY-ESF transgenic tree in the future. Lastly, the trees could be used for creating new backcross lines if needed.

TreeSnap

To start using TreeSnap, download the app to your iPhone or Android device, make an account, and sign in to the app. We hope to get more people involved with finding American chestnuts, so if you have questions or want to get your group involved, contact your chapter or your regional science coordinator.

TACF WELCOMES

David Kaufman-Moore

DONOR RELATIONS MANAGER

Having lived in Asheville for nearly 10 years now, I often get questions from donors or tourists (sometimes they happen to be both) about what brought me to this mountain city, what I like the most about Asheville, and recently, why The American Chestnut Foundation?

Often the answer I give is short: University. While it is a fact, I did graduate from the University of North Carolina at Asheville (UNCA) with a degree in Business Administration, it isn't entirely what brought me here. My first experience of the outdoors, Asheville, and Pisgah National Forest, dates back to my junior year of high school at Greensboro Day School in Greensboro, NC. It was there that I was given the opportunity to take part in experiential education by backpacking for a week in Pisgah Forest. That week forever changed my life, fostering my passion for the outdoors, preservation, and solidifying my decision to attend UNCA.

Other than riding a bike on the Blue Ridge Parkway or being knee-deep in a stream, what I enjoy most about Asheville is the hospitality, especially in regard to non-profits. With more than 2,500 registered non-profits in Buncombe County, a population of more than 250,000, it's inevitable that you will come across others working for and passionate about their respective non-profit organization. While one might assume this leads to competitiveness in the region, it's almost the opposite! We actually share our successes and challenges, as well as ideas about events, communications, fundraising, etc., to increase philanthropy in this region and other regions across the United States.

Prior to joining TACF in January as donor relations manager, I was employed by UNCA and performed a similar role. Working for an organization which has so many passionate volunteers, scientists, and donors all dedicated to reviving a single species of trees is simply magnificent. Though I've never witnessed the American chestnut in its grandeur, I look forward to the day I can share with my children or their children the story of how we, as a community, brought it back from near extinction.

Working for an organization which has so many passionate volunteers, scientists, and donors all dedicated to reviving a single species of trees is simply magnificent.

Q & A WITH DAVID:

What is your role at TACF?

I believe my colleagues would reflect the same sentiment about their roles at TACF, but mine is without a doubt the best. I get to use various analytics and critical thinking in order to determine and change how the organization interacts with its various constituents. What is so fun and rewarding about my role is that I get to look internally at the organization, work with staff to strengthen what we do well, help change what we could do better, think about new ideas, then connect with our supporters to see if we are on the right path.

Can you share a unique story or fact about yourself?

I've always been one to tinker, fix, modify, or change something. I was the kid growing up who would "destroy" their toys all in an effort to find out how they worked. While I still enjoy fixing what is (or might be) broken, I've recently gotten into woodworking and am enjoying that newfound hobby.

How would you define your experience at TACF thus far?

Simply amazing – enthusiastic and friendly members, volunteers, donors and staff, all of whom are coming together to lend whatever resource/talent they have to help restore this majestic tree!

Why Does the Maryland Chapter Collect Pure American Seeds? Because They are the Promise for the Future

By Gary Carver, MD Chapter Board Member

Each year, the Maryland Chapter collects and distributes thousands of pure American chestnut seeds and seedlings to many individuals and organizations. The seeds and seedlings promote awareness of TACF's program, educate people about growing chestnut trees, reforest mined lands, and encourage public awareness and appreciation of trees and forests. The promise is that when blight-resistant American chestnut trees are available in the future people will understand how chestnut trees will improve the forest environment and will know how to grow them. Of course, we all hope that it will be the near future.

Where do the seeds come from?

There are several groves of American chestnut trees in Maryland that produce large numbers of seeds. Almost all the trees in these groves are descendants of non-native Maryland trees that were planted in Maryland. The trees that have continued to produce seeds likely have some enhanced resistance, hypovirulent cankers, what some call “cruddy bark,” or a combination of these conditions.

Two of the groves are at the base of Sugarloaf Mountain. The seeds planted in these groves were irradiated in nuclear reactors and accelerators in the hope of causing mutations, one or more of which would confer blight resistance. One thousand of the irradiated seeds were planted at the “Sugarloaf Mountain American Chestnut Research Area – East Field” in April 1970. The following April, almost 600 second-generation irradiated-nut seedlings were planted at “West Field.” The original seeds for these experiments are thought to have been collected in Virginia, along Skyline Drive.

Today, fewer than two hundred of the Sugarloaf trees survive. They are all in serious decline and only a few possibly original stems remain. Among the reasons these trees survive, despite being decimated by blight (and *Phytophthora* in certain areas), may be that the grove in West Field was inoculated and rogued in the 1980s and some of the surviving trees were treated with hypovirulence in the 1990s. We have used trees from the Sugarloaf groves as mother trees in our breeding program.

About 25 years ago, Tom Scrivener, who is one of our chapter’s founding members, planted two dozen American chestnut seeds behind his company’s parking lot in Hagerstown. He obtained the seeds from the American Chestnut Cooperators’ Foundation (ACCF). While the ACCF grower agreement requires a pledge that no genetic material from the trees be used in “hybrid or other breeding programs,” it is permissible to distribute seeds from the trees. Each year these trees produce more burs than we can harvest.

Another grove, this one of eight trees, is on the grounds of the Western Maryland Research and Education Center in Keedysville. The director of the center obtained seedlings in 1986 from the Wexford County Soil Conservation District in Cadillac, Michigan. We have used two of these trees as mother trees in our breeding program.

Two other American chestnut groves also contribute seeds: Gary Carver’s personal orchard in Frederick County and one of the chapter’s Germplasm Conservation Orchards (GCOs) in Montgomery County. Gary collected and planted on his property seeds from Sugarloaf West Field about 20 years ago and, in subsequent years, from native Maryland trees. Some of his trees are third- and fourth-generation irradiated-nut trees (still not showing any mutations). Others are from open pollinated nuts from surviving native Maryland trees. This GCO, the chapter’s first such orchard, is at the Rockville chapter of The Izaak Walton League of America in Germantown. It recently began producing many seeds and should be a major source of seeds in coming years.

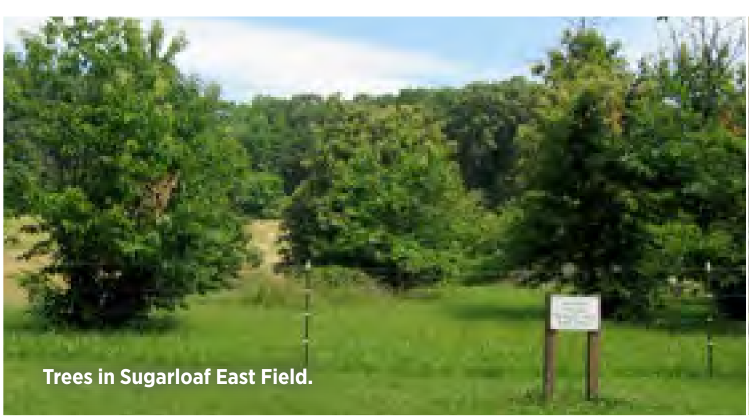
Who receives the seeds?

Each year, the Maryland Chapter sends several thousand or more (one year it was 8,000) pure American seeds to forester Michael French for his programs, strip-mined land reclamation and the TACF “Pure American Program.” A nursery in Georgia receives the seeds and grows bare-root seedlings for Michael and TACF.

For many years, we have been sending about one thousand seeds to Maryland’s John S. Ayton State Tree Nursery. The nursery grows seedlings that are distributed to the public by county forestry boards.

Also, each year, we send several hundred, or sometimes more than one thousand seeds, to Dennis Bittenger, a chapter member in Allegany County. He gives talks and instructs students in the Allegany County Public Schools on planting chestnuts. As a result, most schools in the county have chestnut trees growing on their grounds. Dennis also works with the Western Maryland Correctional Institute in Cumberland where inmates grow and plant chestnut trees on the prison grounds.

In past years we have provided seeds to organizations such as public and private schools, the Pennsylvania Fish and Game Commission, the Appalachian Region Reforestation Initiative, The Nature Conservancy, the


Trees in Sugarloaf East Field.


The Germplasm Conservation Orchard at the Rockville Izaak Walton League of America.

Maryland Environmental Trust, other TACF state chapters, TACF's Meadowview Research Farms, and colleges and universities.

One year, we gave 200 seeds to a retired Army officer who owns a camp in northern Pennsylvania that hosts the Sierra Club's Military Outdoors program. Our seeds were planted by wounded veterans who participate in the camp's programs.

Never enough, but it helps

Each year, several of our members grow potted seedlings. Also each year, the inmates at the Maryland Correctional Training Facility in Hagerstown grow containerized seedlings, usually 100 or more. We give these seedlings out at nature events, chapter meetings, and workshops where one of us presents a talk on chestnut trees. No matter how many we bring to a meeting or event, rarely are any seedlings left over.

Despite all the efforts of our chapter's seed harvesters, seed de-burers, seed stratifyers, and seedling growers, we usually have more requests for seeds and seedlings than we can satisfy. The demand for American chestnut trees is great and the demand for blight-resistant trees is even greater. Meanwhile, the pure American seeds and seedlings we provide are getting many people involved in growing chestnut trees, thereby helping to create what we believe will be an informed and experienced groundswell to support the restoration of our favorite tree. That's the promise.

Tom Scrivener's tree in Hagerstown.

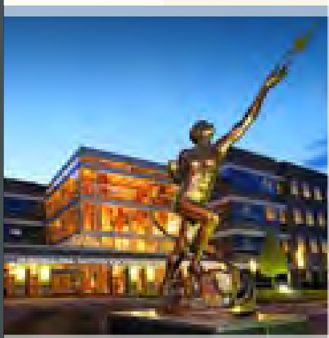
TACF's 35th Anniversary

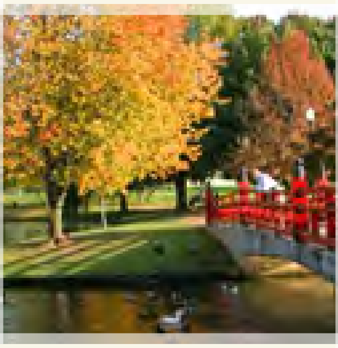
CELEBRATION AND ANNUAL MEETING

October 26 – 28, 2018

Celebrating 35 years of commitment to return the iconic American chestnut to its native range

Each year, TACF hosts its annual fall meeting, located within the native range of the American chestnut. This year's event will be held in Huntsville, Alabama, also known as Rocket City. We'll celebrate our journey alongside the U.S. Space and Rocket Center, Redstone Arsenal, and HudsonAlpha Institute for Biotechnology, a leader in genomic research.





Join us as we tell the stories of where we've been, where we are, and where we're headed. Events will be highlighted by amazing keynote speakers, special award ceremonies, and a host of extraordinary educational sessions. Share your chestnut stories at this special 35th anniversary gathering in October, with those who share the passion to restore this magnificent tree back into its native eastern forests.

Look for additional details in our *eSprout* electronic monthly newsletter, on our Facebook and Twitter pages, and in the fall issue of *Chestnut*.

facebook.com/americanchestnut • twitter/chestnut1904

PART 3
of a 4-part series
on preserving
American chestnut
genetic diversity in Maine

PRESERVING AND MAKING ACCESSIBLE

American Chestnut Biodiversity in Maine

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

The American Chestnut Foundation's "2017-2027 Strategic Plan" encouraged state chapters to create Germplasm Conservation Orchards (GCOs; the plan is online at acf.org/about-us). These orchards are to be planted with the progeny of wild, pure American chestnut trees. The aim is to capture and preserve American chestnut genetic diversity in easily-accessible locations.

Figure 1
Ecological Restoration students tend to month-old American chestnut seedlings they've sown in the University of New England (UNE) greenhouse. l to r: Caroline Cooper, Charlotte Rantz, Paige Dungan, Jes Szetela and Renee Roth.

Figure 2

UNE Ecological Restoration students, Flynn Willsea and Corey Ackerson, label aluminum tags they attach to each American chestnut seedling identifying the mother tree and her location.

Figure 3

Wild Meadow Farm's Noah Hallward-Rough distributes slow-release fertilizer, while UNE student Christina Hattaway and other volunteers plant seedlings.

While some state chapters, particularly Pennsylvania and New York, have been long-term GCO leaders, others such as Maine are now heeding the GCO call. Four GCOs were installed in 2017 in Maine with another three planned for 2018.

Early October in Maine is when we gather wild chestnuts for GCOs and other purposes, including eating! Harvest time is later than in more southern states. Yearly weather variation can shift the harvest timing throughout its range. In the southern half of Maine, which is the northernmost extent of the American chestnut's native range, volunteers search for and gather burs and seeds from wild mother trees from diverse locales. Articles like this one often prompt readers to inform TACF about a previously unknown large, wild chestnut tree – please let us know! (See TreeSnap article on page 5 for information on how you can do this.)

Thanks to a growing seed-exchange network among TACF volunteers from throughout the eastern US, Maine's wild seed inventory also includes a sampling of pure American seeds from other states. While northeastern states have been prioritized, we have also included seeds from Georgia, North Carolina, and other southern sources in our GCOs. We are monitoring and measuring the comparative performance of these geographically-diverse seedlings under different growing conditions and as climate changes.

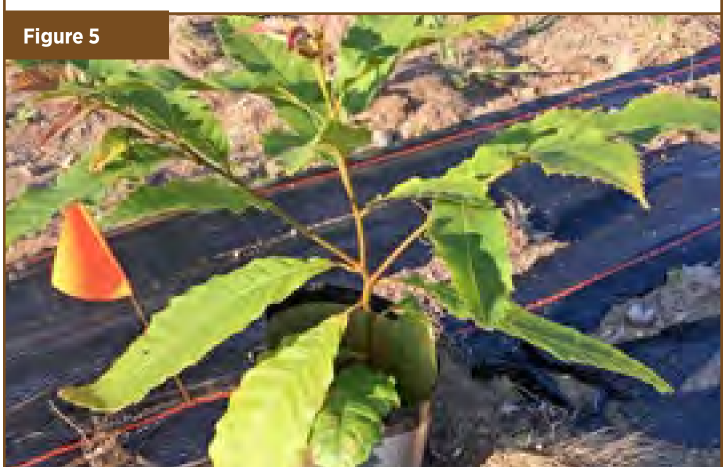
Each spring semester, undergraduates enrolled in my Ecological Restoration course at the University of New England in Biddeford Maine, like students of other professors-cum-chestnut enthusiasts, get their hands dirty. In February, my students are introduced to the chestnut seeds. They had been refrigerated in moistened peat for the previous three months to simulate winter. Students sow the seeds, and then over the following months tend to the seedlings in the campus greenhouse (**Figure 1, 2**). Improving seedling production is itself an on-going experiment that engages students. Variables include the growing medium ingredients (we've settled on mostly peat), the size of the seedling pot, and the amount of light, water, and fertilizer. Joint TACF-university projects like this are synergistic. The Foundation's experimental science advances and the chestnut gene pool expands. Students get hands-on experience with a real-world ecological challenge. The project illustrates concepts such as "functional extinction" (a species too rare to perform its ecological role) and "carbon sinks" (net absorbers of carbon dioxide).

We now turn to the story of a particular Germplasm Conservation Orchard. By late May in Maine, the threat of frost has past and the greenhouse-reared seedlings can be hardened off outdoors. In 2017, one hundred diverse seedlings were selected for a new orchard at Wild Meadow Farm. This organic farm raises sheep, turkeys and vegetables. It is located in rural Saco, Maine, about 30 minutes' drive south of Portland. Landowners Jim Rough and Ann Hallward, and their son Noah, enthusiastically welcomed the opportunity to host our pure American chestnut seedlings and thereby contribute to our multi-pronged national effort to restore this iconic species. "We're totally excited to be able to support the preservation of these trees," said Jim

Figure 4

Landowners Ann Hallward and Jim Rough (back right) plant seedlings with volunteers of all ages.

Figure 5



By mid-summer in Maine, seedlings show solid 1st-year growth. The aluminum collar offers considerable protection against winter browsers.

Rough. “It’s been a fun project to be a part of.” Volunteers assisted the landowners with the seedling planting and their protection with aluminum collars (**Figures 3, 4**).

Completing the installation of a chestnut orchard is a cause for celebration. Planting as early as possible in the summer gives them a full outdoor growing season (**Figure 5**). But the work hardly ends with orchard planting. Grass and weeds need to be kept at bay by mowing and/or pulling by hand (elsewhere herbicides are used). The deer-exclusion electrical fencing requires regular monitoring and maintenance to ensure it continues to emit a charge of at least 4k volts. Other critters, including mice, voles, and porcupines, can elude the fencing and browse the seedlings, particularly if their protective collars get blown off (**Figure 6**).

On top of these annual maintenance issues are seasonal variations: the summer of 2017 brought the second drought in a row for much of the northeast. In response, I gave each seedling a half-gallon of water eight times during the dry summer. Regular watering helps to ensure a greater share of the chestnut seedlings survive the first year; their deeper roots by the second year reduces the need for continued watering (**Figure 7**). All of these maintenance tasks underscore the advantage of locating GCOs where they can be easily accessed by chestnut stewards, as is the case with Wild Meadow Farm, just 1.5 miles from my home.

Wild Meadow Farm’s GCO illustrates something every chestnut grower knows: there is no end to the learning curve. All of our hands-on experience, trial and error results, and accumulated and shared knowledge will be of great value as we move incrementally closer to a time when we can repopulate eastern forests with pathogen-resistant chestnuts.

For more information on American chestnut biodiversity in Maine, email Thomas Klak at tklak@une.edu.

Figure 6

A solar-powered electric fence surrounds the orchard to exclude deer. However, mice, voles, and porcupines go under the fence to browse seedlings, especially those that have lost their protective collars.

Figure 7

Year-old seedlings emerge from February’s snow cover.

Returning the Mighty Giant

By Bryan Burhans, former President and CEO of TACF

Republished from *Pennsylvania Wildlife*, a publication of the Wildlife For Everyone Endowment Foundation in PA

After four miles of plodding along on the Appalachian Trail just north of Caledonia State Park, I could begin to feel a hot spot developing on my foot. Of all the ailments that can strike a backpacker, blisters are my most feared.

As I sat down to change socks, I saw chestnut burs scattered on the ground. I scanned the trees and found the source; a 10-inch diameter American chestnut. The tree was heavily infected with blight, and now was using its last remaining energy reserves in a useless attempt to reproduce.

This tree was a true American chestnut. Its roots could have been 200 to 300 years old, or more. The same blight fungus killing this tree now, was the same fungus that decimated an estimated 4 billion chestnuts during early 1900s throughout its historic range.

As I continued my hike I kept my eye open for more chestnut trees. And there were many. Although the American chestnut is now lost as a canopy tree in our forests, the sprouts from the root systems of chestnut trees that died over 100 years ago are very common, especially on Pennsylvania's ridges. These sprouts follow a natural cycle; they grow from the existing root system until the blight eventually finds the tree and kills it. But the blight does not kill the root system, so the cycle of sprouting and top killing continues on today.

The loss of the American chestnut was a true ecological disaster. When the blight marched across Pennsylvania, efforts were launched by the Pennsylvania Chestnut Blight Commission to further study this disease with the hopes to stop its spread. These valiant efforts failed. And the impact to wildlife raised an alarm with the state wildlife agency, the Pennsylvania Game Commission.

The agency urged sportsmen to plant food-producing trees, vines and shrubs in an attempt to provide wildlife new food sources. In fact, the loss of the chestnut transformed the way the agency managed wildlife by expanding the agency's effort into habitat management instead of just setting season and bag limits for game species. A heavy focus on wildlife habitat management continues today with almost 50 percent of the agency's budget allocated to improving wildlife habitat.

Rescuing the Mighty Giant

Established in 1983, The American Chestnut Foundation (TACF) was formed with the sole mission of restoring the American chestnut to its historic range. But to accomplish this, the Foundation had to first develop a chestnut that could survive the chestnut blight.

The Foundation has focused their efforts for the last three-and-a-half decades on using a traditional breeding program to incorporate

A blighted tree near State College, PA

blight resistance from Asiatic chestnut species into local American chestnuts. The PA/NJ Chapter of TACF has an active breeding and conservation program which has conserved just over 200 wild American chestnuts.

"We have made many great advances in our breeding program throughout the original range of the American chestnut," says TACF's Director of Restoration, Sara Fitzsimmons, headquartered at Penn State. "Restoring a species to a landscape level will be a herculean task, but we are inching closer and the results are extremely encouraging."

On another front, the New York Chapter of TACF and the State University of New York's College of Environment Science & Forestry (SUNY-ESF) partnered on a program to use biotechnology techniques to develop a chestnut resistant to the blight. Scientists have successfully taken a gene from wheat and inserted this into an American chestnut to produce a tree resistant to the chestnut blight.

"Using these biotechnology strategies, SUNY-ESF scientists have been able to develop a chestnut which – in some cases - is showing levels of resistance higher than that of the Chinese chestnut," says Fitzsimmons. "Genetic modification is tightly regulated by several federal agencies, and these clones will require significant outcrossing to thousands of native American chestnuts to create a diverse population suitable for restoration. Additional testing is needed before these trees will be approved and ready for range-wide planting."

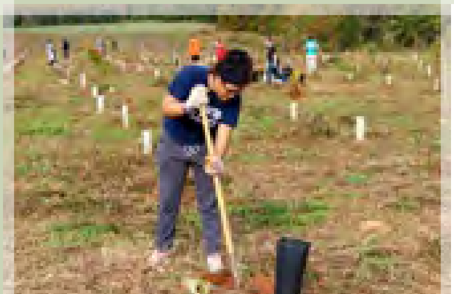
I am confident the American chestnut will someday be returned to Penn's Woods. There is still much work ahead, but the team of scientists working for and with TACF is amazing. Although I may never have the opportunity to lean back on a mature chestnut and listen to the thunder of a turkey's gobble on a spring morning, I feel rewarded that someday this will become a reality.

About the author

Bryan is the Executive Director for the PA Game Commission (PGC). Prior to joining the PGC, Bryan was President and CEO of The American Chestnut Foundation from 2009-2014.

"There's nothing stronger than the heart of a volunteer."

Jimmy Doolittle



NATIONAL VOLUNTEER WEEK

took place on April 15-21. TACF recognizes the essential role volunteers play in supporting our efforts to return the American chestnut to its native range. From planting trees to speaking at various state chapter events and everything in between, our volunteers are committed, passionate, and driven. In acknowledgement of the work these volunteers do year-round, THANK YOU for your support. We truly couldn't do it without you!

Clockwise from top right:

Long time PA/NJ Chapter member Les Nichols assists a student in planting a tree at Pohatcong Township School in Phillipsburg, NJ. Photo courtesy of Pohatcong Township School

GA Chapter: GA Chapter board member Mark Stoakes makes a clearing in the ground to plant a seedling along the Confluence Trail in Atlanta, GA. Photo by John R. French

MD Chapter members Mike Yost and Jim Curtis prepare the ground for a future planting at the Central Maryland Research and Education Center seed orchard. Photo by Dave Gill

VT/NH Chapter member Carol Wallace introduces students to American chestnut burs at an educational planting in Londonderry, NH. Photo by Curt Laffin

Virginia Chapter volunteers practice their newly acquired skills by culling trees at the Stony Brook Farm in Madison County, VA. Photo by Tom Saielli

Volunteers prep pots at the Bent Creek Resistance Screening Center in Asheville, NC. Photo by Ben Jarrett

Catawba College student digs a hole, preparing to plant a B_3F_3 seedling at Frog Hollow orchard, near Salisbury, NC. Photo by Kim Marmon-Saxe

TERRY GULICK'S Deep Roots

By Scott Carlberg, Carolinas Chapter

Terry waving goodbye as he stands with his lunch pail, waiting for the school bus on his first day of 1st grade.

Terry volunteers at TACF's VT/NH booth during the Vermont Farm Show this past January.

"I heard about chestnuts when I was a kid, just like thousands of kids did," says Terry Gulick, TACF volunteer from Springfield, Vermont. "It was 1944, when I was 12, when I found a chestnut tree." While wandering across his family's 110 acres in eastern Vermont, he saw a tree with a long fuzzy leaf. "I took it to a local forester, who verified it was an American chestnut."

Terry was hooked.

Those native chestnut trees died from the blight, as so many have. "I went through catalogs and saw Chinese chestnuts for sale. Ordered and planted them. I lost one, so I had lots of burs but no chestnuts. I didn't know why." He bought more. Of course, this time the trees cross-pollinated. Years became decades as Terry honed his chestnut skills. "Eighty pounds have been the best yield we've had, about three years ago." Production has been down since, though. "Rainy weather and squirrels," says Terry, who now has 25 of his own hybrid trees.

TACF has a dozen orchards across Vermont. The 12-year-old trees were blossoming this last spring. "Good height. Flowers and burs this last summer. Getting ready to be inoculated," he says.

Terry was a catalyst for the Vermont/New Hampshire Chapter of TACF. It was early 2008. "I read about the Foundation in the Rutland Herald. I got in the car to visit the national TACF office, then in Bennington, Vermont." Terry offered his help. By September, the region had the 80 members needed to be a chapter, the 15th chapter of TACF.

His given name is Charles B. Gulick (pronounced GUE-lick). Same name as his dad and grand-dad, but "Call me Charles and no one up here will know who you are talking about," he says. "Terry is my nickname, and I got it as soon as I was born. Didn't have much voice in the matter."

Terry has deep roots on the 110 acres of family land he still occupies in Vermont. "Dad came from Massachusetts and bought the place in '38." Being in sales, Terry's dad could work from anywhere in New England. "Been in Vermont for two generations. I think you need three to be a native," he says.

Terry's original house on the family land was built in 1791. "That's like a lot of homes up here, because that was when Vermont became the 14th state. Maybe people wanted to be sure statehood was going to happen first," he says. Though the house has had an addition built to match family needs, the purpose of the land there has not changed - grow things. "It has been a self-sustaining farm - dairy, some sheep." Now there is also a stand of chestnuts.

He is really more at home in the open air, not inside; a jack-of-all-trades outdoors.

For instance, maple syrup. Terry has been tapping syrup from sugar maples for more than 20 years. In a good year, he can harvest 80 taps for about 12 gallons. "It keeps our family in syrup for the year." He says that successfully tapping maples depends on weather. "The sap has to run just right, best after a night in the 20's and during a day maybe 40 degrees."

Evergreens are important, too. Terry has made the holidays a lot brighter for many families by selling balsam trees and making wreaths, more than two dozen years doing both. "The secret of a good Christmas tree is to shape them in May or June before new growth hardens off, not when they are sold at the holidays." For each of the more than 2,000 trees he sold over 20 years, he planted two trees the next spring. In ten years, the rotation was going full steam.

There is landscaping, too. For 35 years, he tended the yards of retirees or part-time Vermont residents. He still works a few of those accounts.

Terry helps people grow, too. For years Terry has been a best friend of elementary school children from troubled families at the regional Kurn Hattin Homes in nearby Westminster. It is a place where children have a secure and supportive haven during difficult periods in their families' lives. "An oasis within the real world for the kids," he says. He has mentored dozens of boys and girls in first and second grade. "I am 83, but a kid's person."

Terry is a solid example of the Vermont culture and ideals, though he spent some younger years out of state in the military, 1956-57 in the U.S. Army, first at Fort Dix, New Jersey. "When we got to camp, the sergeant asked, 'Who knows how to type?' I took typing in high school, so I raised my hand. They made me company clerk for basic training." For eight weeks he never saw a rifle because he helped process everyone else through the system.

Then it was off to Fort Knox in Kentucky, and a legacy of his youth that connected Terry to his Kentucky military home. That was 4H. As a kid in 4H he had chickens and kept a garden, even earning a blue ribbon at the state fair in Rutland around 1946. He was president of the local 4H club and stayed with the program through high school. "So, I contacted the 4H leaders in Kentucky when I joined the Army." He volunteered throughout his Army service.

Terry Gulick is pointed in his beliefs about our environment. "We've got to take steps to protect open land and our resources." He knows it is a long-term proposition. "I'll be dirt-napping when it is successful."

Though he has been a committed member and volunteer with TACF for many years, the Christmas tree is a symbol of Terry's character. He has grown and sold Christmas trees, and just like Christmas trees, he makes our world happier.

Terry pollinating a wild American chestnut tree in Farilee, VT in 2008.

Terry works with a visitor on a wreath at the Coolidge homestead in Plymouth Notch, VT, this past holiday season. Gulick's family have been friends of the Coolidge family beginning with the President's son, John.

STEM, Chestnuts and the Classroom

INDIANA CHAPTER

By Ben Finegan, Indiana Chapter

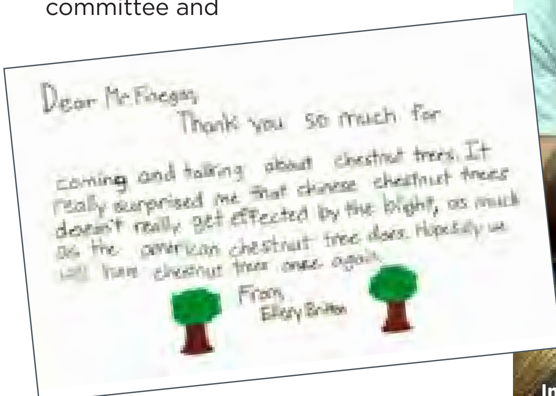
STEM. No, not the part of the plant that holds the leaves to the branch. "STEM" stands for "Science, Technology, Engineering and Math" and it is a big deal these days. Schools, service organizations, the news… everyone is talking about STEM. The American Chestnut Foundation (TACF) adopts many concepts and approaches to STEM education, as described in a recent classroom experience.

St. Pius X students in Maureen Sitzman's class gather around a display of chestnut burs, wood and leaves.

Indiana Chapter volunteer Ben Finegan recently gave a talk for 5th grade STEM classrooms at St. Pius X school in Indianapolis, IN. Mr. Finegan's children attend the school and the STEM teacher, Maureen Sitzman, asked parents if they would like to speak about STEM careers.

Maureen suggested that the talks tie into the class's recent social studies work on Native American tribes, the 13 colonies and early life of European settlers. As many *Chestnut* readers may already be aware, American chestnut featured prominently in all of those cultures.

Mr. Finegan had several prior years' involvement with TACF's education committee and was able to look through educational resources in the learning kit that had been developed for 6th, 7th and 8th graders. He also drew from the work that Indiana Chapter's Carroll Ritter had been instrumental in; setting up outdoor learning labs where students helped to plant a mix of chestnut trees in what would have been their natural habitats and create ongoing study plans for it.

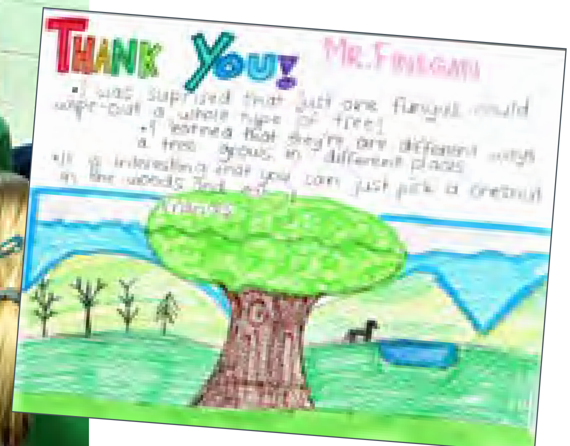

Dear Mr. Finegan,
Thank you so much for coming and talking about chestnut trees. It really surprised me that chinese chestnut trees doesn't really get effected by the blight, as much as the american chestnut tree does. Hopefully we will have chestnut trees once again.
From

Almost all the school children had heard of American chestnut by way of the familiar "Chestnuts roasting on an Open Fire" Christmas carol. Few, however, knew what a chestnut was or the prominence it played in our history. A brief introduction to the tree was accomplished by way of PowerPoint slides and Dean Cornett's Youtube video (link follows article). Familiar pictures of large trees, log cabins, American statesmen like Abraham Lincoln splitting fences, street vendors selling chestnuts, and all the wildlife that relied on chestnuts filled the classroom. They saw pictures of the "ghost forests" and imagined what a dramatic change in people's lives that would have been. The first part of the chestnut story is always a little nostalgic and a little sad. That left the classrooms with the question of "so why are you here?" and as they might have guessed, that is not where the story ends! After a very brief jump to the 1980's and the beginning of TACF, the group got into the hands-on portion. The children quickly recognized what blight can do to a tree when holding an affected American chestnut log in their hands and comparing it to a Chinese chestnut log. They were also able to experience and compare chestnut burs, nuts and leaves. More than one wanted to eat them!


Indiana Chapter volunteer Ben Finegan describes the differences between a Chinese chestnut and an American chestnut to a 5th grade STEM class.

The two classes were broken up into a purple and gold group. Both had very different but very engaged reactions to the talks. Common questions from both groups were "why don't we just plant Chinese chestnuts" and "why don't we just plant all the trees in greenhouses?" It was refreshing and inspiring to hear the varied questions and the "unlimited" perspectives of children.

The talk then switched to looking to the future and ways that TACF is using STEM to work on the chestnut. Biotechnology plays a prominent role in our work and will undoubtedly touch these children's lives one day. Students were able to look at Indiana and TACF professionals in a variety of careers, from geneticists in the lab to foresters planting trees, to getting a feel for the possibilities in a STEM career. The class also got a chance to see Dr. William Powell's TED talk about restoring chestnut using both breeding and transgenic programs (link follows article).



The discussion ended with a chat about why people get involved with TACF. Many people have day jobs but volunteer for the Foundation because they are passionate about its mission and hope to help make the world a better place for children, just like those sitting in that very classroom.

Ms. Sitzman had the following to say about the presentation, "Sharing TACF's work with students gives them an example of continuous innovation in the STEM field. It's so important to have parents share learning experiences with us. Students are able to make connections to relevant work that's making a difference in our current and future environment."

Dean Cornett video: youtube.com/watch?v=-xgbedXnbfw
Dr. William Powell's TED talk: youtube.com/watch?v=WYHQDLCmgyg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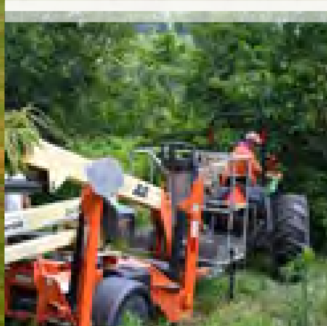
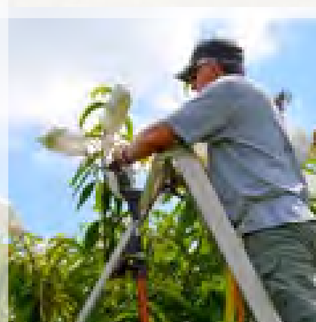
THE AMERICAN CHESTNUT FOUNDATION®

As our new and traditional research of developing a blight-resistant American chestnut tree comes closer to fruition, we now need, more than ever, to focus on investing in new farm equipment that will allow us to take our work, and our mission, on its next journey. Some of this equipment has weathered decades of use at Meadowview Research Farms and urgently needs to be replaced. Not only will new equipment improve the safety of our volunteers and staff, it will also aid in increasing our efficiencies moving forward.

What we need, more than ever, is to come together with our resources, making sure we are ready for the next chapter of chestnut restoration. Last year we came together to build a greenhouse. This year, our goal is for equipment upgrades such as bucket lifts, a new 4WD truck, and greenhouse and lab supplies. Glamorous? No. Mission critical? Yes! Can we count on your support?

Donations can be made through our website at acf.org or by calling the National office of The American Chestnut Foundation at (828) 281-0047.


‘A Foe, Insidious and Merciless is Advancing:’

HISTORICAL NEWSPAPER ARTICLE FROM AUGUST 23, 1923

By Jim Buchanan, Editor of the *Sylva Herald*
Reprinted in the *Sylva Herald* on February 15, 2018

This article relates warnings of the chestnut blight’s approach on the front page of the *Jackson County Journal* in Jackson County, NC – how efforts to stop it failed in spite of efforts born of such warnings, and the hopes for the return of healthy chestnut forests.

We can’t say we weren’t warned.

And we can’t say the warnings weren’t heeded.

But in the case of the chestnut blight, warnings, vigilance and the best-laid plans proved to be no match for people in these mountains trying to protect a treasured cultural lodestone and economic engine.

The blight won.

Or at least it won the battle. We’re still fighting the war.

The front page of the *Jackson County Journal* laid out in stark detail what was heading our way in an article appearing Aug. 24, 1923, headlined “Real Danger Threatens Our Homeland: An Insidious and Merciless Foe is Approaching,” which reads as follows:

"There is real danger just ahead of us, and only the most careful and patriotic efforts of all our people can save a large part of our region from becoming a barren wilderness, and many of our industrial enterprises from closing down and leaving our people out of employment.

"This sounds like a woeful picture, but it was made so for the purpose of arousing the people of Jackson County, as well as the whole of the chestnut region of Western North Carolina, to the catastrophe that is just ahead of us.

"A few years ago, there were brought to Central Park, in New York City, a few Japanese chestnut trees, which, without the knowledge of the park authorities, were affected with chestnut blight, a tree disease hitherto unknown in America. And thus, through carelessness, was brought to this country a foe that threatens us with real and appalling danger. From Central Park the disease spread until it has completely annihilated the chestnut timber throughout New England, and a large part of Pennsylvania, New York, Maryland, and West Virginia, and is gradually encroaching upon North Carolina, having recently made its appearance near Old Fort.

"As the wind blows from South to North in the path of the chestnut blight, it was at first thought that it would not reach North Carolina, but aside from the carrying of the fine dust spores, the disease is spread by birds, and is thus likely to break out in isolated places, as was the case near Old Fort, at most anytime.

"It is thought that the menace can be averted by concerted action of foresters, and people who live in the country, who are warned to be on the look-out for the blight, and immediately take steps to stamp it out, by cutting infected trees and burning them.

"Only by enlisting the aid of the people can this real menace to the happiness of our people, and to the beautiful handiwork of the Creator, be averted. The Champion Fiber Company is leading in organizing the timber and acid wood interests of this region in a fight to combat the disease, and has sent out letters to the Armour Leather Company, at Chicago, Sylva Tanning Company, Sylva, Union Tanning Company, Old Fort, Blackwood Lumber Company, East La Porte, W. M. Hitter and Company, Columbus, Ohio, International Shoe Company, Morganton, Gennett Lumber Company, Asheville, and other industrial enterprises that are most vitally interested, calling for a meeting to organize the forces to wage the battle.

"It can be seen at a glance the real danger that confronts this region, and in Jackson County, where perhaps is left the greatest acreage of chestnut timber in the world, we are particularly and vitally concerned. With proper lumbering methods, the chestnut timber in this country will be an everlasting source of revenue and beauty, if we can keep the blight from upsetting all calculations that have been made for the future development of Jackson County. This disease is a menace that threatens every activity of our people. Every man and woman in this region is vitally concerned.

"And this paper calls upon all the people to keep on the watch for the blight, report it, and cut the trees that become affected. The next general assembly will be asked to enact legislation allowing an affected tree to be cut on anybody's land, anytime, anywhere, and to pass other legislation to assist in the fight. At present, we can only depend upon the good sense of the people, who, if aroused to the danger to their homeland, will turn the trick."

It did not.

Despite efforts such as felling millions of trees in Pennsylvania and New York to create a firewall, the blight jumped ahead of efforts to contain it and spread at a rate of 50 or more miles a year. Within a generation all that remained of the vast chestnut forests that provided food, forage and highly valued timber were stands of ghostly sentinels.

Chestnut blight does kill the tree, but it doesn't kill the roots. Trees will send up sprouts that survive until falling again to the blight.

The American Chestnut Foundation, based in Asheville, has been the tip of the spear in the ongoing war against blight through decades of research dedicated to finding blight-resistant cultivars. Test plots of blight-resistant trees have been planted in the area.

TACF volunteers send in over 2,000 leaf samples annually for testing and identification; volunteers help locate sprouts for entry into a database and help plant trees and monitor test sites. The Foundation also offers seedlings to boost the effort to reestablish this mountain treasure.

The battle to stop the spread of the blight in the 20th century was lost.

But the war is not over.

About the author: Jim is a Sylva native and WCU grad. He served as editor of the *Cashiers Crossroads Chronicle* before putting in 29 years at the *Asheville Citizen-Times*, where he was on the editorial board for 20 years and editorial page editor his last eight. Currently, he is editor of The *Sylva Herald*. Jim also serves as Vice President of the Western North Carolina Historical Association.

Direct-Seeding Chestnuts

By Sara Fern Fitzsimmons, TACF Director of Restoration

Ungerminated chestnut hulls on the ground seen through wire mesh. Many nuts remained on the ground from the failed State Gamelands 205 study established in the spring of 2016. Vegetative competition proved too much for the direct-seeded chestnuts to overcome.

When starting a chestnut planting, one of the first dilemmas a grower faces is the decision on whether to plant a seed straight in the ground, or plant a seedling. The answer depends on many factors, including plain ole' personal preference. Rather than debate the question, let's look at information available about direct-seeding.

The holy grail of planting chestnuts is to put a chestnut in the ground and walk away. But there are thousands of critters and pests and diseases all lurking, waiting to get at that chestnut you just planted. A review of the literature on direct-seeding trees suggests that the methodology holds promise as an economical means for reforestation across the globe, but cautions that proper management beyond just putting seed in the ground must be followed (Grossnickle and Ivetić 2017). In a series of studies, Phelps et al (2005) suggest that protected, direct-seeding of chestnut can be successful, even efficient and economical, as long as deer predation and vegetative competition are controlled. There are three primary means of direct seeding: physical barriers, chemical coatings, or having no protection at all.

PHYSICAL

The most commonly employed strategy for direct-seeding chestnuts is using a physical barrier. One of the first published protection methods for direct seeding of chestnut was one that used a tin can around planted nuts as documented by USDA chestnut breeder, Jesse Diller (1946, 1959). In TACF, a majority of direct-planted seeds are protected with a cylinder of aluminum flashing or plastic, mimicking the action of the tin can (**Figure 1**).

These methods are tried and true, and work in many situations with a variety of hard mast species, including chestnut (Farlee 2013). Disadvantages of this method involve both the expense and labor required to install and eventually remove the materials. Another shortcoming is that these methods will not protect from predation from larger varmints such as raccoons or turkeys, both of which have been implicated in the poaching of thousands of nuts from a few TACF plantings. If you have a high raccoon population, you will need to plant seedlings.

Seedling planting is not without its shortfalls, but we'll save that for another discussion. Still, for seedling production, the seeds have to be planted somewhere. In the nursery bed, chestnuts are typically planted inches apart in a raised bed (**Figure 2**). Snow fencing, hardware cloth, or some other sort of tight fencing is then placed on top to prevent predation. TACF's nursery cooperators see an average of 50% germination and survival in their 1-0 bareroot chestnut seedlings.

A type of protection not often employed for chestnut plantings is a seed protector. Grossnickle and Ivetić (2017) document several types of seed protection devices, most of which have not been trialed on chestnut.

Researchers at Penn State developed a seed protector out of a 6″ piece of PVC pipe (Bowersox 1993). Phelps et al (2005) demonstrated that in areas with reduced deer pressure, trees established from seed in the PVC seed protectors grew taller than those protected by 5′ tree shelters. In practice, those PVC seed protectors can be difficult to use, are not commercially available, and must be manually removed to prevent root and root collar constriction over time (**Figure 3**).

Direct-seeding with protection raises another suite of oft-debated topics among chestnut growers: What brand of tree shelter to use? How tall? What diameter? Vented or unvented? What's the best way to hold up the shelter? We'll have to save those questions for another article!

CHEMICAL

A commonly used repellent for seeds is capsaicin, the active ingredient in chili peppers that makes them hot. Many squirrel repellents branded for bird seed contain capsaicin. It's effective against squirrels, but birds are undeterred. Curtis et al (2000) found capsaicin coatings repelled squirrels from seed at birdfeeders, but chipmunks were unaffected. For protection of longleaf pine seeds in direct-seeding, Nolte and Barnett (2000) found that a combination of capsaicin and Thiram was most effective in reducing mouse damage.

Through the late 1930s and early 1940s, Jesse Diller (1946) trialed coating chestnuts with strychnine, a commonly-used "repellent" of the day. The resulting treated seed never made it out of the laboratory; less than 6% of the treated seed germinated, and the resulting seedlings were in poor health.

In 2003 and 2004, long-time TACF member Tim Phelps installed several trials with concentrated habanero extract produced by Global Source Advantage. Tim coated a few dozen chestnuts and planted them in a field outside his office at Penn State. The product measured in with 675,000 Scoville Units (by comparison, a jalapeno maxes out around 100,000). Even when the undiluted product coated the seed, predators pilfered the entire planting overnight. We can't know if the nuts were eaten – they may have been dropped somewhere or re-cached – but they didn't stay where they were planted.

Tim built his study on chestnuts around a sister study on acorns performed in 2002. The acorn study was a bit more formalized: four replicated sites, each with 60 acorns. Ten acorns each were coated in various dilutions of capsaicin coatings (100%, 75%, 50%, 25%, 10%, and uncoated control). Overnight, all of the untreated acorns were gone. Within two months, the remaining treated acorns were gone, with the exception of 5 seeds from one undiluted treatment in a single location.

In the spring of 2010, long-time TACF members Gary Carver and Tom Scrivener tried a mixture of capsaicin, Bitrex, and ethyl alcohol. Gary and Tom found that combining the three ingredients as per label instructions (exact specifications unavailable) and soaking for up to 15 minutes was not effective in deterring predation of chestnuts (soaking longer than 15 minutes killed the seeds); all the treated chestnuts were gone the day after planting. At 10x recommended concentrations, most seeds were taken. Finally, Gary decided to go "all in." He used a "heaping teaspoon to a ½ cup of alcohol" and that seemed to do the trick – hardly any of those seeds were taken. Resulting germination of these seeds was not followed, so the effect of this concoction on seed or seedling health remains unknown.

For full protection against all possible seed consuming critters with chemical deterrents, a mixture will be required, as different animals are repelled by different chemicals. From these few case studies, chemical means of protecting seeds from predation do not appear the most economical or effective means of direct-seeding, but there's a lot of

Figure 1

Direct-seeded American chestnuts in a Germplasm Conservation Orchard (GCO) at the Carbaugh Farm in Danville, PA. The seeds planted represent a variety of American chestnut provenance from Pennsylvania and beyond. This picture illustrates best management practices promoted by TACF for direct-seeding. The seeds are planted and then protected by 18″ – 24″ plastic tree shelters. The planting is protected from deer browse by a fence, in this case a plastic fence. Vegetative competition is controlled through the application of herbicide in a 3′ wide band along the trees.

room for experimentation. This brings us to final strategy, the one employed by nature – strength in numbers!

NONE

In nature, the amount of seed produced by trees works to overwhelm the predator base. Such a strategy could work for artificial regeneration plots, but if you want to be sure that the exact nut you planted in a certain spot will stay there and germinate, this is not the method for you.

I could find only one published account of unprotected, direct-seeded trials of chestnut. Jesse Diller (1946) planted 4000 bare chestnuts across 12 locations and 2 years. Although not specifically documented, this suggests 166 seed per location per date. These plantings resulted in less than 4% germination to seedlings, of which only a handful survived additional years. The author concludes that seedling planting is the only way to go for planting chestnuts.

There are more studies available for review on oaks. Based on observed values of multi-year survival of direct-seeded oak, Johnson and Krinard (1985) recommended planting densities of 1500 acorns/acre across a minimum of 2.5 acres to minimize losses from rodent predation. In a review of artificial regeneration of oak, Dey et al (2008) suggest that while direct-seeding of oak can be successful, most land managers continue to favor seedling planting of oak, citing better predictability for long-term success of oak seedlings vs. direct-seeded acorn plantings.

Still, these unprotected direct-seeding trials can be successful provided appropriate measures are taken to reduce vegetation competition. On October 31, 2014, representatives from TACF, the Pennsylvania Game Commission, and Moravian College planted a mixture of 20,000 chestnuts and acorns (both of various species). These seeds were planted in three replications across five treatment blocks each being 50′ x 50′ in size (**Figure 4**). The total planting area was less than an acre (37,500 sq. ft.[2]). The site had been previously treated with glyphosate. Seeds were evenly distributed by hand and then disked in with a tractor.

The following summer, the resulting seedlings were counted. On average, oaks saw a 7.5% survival rate while chestnuts exhibited 10.7%. Chestnut germinated and survived best when it was on its own (**Figure 5**).

Figure 2

Planting at the Pennsylvania Game Commission's Howard Nursery in 2010.

Figure 3

This 13-year old tree was direct-seeded in a seed protector as part of silvicultural trials established in 2005 in Penn State's Stone Valley Experimental Forest. The tree has grown in to the PVC protector which can no longer be removed.

Figure 4

Map showing configuration of direct-seeding blocks on State Gamelands 205 study established in the fall of 2014.

In the spring of 2016, volunteers and staff from PA/NJ Chapter of TACF and the PA Game Commission tried replicating the success of the trial above. This time, 40,000 chestnuts were manually distributed across an open field 0.66 acres in size. At a resulting count in July of 2016, only 190 resulting chestnut seedlings could be found. There were still seeds, but they had either not germinated, or germinated and died (We know the seed were sound as greenhouse plantings of the same seed showed 90% germination). Those seedlings which were there were greatly overwhelmed by the competing vegetation (**Figure 6**).

The major difference between the 2014 and 2016 studies were in the preparation of the site. While both plantings took place on bare soil, the 2016 site had not been treated with herbicide, and the resulting spring grasses quickly overwhelmed the trees in the open site. This result echoes that of many cited for other species (Grossnickle and Ivetić 2017).

Direct-seeding is certainly a viable option for many chestnut plantings, given that appropriate installation and maintenance practices are followed. How one goes about establishing and maintaining a chestnut planting requires an analysis of site selection, budget, types of local predators, types of competing, types of vegetation control available, etc. Several resources cover best management practices for planting chestnuts[1], but they won't necessarily optimize the efficiency of efficacy for every grower in every location. For that, each grower will need to evaluate their own given situation.

[1]Please visit the Chestnut Growers Website for access to many of these resources: http://ecosystems.psu.edu/research/chestnut

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Figure 5: Germination of Direct-seeded Chestnut and Oaks Seeds at Gamelands 205

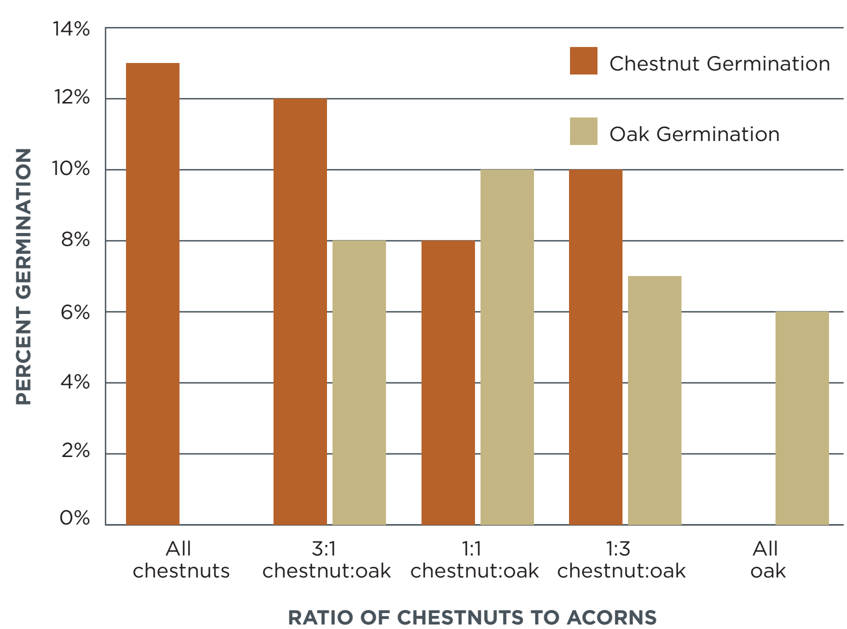


Figure 6

Small seedling getting choked by grasses and other vegetation.

PART 3

Part one of this three-part series described the biology of the fungus (*Cryphonectria parasitica*) that causes chestnut blight. Part two focused on what occurs at the chromosomal level during traditional breeding methods with Chinese (*Castanea mollissima*) and American (*Castanea dentata*) chestnuts to explain why this process generates millions of genetic variants rather than genetically-identical clones. Part three describes in detail the methods being used to create genetically-modified, blight-resistant American chestnut trees starting with one gene from wheat and one cell from an American chestnut.

The American Chestnut Tree Returns

WITH SOME NEW PAIRS OF NEW GENES

By Kent Wilcox, Carolinas Chapter

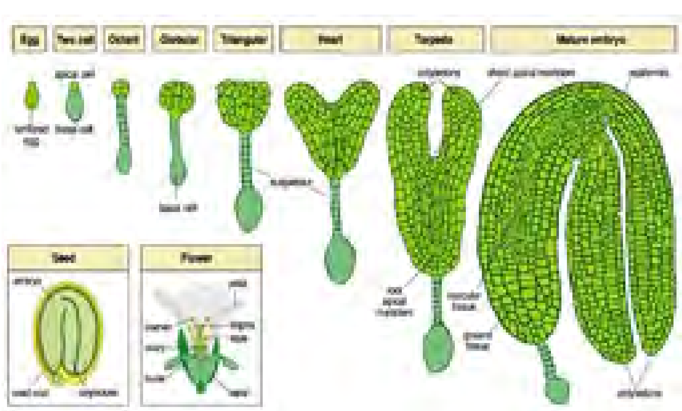
As described in Part Two of this series, using traditional breeding methods to create a hybrid Asian/American chestnut tree that is resistant to chestnut blight is a long, tedious process. The distribution of multiple genes that confer blight resistance into the sperm and eggs of hybrid trees at each step in the breeding process is entirely random. Consequently, thousands of nuts from each generation of the breeding process must be planted and the resulting trees must be screened for resistance and other properties. Most are discarded.

The advent of molecular and cellular technologies to engineer genetically modified organisms (GMOs) in the laboratory has led to the rapid creation of commercially-important plants such as corn, cotton, and soybean with introduced genes that confer resistance to pests and disease. Can blight-resistant chestnut trees be created by the same technology? To address this question, let's consider methods and problems associated with genetic engineering of plants.

In the case of chestnut trees, the end goal is to create a diverse population of trees that produce both sperm and eggs that contain the desired genes. Cross-fertilization between first generation GMO trees will yield some chestnuts that contain two copies of each of the desired genes, thus ensuring that successive generations will be blight resistant. To reach this goal, genetic engineers must have DNA that contains the genes of interest, a method to transfer DNA with the genes of interest into an individual cell to create a genetically-modified cell, and a method to produce a mature, fertile tree from one genetically-modified cell. A quick note on terminology. The term "transgenic" is used for organisms that have been genetically modified by introduction of DNA from an unrelated species (such as introducing DNA from wheat into an American chestnut) and the term "cisgenic" is used for organisms that have been genetically modified by introduction of DNA from a related species (such as introducing DNA from a Chinese chestnut into an American chestnut). GMO is a broader term that applies to any organism modified by genetic engineering, regardless of the DNA source.

Identifying the genes in Asian chestnut trees that confer blight resistance is a difficult process that requires comparing the genetic makeup of blight-resistant and non-resistant chestnut trees. Sequencing the DNA from the twelve different chromosomes (designated A through L) in Chinese chestnut trees is nearly complete (Hardwood Genomics Project, funded by the Forest Health Initiative). The corresponding project for the American chestnut tree has been initiated (HudsonAlpha Institute for Biotechnology, funded by The American Chestnut Foundation) but is far from complete. When that sequencing project is finished, it might be assumed that a direct comparison of the genes in Chinese and American chestnut trees would reveal the genes that confer blight resistance, but it's not that simple. Chinese and American trees differ in growth morphology, flowering time, and many other

Figure 1



Developmental stages during natural plant embryogenesis from zygote (fertilized egg) to mature embryo. Insert shows parts of a typical flower, including several ovules. Diagram courtesy of the University of Nairobi, Kenya

Figure 2

A callus (disorganized cluster of somatic cells) produced from fern tissue. Photo courtesy of Blahnais, Wikimedia Commons

Figure 3A

Figure 3B

Embryogenic cells (well-organized globular clusters of cells) produced by somatic cell embryogenesis from (A) American chestnut tree and (B) sweet orange tree. Photos courtesy of (A) Linda Polin McGuigan, State University of New York College of Environmental Science and Forestry, (B) Randall Niedz, Agricultural Research Service, USDA

characteristics that are due to genetic differences. Low resolution chromosomal analyses of blight-resistant hybrid trees created by traditional breeding suggest that genes located in specific regions on chromosomes B, F, and G in Chinese chestnuts confer some degree of blight resistance (Rebecca Hirsch, "Blight Resistance: It's in the DNA," *Journal of The American Chestnut Foundation* (*JTACF*), March/April 2012). Thus there are at least three and probably more genes that contribute to blight resistance. Once identified, acquiring the DNA for the desired genes will be relatively easy, thanks to advances in synthesizing DNA.

A brief refresher on plant cell biology is imperative to understand what follows. Most plant cells are diploid, which means they have two copies of each chromosome. The term "somatic cell" is used to distinguish diploid cells from reproductive cells (sperm and eggs) which are haploid (one copy of each chromosome). Fertilization of an egg with a sperm creates a diploid cell called a zygote, which undergoes multiple rounds of cell division to become a mature embryo within a nut. The mature embryo consists of differentiated somatic cells that form the shoot apical meristem, the root apical meristem (radical), and two leaves (cotyledons) (**Figure 1**). During germination, these components emerge from a nut and form a seedling (Amy Miller et al., "How a Flower Becomes a Chestnut," *JTACF*, March/April 2014).

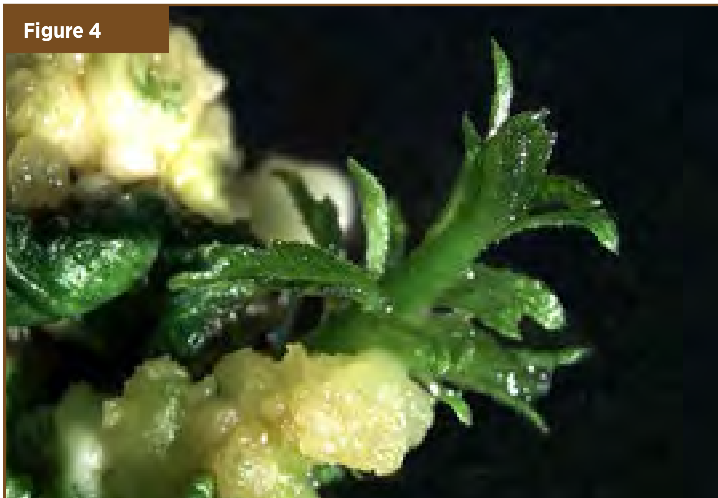
Because reproductive cells are haploid and have the capacity to generate an entire tree after fertilization, one might assume that the best method to create a GMO chestnut tree would be to transfer the desired genes directly into the nuclei of sperm and eggs, use in vitro fertilization to make a diploid zygote, and culture the zygote to create a mature embryo that becomes a seedling. However, the tough outer wall of pollen grains is a barrier that blocks DNA transfer to the sperm inside. Sperm could be extracted from pollen, but DNA transfer would probably fail because sperm DNA is highly compact and sperm nuclei lack mechanisms required for DNA recombination. There is an easy method to transfer DNA into the ovules (structures that contain eggs, Figure 1) of some flowers, but thus far this floral dip method has succeeded with only a few species (not including chestnut flowers), primarily because most plant ovules are relatively inaccessible to DNA transfer methods. Given the difficulties of introducing DNA into haploid reproductive cells from chestnut trees, investigators have chosen to begin with somatic cells and induce them to undergo somatic cell embryogenesis, an artificial process in which a somatic cell from a plant is induced to form an embryo.

Obtaining somatic cells from mature trees would allow genetic engineers to choose a tree with the desired characteristics before starting the process of somatic cell embryogenesis. However somatic cell embryogenesis using tissues from mature American chestnut trees has not succeeded. Fortunately, since 1985, investigators working with several species of chestnut trees have reported successes in generating embryonic cells from somatic cells obtained either from immature embryos within chestnuts or from cotyledons in newly-germinated seedlings. These successes reveal that young, newly-differentiated somatic cells are receptive to reprogramming to become non-differentiated embryonic cells, which is the key step in somatic cell embryogenesis. This step is accomplished by placing the somatic cell tissues in culture medium that contains nutrients and 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic analog of the plant hormone auxin that induces somatic cells to dedifferentiate as they grow and divide. (Ironically, 2,4-D is used as an herbicide that kills plants by inducing uncontrolled cell growth.) Under the influence of 2,4-D, the dividing cells form an aggregate that consists of disorganized clusters of cells called a callus (**Figure 2**) or a proembryogenic mass (PEM) that consists of embryogenic and nonembryogenic cells (**Figure 3**). After PEMs are observed, the culture conditions are changed to stimulate embryogenic cells to transition to an immature embryo. Further changes in the culture conditions induce the immature embryo to differentiate and form a mature embryo with two cotyledons and components of the apical meristem (the shoot that grows above ground) (**Figure 4**). Refrigeration (40°F) of the mature embryo for two or three months is required to induce germination, which occurs when an initial root called the radicle extends from the embryo. This delicate assemblage of differentiated cells can be placed in potting soil to form a plantlet (since there is no outer coat around the mature embryo to form a seed, scientists prefer the term plantlet rather than seedling). Establishing and optimizing conditions for each of these steps required a lot of time and effort. Dr. Scott Merkle and his colleagues in the School of Forest Resources at the University of Georgia worked for six years to find conditions to induce American chestnut somatic cell embryos to germinate and another eight years to increase the germination frequency from 0.4% to 40%.

While working on methods to achieve somatic cell embryogenesis, scientists were also working on methods to transfer DNA into somatic chestnut cells. The first attempts used gene guns that shoot tiny gold particles covered with DNA into cells. The method works, but is very inefficient because most of the cells are killed. Currently the preferred method is to use a bacterial species called *Agrobacterium tumefaciens* that naturally mates with some species of plant cells. The term "mate" is used rather than "infect" because *Agrobacterium tumefaciens* itself does not enter the plant cell. What actually happens is that a bacterial cell makes contact with a plant cell, creates a pore in the plant cell wall, and injects a very specific complex of bacterial DNA and proteins through the pore into the cytoplasm. The proteins associated with the bacterial DNA guide the DNA into the cell nucleus where the DNA is recombined with (integrated into) a plant chromosome at a random site.

Agrobacterium tumefaciens (**Figure 5**) exists naturally in soil and has the capacity to mate with more than 140 species of plant cells and induce a tumor-like disease commonly referred to as crown gall. The integrated bacterial DNA encodes enzymes for the production of hormones (including auxin) that stimulate plant cells to grow and divide profusely as a benign tumor and also induce plant cells to produce nutrients needed by the bacteria to propagate. The net result is a large growth on the plant called a gall (**Figure 6**) that provides a nutritious habitat for A*grobacterium tumefaciens*. Scientists have cleverly modified (some use the term "disarmed") the DNA injected by *Agrobacterium tumefaciens* so that it lacks the genes for production of hormones and nutrients, but retains the sequences needed to integrate a DNA fragment

Figure 4



American chestnut shoot and leaves emerging from a mature embryo created by somatic cell embryogenesis. Photo courtesy of Linda Polin McGuigan, SUNY College of Environmental Science and Forestry

Figure 5

Image taken with a scanning electron microscope of ***Agrobacterium tumefaciens***. The rod-shaped bacterial cells are about 0.003 mm long. Photo courtesy of Shirley Owens, Microbe Zoo Project, Comm Tech Lab, Michigan State University

that contains "genes of interest" into plant chromosomes. In the case of GMO corn and soybeans, those "genes of interest" include a gene that confers resistance to a pesticide or herbicide plus one or more marker genes to identify and/or select the cells with integrated DNA. Because gene transfer is successful in less than 10% of the cells, these marker genes provide a method to eliminate the cells that lack the genes of interest. Scientists have tweaked the process to expand the range of plant species that will "mate" with *Agrobacterium tumefaciens*. That's the good news. The bad news – and this can be a deal breaker in some cases – is that the injected DNA integrates into random sites in plant chromosomes. Insertion of "genes of interest" at a site that causes inactivation of a chestnut gene required for flowering or drought resistance would create an undesirable GMO chestnut tree. The other bad news is that the injected DNA contains sequences required for the integration process and marker genes for selecting modified cells that some GMO opponents consider to be undesirable foreign material.

In spite of these difficulties, in 2006 Dr. William Powell and his colleagues in the College of Environmental Science and Forestry at the State University of New York in Syracuse succeeded in using somatic cell embryogenesis and *Agrobacterium*-mediated gene transfer to create American chestnut trees with increased resistance to blight. But since the Chinese chestnut genes that confer resistance to chestnut blight were not known in 2006, what gene did Dr. Powell's group use? To answer this question, we need to back up a bit. The detrimental effects of some fungi (including *Cryphonectria parasitica*) are due in part to the production by fungi of oxalic acid (an organic acid originally isolated from the wood sorrel plant *Oxalis* and a toxic component of rhubarb leaves). Oxalic acid promotes fungal invasion by causing degradation of the lignin and cellulose in the rigid walls that surround plant cells. To counteract the destructive effects of oxalic acid, some plants (but not the American chestnut) produce an enzyme called oxalate oxidase (*oxo*), which converts oxalic acid into carbon dioxide and hydrogen peroxide. An extensive investigation in the 1990s of the oxalate oxidase enzyme produced by wheat convinced scientists that this enzyme plays an important role in conferring resistance against fungal infections in wheat. The rush was on to see if the same might be true for other plants. Dr. Powell's group used the wheat *oxo* gene to create GMO popular trees and American chestnut trees while other groups used the same gene to create GMO soybean, tomato, and peanut plants that exhibit increased resistance to fungal infection.

It's been more than ten years since the first transgenic, partially blight-resistant American chestnut trees were created (**Figure 7**). Several thousand transgenic American chestnut trees are now growing on test plots in New York. Do these first generation transgenic trees make the sixth generation of Chinese-American hybrids obsolete? The short answer is "no." A thorough answer requires a separate article, but in a nutshell, the first generation transgenic trees exhibit only moderate resistance to the blight. Resistance equivalent to that exhibited by Asian Chinese chestnuts will probably require elevating the amount of *oxo* produced and/or adding additional genes that contribute to resistance. Even with such improvements, first generation GMO trees are essentially clones that lack the genetic diversity required for optimal growth in different ecological habitats from Alabama to Maine. The American Chestnut Foundation is cross-breeding transgenic trees with wild-type American and hybrid Asia/American chestnuts to create trees with increased diversity and resistance (Jared Westbrook, "Merging Genomics and Biotechnology with Breeding," *JTACF*, fall 2016).

However, until sufficient studies establish otherwise, there are concerns that integration of the *oxo* and other foreign genes at random sites in the twelve chromosomes of American chestnut trees could have adverse effects on the long term survival and reproduction of GMO chestnut trees in the wild. Research is ongoing to determine whether high levels of oxalate oxidase production in the roots of chestnut trees might inhibit some mycorrhizal fungi that establish symbiotic relationships with and promote growth

Figure 6

Gall at the base of an oak tree. Photo courtesy of Kent Wilcox

Figure 7

Wild-type (left) and 'Darling 54' transgenic (right) American chestnut trees 30 days after infection with the blight fungus *Cryphonectria parasitica*. Photo courtesy of Andrew Newhouse, SUNY College of Environmental Science and Forestry

of chestnut trees. There are concerns about the potentially harmful effects of high levels of oxalate oxidase on organisms that collect pollen or consume the nuts or leaves from chestnut trees. One interesting study revealed that gypsy moth caterpillars that fed for three days on leaves from first generation transgenic American chestnut trees grew 16% faster than caterpillars that fed on leaves from wild-type American chestnut trees, but there was no follow-up study to determine whether bigger caterpillars turn into more prolific moths. Because white rot fungi secrete oxalic acid to promote the degradation of leaves and woody tissues, scientists are testing whether decomposition of leaves with high *oxo* levels takes more time. To alleviate some of these potentially detrimental effects, scientists are exploring options to replace the first version of the *oxo* gene that produces the enzyme in all tissues of chestnut trees with a version that produces oxalate oxidase only in the trunk and limbs, which are the portions of the tree that are most susceptible to infection by the blight fungus.

Will genetic engineering replace traditional breeding as the method to create blight-resistant trees? The short answer is "yes, but not entirely," with some caveats. If regulatory agencies or members of the public are reluctant to endorse transgenic trees that contain a gene(s) from an unrelated organism, repopulation of Appalachian forests with American chestnut trees may not occur until cisgenic GMO trees with the three or more Chinese chestnut genes that confer blight resistance are created. The fact that DNA transferred by Agrobacterium is integrated at random sites in plant chromosomes is problematic, because every new transgenic tree made by this procedure will have the blight-resistant gene(s) inserted at a different site. Deleterious effects of some integration events may not become apparent until years after GMO nuts are planted. This random-site-integration problem may be solved using a new technology called CRISPR, which allows investigators to precisely insert a gene at a predetermined chromosomal site that does not disrupt nearby genes. In terms of production time, genetic engineering has a huge advantage over the thirty or more years required for six generations of traditional breeding. The current method used to create a transgenic plantlet from one somatic cell takes approximately 14 months and can be scaled up to produce hundreds of plantlets. Diversity can be achieved by creating somatic embryos using wild-type American chestnuts collected from a variety of geographic sites. Because first generation transgenic trees contain only one copy of a transgene, it will be necessary to interbreed GMO trees to create a population of trees that contain two copies of the transgene. Crossing cisgenic trees with three different genes for blight resistance will require screening several hundred progeny trees to find a tree with two copies of all three genes. Fortunately, testing tissues for specific DNA sequences can be done in two days (Jared Westbrook, "Genomic Selection for Disease Resistance," *JTACF*, fall 2017), compared to the six to nine months required to test trees for blight resistance.

Since the first attempts to create blight-resistant American chestnut trees began nearly 100 years ago, there has been a lot of progress towards the goal of repopulating Appalachian forests with American chestnut trees. Much has been learned from the traditional breeding programs about methods to pollinate, propagate, and evaluate chestnut trees. More emphasis is now being put on the importance of diverse strains that are adapted to specific habitats. This information is essential for current projects that combine traditional breeding methods with advances in genetic engineering and cell biology to create a diverse population of trees that, other than increased resistance to fungal diseases, are American chestnut trees in all respects.

The author wishes to acknowledge North Carolina State University for providing access to many outstanding research papers on this topic, especially those authored by Dr. William Powell and Dr. Scott Merkle and their colleagues. For more information, readers are encouraged to explore the resources available at esf.edu/chestnut.

American Chestnut and Fire:

IMPLICATIONS FOR RESTORATION

By Matthew Vaughan, PhD Student, Forest Resources, Clemson University

In our efforts to develop blight resistance and restore the American chestnut to eastern forests, we often focus on the effectiveness of breeding and genetic modification to produce chestnuts with the desired traits and adaptations that will allow them to thrive. But how often do we consider what the optimal strategies and forest conditions would be to ensure their long-term success upon reintroduction? How can management prescriptions create conditions conducive to chestnut growth and survival? Without an endgame, landscape-scale approach to restoration that relates chestnut vitality to the diverse mosaic of disturbance regimes, stand structure, and topography throughout its range, will be difficult for us to be effective stewards of this magnificent tree moving forward.

Abundance of American chestnuts taking advantage of high light availability in an open stand following an April 27, 2013 controlled burn in the Catback burn unit on Massanutten Mountain in the George Washington National Forest in western Page County, Virginia. Photo taken October 27, 2016 by Matthew Vaughan

Historical observations and current insights suggest that American chestnut was associated with a variety of forest types and is adapted to a broad range of environmental conditions throughout its range in the Appalachians (Hawley & Hawes 1912, Russell 1987, Jacobs et al. 2013). However, recurrent chestnut sprouts today are most commonly found on xeric upper slopes and ridges dominated by oak and with a historically frequent fire regime (Stephenson et al. 1991, Anagnostakis 2001, Nowacki & Abrams 2008, Anagnostakis 2012, Brose et al. 2001, Lafon et al. 2017). Chestnut's persistence at these sites may be explained by ecological adaptations that provide a competitive advantage following fire.

Physiological traits and life history characteristics that make oak species fire-adapted (e.g. thick bark, large roots, and vigorous sprouting ability) have been extensively studied and well documented in upland forests (Nowacki & Abrams 2008, Johnson et al. 2009, Brose et al. 2013), with indications that chestnut may share similar traits (Belair 2014, Russell 1987, Foster et al. 2002, Wang et al. 2013). Nevertheless, *Castanea dentata* traits such as its bark thickness and shade tolerance appear to distinguish chestnut disturbance ecology from that of oak (Hawley & Hawes 1912, Russell 1987, Paillet 1982, 2002, Wang et al. 2006). Whereas chestnut sprouts can grow prodigiously to exploit canopy gaps created by fire (Boring et al. 1981, Paillet 1982, 1984, Griffin 1989, Paillet & Rutter 1989, Billo 1998, Paillet 2002, Clark et al. 2010, Clark et al. 2012), it may also be less adapted and more vulnerable to fire overall (Belair 2014, Clark et al. 2014). Since 2005, a few studies have begun to evaluate the effects of fire on chestnut sprout regeneration, but with largely inconclusive results to date (McCament & McCarthy 2005, Belair 2014, Clark et al. 2014, Jarrett et al. unpublished).

Does American chestnut sprout regeneration benefit from controlled burning? How does its response to burning vary according to topography across a diverse landscape? With our understanding of chestnut-fire interactions incomplete, the study presented here aimed to evaluate the vitality of chestnut sprouts in response to controlled burning by sampling and measuring wild American chestnut trees along transects in recently burned forests of the central Appalachian Mountains of Virginia. Fieldwork to sample these chestnuts was conducted May-October 2016 with a team of undergraduates and recent graduates from Texas A&M University and Virginia Tech at three areas within the George Washington and Jefferson National Forests, with assistance from and cooperation with the U.S. Forest Service and the Allegheny Highlands Program of The Nature Conservancy in Virginia. All three study areas chosen for this project provided accessible burn units with documented, diverse fire history and encompassed a wide variety of canopy conditions and terrain features of interest. Our hypotheses were that increased fire occurrence, frequency, or severity would not negatively affect chestnut sprout vitality upon regrowth following the burn, and that chestnut sprout vitality in response to fire would be greater on drier upper slopes and ridges receiving more sunlight.

With controlled burn units identified, transects within and adjacent to the units were proportionally stratified according to landscape features of interest and subdivided into sampling sections. All chestnut trees within the transect width were tallied, and the first chestnut in sight of each transect section was measured, using height, stem count, stem diameter at root collar, stem mortality, presence of chestnut blight, and shoot-to-root ratio to gauge chestnut vitality. A total of 1,782 American chestnut stems from 230 trees in 438 sections of 39 transects within and outside of 16 burn units across the three study areas were measured for this project. Chestnut vitality variables were related with factors of fire occurrence, frequency, and severity, including categories of burned vs. unburned, number of times burned, time since last burn, time between successive burns, and canopy cover as a proxy for burn severity. Additional explanatory variables derived in a geographic information system (GIS) were also used to determine how varying incident radiation, topographic moisture, and slope position affected chestnut response at a landscape level.

The results of this study suggest that increased fire does not negatively

Large juvenile American chestnut that has vigorously re-sprouted in full sunlight following a March 11, 2014 controlled burn in the Fenwick burn unit in the Fenwick Mines area of the Jefferson National Forest in northern Craig County, Virginia. Photo taken May 16, 2016 by Matthew Vaughan

Field team posing for a photo after visiting The American Chestnut Foundation's Meadowview Research Farms in Meadowview, Virginia. From left to right, Jose Silva, Daniel Huseman, Brian Thomas, Chris Moore, John Timberlake, and Matthew Vaughan. Photo by Eric Jenkins

affect chestnut re-growth, but that canopy openings created by fire and other disturbances may hinder chestnut's ability to resist chestnut blight. Tree height and stem diameter were significantly positively correlated with relative light availability, but presence of chestnut blight was as well. The capacity of chestnut sprouts to resist chestnut blight may be impaired by the increased proportion of resources devoted to stem grown and development in high light environments (Latham 1992, Griffin 1989). This impairment may lead to decreased competitive ability in later stages of succession, as there were generally fewer chestnuts in units with a longer time since last burn. The picture that emerges of chestnut growth following fire is one of initial vigorous re-sprouting with many stems, but eventual concentration of growth in the main stem with greater susceptibility to chestnut blight under more open canopies.

From a landscape perspective, chestnut abundance was highest on xeric upper slopes and ridges, but with no preference for slopes and aspects receiving more sunlight. This pattern suggests that chestnut is better suited to the more exposed parts of a mountainous landscape and underscores the importance of canopy openness to chestnut sprout success, as chestnuts cannot benefit from increased sunshine under closed canopies. Even if chestnut sprouts on upper slopes and ridges do not grow taller and larger than chestnuts at lower slope positions, their increased abundance in these portions of the landscape suggest the importance of implementing management prescriptions there (cf. Griscom & Griscom 2012).

Controlled burns can be beneficial to American chestnut by stimulating re-growth with more light availability and removing competition from other species. However, if controlled burning does not open the canopy enough such that chestnut can capitalize with its aboveground response, there may be little to no benefit of fire to chestnut. In some cases, other treatments may be preferable to controlled burning as a means for managing canopy conditions conducive to chestnut growth and survival, as there are often limitations on how much of an effect controlled burns can have on the overstory. Ultimately, the light environment both before and after controlled burning must be carefully considered when determining how and when to implement fire as part of restoration efforts. To maximize the chances of successful blight-resistant chestnut establishment, management strategy should focus on creating canopy gaps to stimulate initial chestnut growth while keeping competing vegetation in check through periodic controlled burning.

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A Look at Chestnut Mold

IN MARYLAND

By Bruce Levine, Maryland Chapter

If you grow chestnuts, whether for eating or planting, mold on the nuts is a problem you've no doubt experienced. Here in the Maryland chapter, our stored nuts seem to get infected each year with the same white and green molds. Some nuts have both types growing on them, and at harvest time, I occasionally see green mold growing on burs in the trees (**fig 1.**).

Figure 1

Fig. 1 Top: chestnuts removed from winter storage, infested with *Trichoderma viride* hyphae (white) and spores (green). Center: *Trichoderma viride* spores on a chestnut bur at harvest time. Bottom: *Pestalotiopsis* mycelium coats nuts and fuses them together. Photos by Bruce Levine

This year, I decided to find out what these fungi are, and whether they harm the nuts or affect germination in any way. I took fungal samples from the shells and insides of Chinese, American and hybrid nuts that the Maryland chapter harvested in 2016 from three different orchards. I grew the fungi in Petrie dishes and extracted DNA to identify the species. (I used the nucleotide sequence of section of the genome called the internal transcribed spacer (ITS), a common reference for identifying fungi by their DNA.) I was rewarded with two surprises: first, the white fungus turned out to be two entirely different white fungi, even though they looked the same, and second, one of them was the same fungus as the blue-green mold. I also isolated two other fungi from the meat of some nuts.

The blue-green mold, *Trichoderma viride* (*Tv*), was pretty much everywhere. I found it on the surface of all three nut types, and also isolated it from the meat of Chinese and hybrid nuts. *Tv* is a very common soil-borne fungus that produces abundant asexual spores. It can also grow and sporulate on above-ground parts of plants, evidently including ripe chestnut burs. This could explain how the fungus was able to infect American nuts that we never allowed to come into contact with the soil. *Tv*'s ability to grow on chestnut burs may help it colonize the nuts, and from there, the roots of new seedlings. Like other *Trichoderma* species, *Tv* is normally associated with plant roots and can be a parasite of other fungi. By the way, I did test to see whether *Tv* would inhibit chestnut blight in a Petrie dish, but saw no evidence that it did. Still, *Tv* may help protect its chestnut hosts against soil-borne pathogens. In fact, it is an ingredient in several bio-control products marketed to gardeners to protect against soil-borne fungal pathogens like *Rhizoctonia* and oomycetes like *Pythium*. It is also used to make "stone washed" blue jeans feel soft and look well-worn, because of its ability to degrade cellulose. (1)

The other white mold came only from our Chinese nuts, some of which had been gathered from the ground. It is a species of *Pestalotiopsis*, a large genus known for making complex, five-celled asexual spores with little branches coming off the ends (**fig. 2**). Many *Pestalotiopsis* species are endophytes, meaning that they can live, generally harmlessly, inside plants, interspersed with plant tissue. Many *Pestalotiopsis* species are plant pathogens, responsible for leaf and

Figure 2

The distinctive spores of *Pestalotiopsis* isolated from chestnut. Photo by Bruce Levine

fruit diseases on a long list of woody plants including chestnut. (2) *Pestalotiopsis* fungi are a favorite subject of biochemical research because they produce a wide variety of unusual chemical compounds not found in other fungi. One species, *Pestalotiopsis microspora* has even been found to produce the anti-cancer drug taxol as well as enzymes that can break down plastic. (2,3) The ability to produce unusual chemical compounds may help *Pestalotiopsis* species suppress competition on the plants where they live.

By sheer coincidence, I had already separately isolated *Pestalotiopsis*, along with chestnut blight fungus, from a blight canker on a chestnut seedling nowhere near where we collected our nuts. Interestingly, the *Pestaloptiopsis* appeared to inhibit the growth of the chestnut blight fungus in a petri dish (**fig. 3**). The effect was not dramatic, however, and the fact that *Pestalotiopsis* may be pathogenic to chestnut in its own right does not recommend it as a potential bio-control against blight. I suspect that *Pestalotiopsis* causes nut rot, though I could not prove it. I did not find it inside any of the nuts sampled, but I did notice that the batch of Chinese nuts on which I found it had a much lower germination rate (about 75%) than the American nuts that only sported *Tv* (over 95%).

This raises the question of mutualism. The presence of these molds on chestnuts is not random, but the result of long-established associations between the fungi and the plant hosts. Like all relationships, it is a two-way street. For example, while *Tv* may help protect chestnut seedlings from soil pathogens, it seems to have a cost in terms of germination. Two thirds of the heavily *Tv*-infested hybrid nuts were completely rotten and non-viable by the end of the winter storage season. A third fungus I found on the inside (but not the surface) of our Chinese nuts, *Gnomoniopsis castanea* provides another example of mutualism. As you can guess from the name, this endophyte is normally associated with chestnut. It is a pathogen in its own right, known to cause bark cankers, die-back and nut rot. (4) But even this fungus is not all bad from the tree's perspective, as it has also been identified as the causal agent of chestnut gall necrosis, and may be helpful in controlling chestnut gall wasp. (5)

Figure 3

***Pestalotiopsis* (white colonies on right) appears to inhibit the radial growth of chestnut blight fungus (on left) compared to the chestnut blight-only control plate (upper left) at 20 days post-inoculation on acidified potato dextrose agar medium. Photo by Bruce Levine**

This investigation suggests that chestnut molds are a normal part of chestnut ecology (**fig. 4**), it may be possible to manage them, but control or elimination of these molds is unlikely. A few years ago, in an effort to prevent moldy seeds, and inspired by a *Journal* article on seed treatment (6), I soaked some clean nuts in pure household bleach for 10 minutes before storing them. When I opened the bag in the spring, they were covered with white mold, probably from hyphae that had already grown into the safe interior of the nuts before I bleached them.

The conclusion I draw from this, is that basic sanitation is important: harvesting directly from the trees, store nuts in clean material without too much moisture, do not crowd the nuts in storage (put fewer than 50 nuts per one gallon bag), store at 0-3 degrees

Figure 4

Fungi isolated from chestnuts in storage in Maryland. Left to right: *Pestalotiopsis* species, *Trichoderma viride*, *Pennicilium* species, *Gnomoniopsis castanea*. Photos by Bruce Levine

C, and plant as early as possible. But it is also important to prepare, and harvest more nuts than you need, especially if the orchard or tree you take them from has had mold problems in the past (see TACF fact sheet "Harvesting Chestnuts" at www.acf.org/factsheets.php). If you are growing nuts for consumption, however, you need to do more (see TACF fact sheet "Prepping & Eating Chestnuts" at www.acf.org/factsheets.php). Some chestnut molds can actually be quite harmful to human health. (7) The fourth and final fungus isolated from our nuts was a species of *Pennicilium*, a genus of fungi famous for giving us antibiotics and Roquefort cheese, but also serious post-harvest food spoilage and powerful toxins. It is very important to know what kind of fungi you have growing on your seeds and how to control them. There has been considerable research on the large number of fungi that grow as molds on chestnut, and a lot of material is available online. I would encourage anyone struggling with this problem to tap into it. **A good place to start learning is this website from Michigan State: http://msue.anr.msu.edu/topic/chestnuts/harvest_storage/storage.**

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Chestnut Sunshine Salad

Recipe from: Classic Chestnut Cuisine
Published in 1993 by Citizen Forester Institute

Ingredients

- 2 carrots
- 2 apples
- 1 rib of celery
- ¼ tsp. salt
- ½ cup raisins
- 1 tsp. lemon juice
- ¼ cup sliced chestnuts (fresh, canned, bottled)
- ½ cup vanilla yogurt
- ½ cup frozen whipped cream

Instructions

Grate carrots and put them in a mixing bowl

Cut apples (peel on) into small pieces

Chop celery

Combine celery, apples and chestnuts with the carrots and add a dash of salt and lemon juice

Stir in vanilla yogurt then fold in the whipped cream

Serves 4

My Chestnut Encounter that Kindled a Lifelong Interest in Conservation

By Lawrence Jacobson, TACF Member

It was April 1961. I was 15 and on spring vacation with my parents at the Great Smoky Mountains National Park. It was different than most of our other family vacations where we drove from Chicago to New York, Washington D.C., or New Orleans.

The Smokies were uncrowded in those days. The timing was perfect to see blooming redbuds and dogwoods. This was also the first time I ever saw clear creeks. The creeks and rivers near Chicago were always brown or grey in color. In those days, creek water of the Smoky Mountains was clean enough to drink untreated.

It was heaven for a 15-year-old boy from Chicago who was used to being careful not to walk on the grass areas of our apartment complex. When we stopped at the Chimneys pullout, I was shocked to see a forest of dead bleached standing American chestnuts. I wanted to do something to restore/save them.

That experience kindled a lifelong interest in conservation. 50 years later I learned about TACF and decided to join. You might think there isn't much an elderly person who lives in Washington State could do to help. But there are a couple of American chestnuts in Tumwater, WA. So, I gather the few fertile nuts I can find each September and send them in. This provides a modest expansion of the American chestnut gene pool available for cross-breeding.

I'm grateful for that visit to the Great Smoky Mountains many years ago. It's an experience that changed my life and a memory that lives on.

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