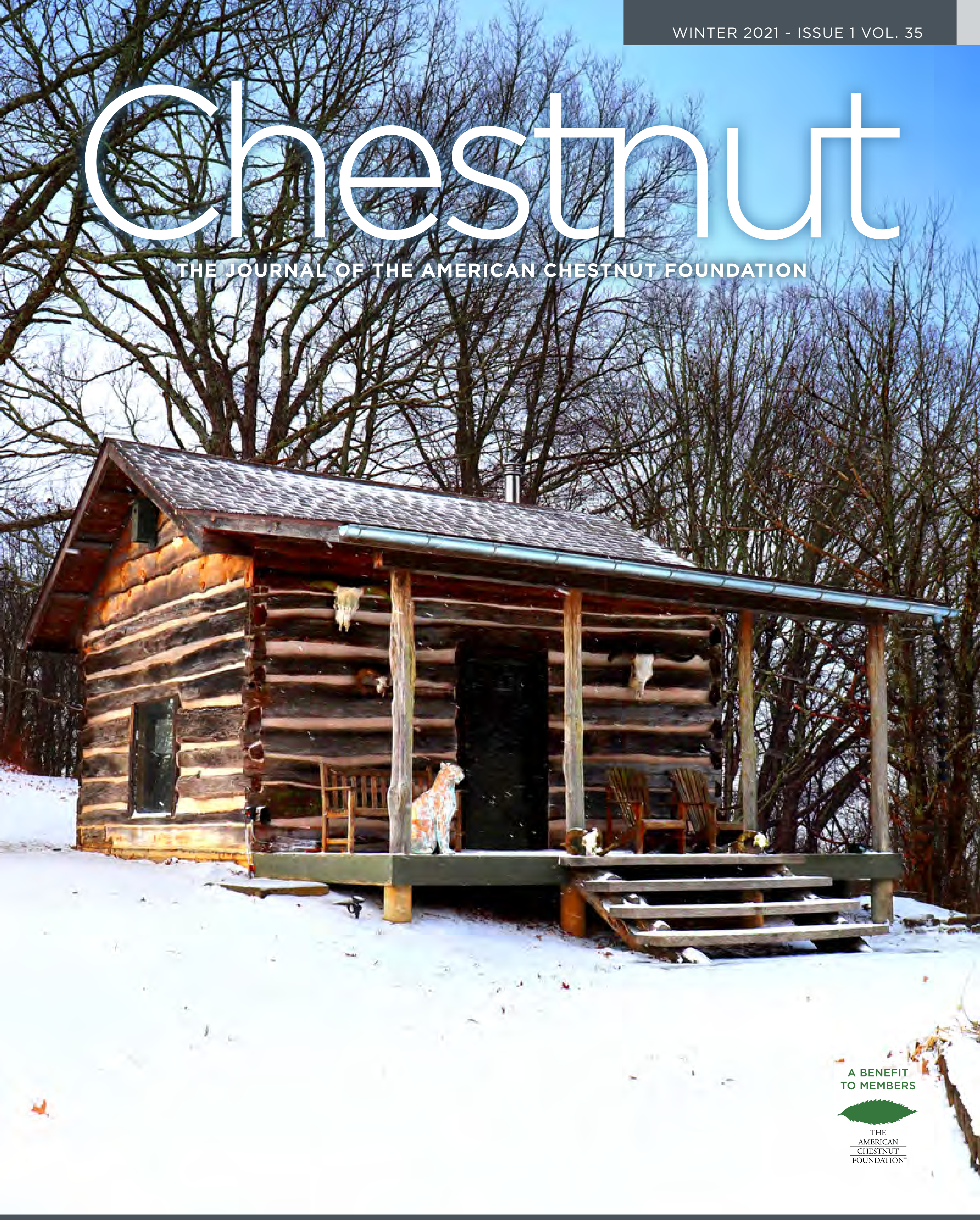
WINTER 2021 ~ ISSUE 1 VOL. 35

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION



A BENEFIT TO MEMBERS

THE AMERICAN CHESTNUT FOUNDATION

Deregulation for Restoration ~ Notable American Chestnut ~ Small Stem Assays ~ Chemical Fingerprinting

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A BENEFIT TO MEMBERS

THE AMERICAN CHESTNUT FOUNDATION

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Lisa Thomson

President and CEO

DEAR CHESTNUT FRIENDS,

By the time you receive this, 2020 will be behind us with better days ahead! Despite the difficulties the year placed on so many lives, we remain optimistic at The American Chestnut Foundation (TACF). It is not lost on our collective family of volunteers, staff, and partners that science innovation and drive will ensure we have normal lives again, just as science ingenuity will bring back an iconic species once thought lost forever. Though all of our lives have been disrupted by the pandemic, if you, your loved ones, or circle of friends were seriously impacted we send compassion and strength.

When government and academic researchers essentially abandoned efforts in the early 1960s to restore the American chestnut, it took a huge leap of faith from our founders to attempt a bold experiment based on the best science at the time. In 1983, Charles Burnham, Phil Rutter, Norman Borlaug, and Donald Willeke, Esq., were among those willing to once again pursue efforts to restore the tree. Phil was hired as TACF's first President & CEO and Don drew up the charter. This determined group of visionary scientists and private citizens began raising funds, giving the American chestnut another fighting chance. Innovative science drove their goals, just as it does now.

Fast forward nearly 40 years and hope abounds in our organization! It is an honor to experience the sincerity of purpose and singular focus of saving an ecologically and economically significant species. It is a compelling story worth telling. I have the opportunity to call and thank those of you who generously donated to our end of year campaign, which was, considering the times, remarkably successful. When I ask what inspires these gifts, I hear the most wide-ranging and heart-warming answers. My personal favorite lately? "Because it's the right thing to do."

May your new year be prosperous and healthy with the hope of many TACF reunions!

With gratitude,

Lisa Thomson

Lisa Thomson, President and CEO
The American Chestnut Foundation

The American Chestnut Foundation has received a 4-star rating, the organization's highest ranking, seven years in a row.

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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Chestnut Cabin

This cabin was built around 1850 in the Newfound Mountains near Sandy Mush, North Carolina and lovingly restored. (Staff photo)

Arrested expansion of chestnut blight canker following successful transmission of hypovirus from super donor strains to naturally occurring virulent strains at initial field release site in Western Maryland. Photo by Mark Double.

BIOCONTROL: ENGINEERED CHESTNUT BLIGHT, ARMED WITH A FUNGAL VIRUS, CAN GIVE THE AMERICAN CHESTNUT

A Fighting Chance

By Brian Lovett, Amy Metheny, and Matt Kasson
West Virginia University

Will the blight end the chestnut?
The farmers rather guess not.
It keeps smoldering at the roots
And sending up new shoots
Till another parasite
Shall come to end the blight.

– Robert Frost (1936)

BREEDING • BIOTECHNOLOGY • BIOCONTROL

When we use biocontrol, we partner with other organisms to limit the spread of pathogens, pests, and weeds that threaten agriculture and natural landscapes. When the chestnut blight fungus is infected with a virus, it is weakened enough to provide American chestnuts a chance to overcome infection and survive another year. Knowing this, our goal is to intentionally introduce this hypovirus – another parasite to end the blight – into naturally occurring populations of the chestnut blight pathogen, *Cryphonectria parasitica*.

This is not as easy as it sounds. Typically, when we think of viruses, we think of respiratory viruses like influenza or COVID-19 that are transmitted outside of their hosts on surfaces and in the air, but hypoviruses are confined to the inside of fungal cells. This means that for chestnut blight fungus to pick up the virus, their cytoplasm (the gooey center of fungal cells) must mix with the cytoplasm of another fungus that is already infected, similar to the formation of a functional graft between scion wood and a root stock.

Unsurprisingly, fungi are picky about who they will swap cytoplasm with through a process called anastomosis. Even within the same species, fungi have genes that help them determine compatibility for anastomosis. These compatibility groups provide protection from cytoplasmic hypoviruses, and for chestnut blight there are over 60 such groups. These are mediated by vegetative incompatibility (*vic*) genes that prevent anastomosis when they don't match (think of outsiders trying to enter your "pandemic bubble"). This is an important defense mechanism for fungi because without such barriers, they would quickly succumb to foreign invaders: long before they can cause chestnut blight. Hypovirus spread is ultimately limited by these genes, so they have served as a hurdle to widespread use of hypoviruses for biocontrol of chestnut blight in the United States.

Fortunately, with the help of genetic engineering, we can remove *vic* genes, thereby removing barriers for widespread virus transmission. In 2016, University of Maryland researchers Don Nuss and Dong-Xiu Zhang did just that, reporting successful disruption of four *C. parasitica vic* genes to create two strains that are "super donors" of hypoviruses. These chestnut blight strains could pass hypovirus to fungi outside of their natural compatibility group to all known compatibility groups. Effectively, these fungal strains are universal donors, like people with an O-negative blood type.

Here at West Virginia University, we have been using these super donor strains in field experiments to better characterize their biocontrol potential. With the support of USDA-APHIS, The Ohrstrom Foundation, and Multistate Project NE-1833 members, our work has revealed that although these super donor strains armed with the right hypovirus are excellent at

Chestnut blight canker treated with super-donor inoculum around the margin of the canker. Reservoirs for inoculum were created using a 0.6 cm-diameter steel punch. Photo by Amy Metheny.

Isolates recovered from micro-sampled wood plugs from super donor-treated canker one-year post-treatment including virulent (orange), hypovirulent (white), and super donor (small brown) strains. Photo by Mark Double.

controlling chestnut blight cankers, they need to be applied to each canker throughout a tree's life to offer full protection. Trees often acquire new infections as they age, and these new cankers also need to be treated. This super donor treatment is like an ointment for an infected cut, rather than an antibiotic pill. Unfortunately, some of these infected cuts are out of our reach: efforts to keep cankers treated within the lower 8-10' of stem will not prevent cankers from killing the tops of these towering forest trees.

We are investigating other factors to improve this biocontrol method, including hypovirus selection and application method. In 2017, our research group introduced a second virus into our super donor strains. In doing so, we were able to compare the efficacy of the super donor strains armed with either of these two hypoviruses. In addition, super donor strains were applied to cankers using various application methods to introduce super donor strains into chestnut blight cankers. We found both the virus and the application method mattered significantly, and we confirmed our earlier findings that super donor strains need to be applied more than once to offer protection. Just as new cankers can form on remaining healthy stem and branch tissue, existing cankers can become colonized by additional *C. parasitica* strains: turning once controlled cankers into actively expanding ones.

Thus far, super donor strains seem to offer an effective strategy for keeping orchard trees alive in transgenic and traditional breeding orchards. In these environments trees are shorter and adequately spaced for treatment and inoculum loads are naturally lower because there are fewer susceptible chestnuts in adjacent forested stands.

Super donor strains represent a promising tool in our efforts to restore American chestnut to the eastern forests. This fungal biotechnology can suppress chestnut blight canker expansion to keep blight-resistant varieties (transgenic or otherwise) around long enough to enter into breeding programs. Biocontrol alone will not end the blight, but together with other TACF-supported tools, these super donor fungi may help us soon turn the page on this dark chapter for American chestnuts.

Laboratory Technician Amy Metheny measures canker expansion at a super donor field release site in Western Maryland following treatment of artificially initiated cankers with super donor strains. Photo by Matt Kasson.

Deregulation for Restoration:

A JOURNEY

By Lisa Thomson, TACF President & CEO

The American Chestnut Foundation (TACF) and its partners at SUNY's College of Environmental Science and Forestry (ESF) have invested tremendous time and resources to educate and build relationships with key personnel at federal agencies that have jurisdiction to regulate the promising "Darling 58" transgenic American chestnut tree.

For several years, we have conducted meetings with colleagues at the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) the U.S. Forest Service (USFS), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA), all of whom are key decision makers and partners affecting its eventual release. Each of these agencies have specific jurisdictions over this genetically engineered tree, currently grown only under strict permitting by APHIS.

Educating these agencies about the nature of TACF's long-term mission and goals of its partnership with ESF has been key to the positive position in which we now find ourselves. An important aspect of cultivating agency interest and enthusiasm has been demonstrating the scientific rigor applied both in developing Darling 58, and in assessing the

Lisa Thomson and Bill Powell at EPA headquarters, April 2019.

potential effects of its reintroduction to forests. To do so included many trips to Washington, D.C. for in person meetings to present findings and discuss steps needed for deregulation. TACF retained the respected D.C. law firm Keller and Heckman, experts in regulatory law, to help navigate the complex processes involved in deregulation. After nearly one year of charging us reduced attorney fee rates, they now represent our interests on a pro bono basis, a tremendous show of generosity and commitment.

TACF's agency relationship-building began in 2015, when USFS chestnut researchers and administrators met with TACF staff at the Southern Research Station in Asheville. This was necessary to begin the renewal process of TACF-USFS' Memorandum of Understanding, which is an important governing document that solidifies TACF's already decades-long relationship with the Forest Service. The USFS is arguably one of the strongest agency proponents of chestnut restoration, and it was at their invitation that I presented a TACF restoration update in September of that year to USFS leaders at their national headquarters. Through the years, subsequent meetings between the agencies and TACF and ESF staff ensued, often thanks to introductions from the USFS, and included leaders at the USDA, the EPA and the FDA.

The most recent was March 6th of this year, when Bill Powell, Jared Westbrook and I traveled to D.C. to meet with USFS leaders and senior advisors to the USDA to update them on the pending petition for deregulation. The city had a worrisome pall over it, as the coronavirus began dominating news cycles. Despite this, we were still asked to present to the USFS, including a meeting with Forest Service Chief Victoria Christiansen and senior aides to USDA Secretary Sonny Perdue.

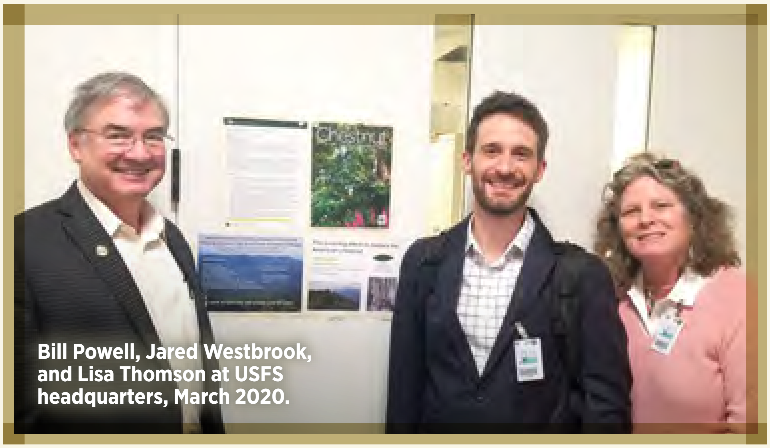
In every instance our agency colleagues were well prepared and took each meeting seriously. There is sincere interest within the agencies for the success of the chestnut restoration project. One high level EPA administrator began a meeting with, "When I left the house this morning, I told my kids I didn't come to work today to kill the chestnut project." A bit tongue in cheek, but it demonstrates how willing the agency professionals are to work within the guidelines of their mandates in order to stimulate innovation and large-scale reintroduction of a keystone species.

The first public comment period on the deregulatory petition before USDA-APHIS was open for 60 days and ended on October 19, 2020. Thanks to a deeply engaged TACF volunteer task force led by board of

Bill Powell and Jared Westbrook co-presenting to USFS leaders, March 2020.

Andy Newhouse and Bill Powell at USFS headquarters, February 2017.

directors member and PA Chapter president Jim Searing, supported by Keller and Heckman and Scott Circle Communications, our highly coordinated campaign contributed to the more than 4,300 public comments weighing in on the merits of the ESF petition. The coordinated and frequent outreach to the vast constituency of TACF members, ESF alumni, and the scientific community resulted in hundreds of thoughtful, substantive comments that validated and expanded on the science assessment of risks and benefits in the petition. It also demonstrated the strong and wide public interest in the restoration of the American chestnut. The comment period also drew written support from important conservation groups such as The Nature Conservancy, Environmental Defense Fund, and the Society of American Foresters. Overall, positive comments outweighed the negative, both in number and in substance. Most comments opposing the petition represented generic opposition to engineered organisms, but a handful of critics challenged aspects of the petition with substantive arguments. Real time monitoring of incoming public



Bill Powell, Jared Westbrook, and Lisa Thomson at USFS headquarters, March 2020.

USDA-APHIS PUBLIC COMMENT PERIOD CAMPAIGN A SUCCESS! OUR HEARTFELT THANKS TO ALL WHO PARTICIPATED.

PETITION TO DEREGULATE ESF'S DARLING 58 AMERICAN CHESTNUT

Supportive Comments by the Numbers:

4,337
total comments submitted with 62% positive responses

358
comments from the scientific community

100+
organizations represented

71
academic institutions represented

1,256
individuals engaged in social media posts from TACF and ESF

Jared Westbrook, Andy Newhouse, and James Votaw (Keller and Heckman) at EPA headquarters, November 2019.

comments by TACF and ESF staff, as well as members of the volunteer task force, allowed ESF and TACF science leaders to carefully address these concerns on the record before the comment period closed. To all of you who submitted supportive comments, we are extremely grateful.

What's next? APHIS will publish a draft Plant Pest Risk Assessment (PPRA) and an Environmental Impact Statement (EIS) after it completes its review of the petition and all accompanying public comments. This could take six months to a year, after which there will be another (shorter) public comment period. Meanwhile, efforts are underway to obtain necessary regulatory approvals from EPA. The transgenic tree, with its protection from the fungal lethalness via the insertion of an oxalate oxidase gene (OxO) from wheat, falls under the jurisdiction of EPA and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as a "pesticide." The team of TACF-ESF was unable to persuade EPA to exercise pesticide jurisdiction over the tree, which is intended to co-exist with the fungus and not kill or mitigate it, EPA nevertheless worked carefully with the team and recommended submitting a FIFRA registration application while also building a case for a full exemption from registration. These are science-based decisions and ESF and its team of experts at the IR-4 Project, based at Rutgers University, together with TACF volunteers supported by Keller and Heckman, are developing both a pesticide registration application and a petition to exempt the Darling 58 from regulation as a pesticide. The registration will provide broad operational flexibility to ESF and TACF, which will be useful if deliberations on the exemption petition are extended. Both those documents are currently in progress and will be considered by EPA in parallel. Each will likely have its own public comment period and it is estimated to take 18 months to two years from submission to approval. As for the FDA, that submission is less complex than the other two agencies and it is hoped that their review will be completed in a matter of months.

Stay tuned for more updates on this fascinating journey of the federal regulatory process. This could be one of the most important milestones for the future of chestnut restoration and indeed, other forest tree species where safe, thoroughly tested genetic engineering could be their hope for survival.

2020 Photo Contest Winners

Judged by the Montreat Tree Board

WINNER
ANDY NEWHOUSE
CHESTNUT SUNSET

2ND PLACE WINNER:
Jon Taylor, Sunbeam and Flowering Chestnuts

3RD PLACE WINNER:
Brian Fox, Old Chestnut Beam

Thanks to all those who submitted pictures to TACF's 2020 Chestnut Photo Contest! Every year we receive a variety of creative chestnut photos, from wild-type trees growing in mountain forests to logs used to build structures still standing today.

Andy Newhouse of Syracuse, NY took this year's winning photo, "Chestnut Sunset." Andy discovered the tree near an open field in Spafford, NY, just as the sun was beginning to set, making the perfect backdrop by highlighting the tender leaves on this young American chestnut. Congratulations, Andy!

ABOUT THE JUDGES:
The Montreat Tree Board, established in 2016, works with community members to protect and maintain healthy forests in the town of Montreat, where TACF recently collaborated on a ceremonial chestnut planting. We appreciate their good work and willingness to judge our 2020 Chestnut Photo Contest!


WINNER: Photo taken in Spafford, NY by Andy Newhouse, NY-TACF Chapter.


2nd PLACE: Photo taken in Albert Mountain, NC by Jon Taylor, Carolinas Chapter.

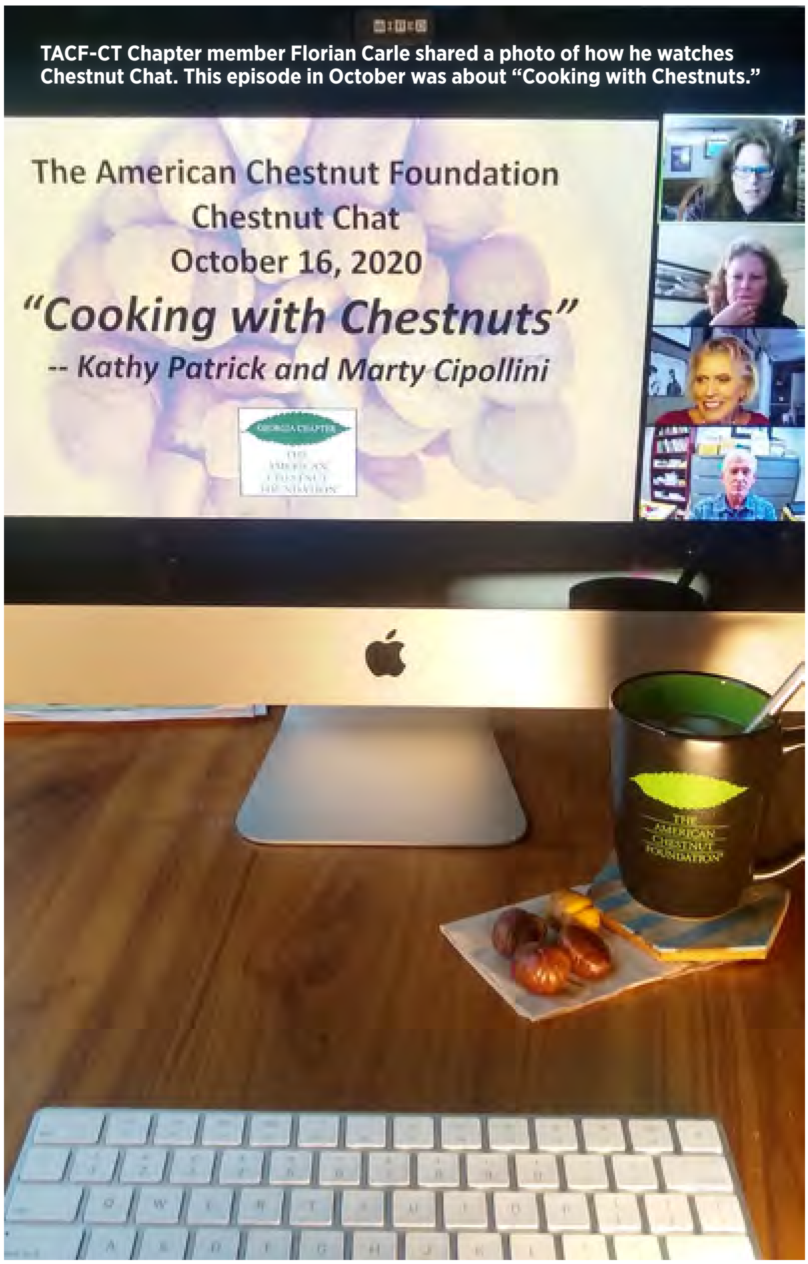

3rd PLACE: Photo taken in Pisgah National Forest, NC by Brian Fox, Carolinas Chapter.

Chestnut Chat Continues

IN THE NEW YEAR

By Sara Fitzsimmons, TACF Director of Restoration

With all in-person meetings cancelled or postponed because of the ongoing COVID-19 pandemic, TACF embraced the virtual meeting platform and developed a live series of presentations, panels, and town halls called "Chestnut Chat."



TACF-CT Chapter member Florian Carle shared a photo of how he watches Chestnut Chat. This episode in October was about "Cooking with Chestnuts."

The first Chat took place on April 17, when we introduced President & CEO Lisa Thomson and the science leadership team; Director of Science Jared Westbrook, and yours truly. We all reported on how different aspects of the Foundation were managing during a time of lockdowns, and answered any and all questions submitted by participants. The session lasted more than two hours and nearly 100 people attended!

Chestnut Chats are held in a Zoom webinar format. Only panelists are allowed to share their screen, camera, and audio. Audience participants can engage using a chat box or Q&A module, making these presentations very interactive.

At the time of this publication, 1,080 unique visitors have attended the Chats. The majority of folks engage via the web (962), though a total of 118 attendees have called in using phones. Chats average about 100 minutes in length with 100 participants, depending on the topic. We have engaged with people from across the country and even internationally, attracting viewers from Afghanistan, Brazil, Canada, France, Greece, Italy, Portugal, Turkey, and the United Kingdom.

In the U.S., folks from 38 states have participated. Of TACF's 16 state chapters, members from Pennsylvania and New York neck-and-neck with the largest amount of viewers and the most amount of time spent on the Chats. Nearly half of the 1,080 unique attendees are members of TACF. One of those members, Carl Meixsell of the PA/NJ Chapter, has attended every single Chestnut Chat and has logged the most time of any other attendee. Many thanks to you, Carl, for your faithful appearance! TACF is lucky to have you as a 21-year member and, as such, will be gifting you with a free one year membership and a new TACF baseball cap.

We encourage the public to contact us with topic ideas for future Chats and have received a number of great suggestions. Most frequently, requests have come in about biocontrol, as well as the origin of certain named chestnut varieties, most particularly "Dunstan." With so much competition for online audiences, and the ever-increasing phenomenon known as "Zoom Fatigue," we will be switching our series from bi-monthly to monthly for 2021, but we will still cover a lot of ground in 12 editions. To the right is a tentative slate for the earlier half of 2021. All Chestnut Chats begin at 11:30AM (EST).

#	DATE	TOPIC	PRESENTER
CC21	2/19/2021	Historic importations and important chestnut cultivars	Sandy Anagnostakis (CAES) and Mike Nave (CA)
CC22	3/19/2021	Controversies of American chestnut restoration	Panel (TACF and ESF)
CC23	4/16/2021	Cruddy Bark and the complex world of blight cankers	Mark Double (WV Chapter) and Laurel Rogers (Shenandoah University)
CC24	5/21/2021	Phytophthora root rot	TBD

If you are not receiving electronic reminders for Chestnut Chat, or other updates and notifications from TACF, please contact us at chestnut@acf.org.

All live Chestnut Chat sessions are recorded and published, along with relevant links and files, on TACF's website at acf.org/resources/chestnut-chat-series/. Even when in-person events and meetings return, we plan to continue offering Chestnut Chat with some regularity. I look forward to "seeing" you both virtually and – hopefully very soon – in person.

IRA CHARITABLE ROLLOVER

THE AMERICAN CHESTNUT FOUNDATION®

AN IMMEDIATE GIFT, ONE TREE'S FUTURE

Originally passed by Congress in 2015, the IRA Charitable Rollover is an easy way to give an immediate, outright gift to support The American Chestnut Foundation. If you are 70 1/2 or older, you can make tax-free gifts to TACF directly from your retirement account. These gifts can be one-time or reoccurring.

Through your traditional or Roth IRA, you can donate up to $100,000 each year. Those distributions go directly from your account to TACF without incurring any income tax. This is especially attractive if you do not need the required minimum distribution (RMD) for supplemental income from your IRA.

Qualified charitable distributions from your IRA can also help reduce or satisfy your RMDs each year. For example, if your 2020 RMD was $10,000, and you made a $5,000 qualified charitable distribution to TACF that year, you reduced your RMD (which is taxed as ordinary income) by $5,000. This arrangement is a win-win for you and TACF!

If you are interested in this easy and tax-savvy way to support the restoration of the American chestnut, please contact Lisa Thomson at lisa.thomson@acf.org or call our national office at (828) 281-0047.

We always encourage you to discuss your charitable giving strategies with your tax advisor or attorney.

Mike and Louise Aucott

VOLUNTEERS WHO PLANT CHESTNUTS AND CARGO VANS

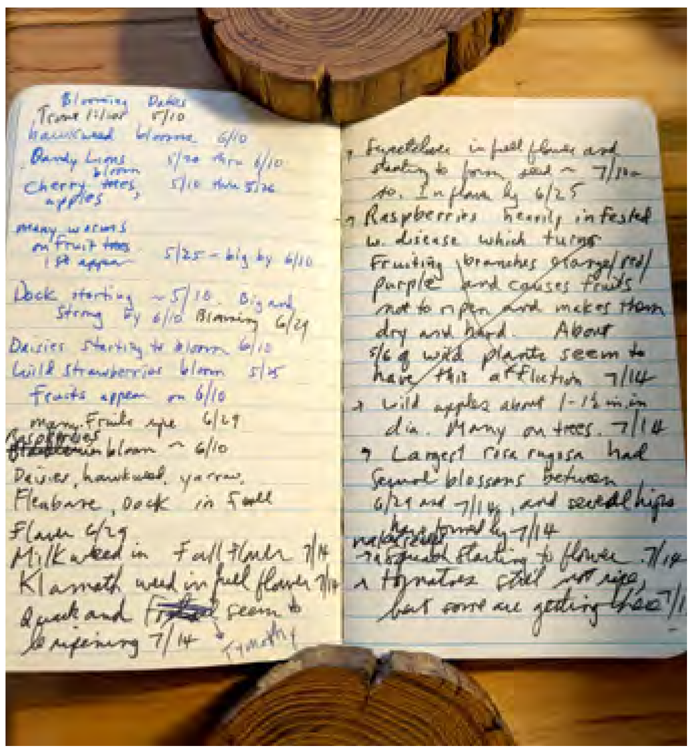
By Scott Carlberg, Contributing Author

Mike and Louise Aucott buried their 1967 Dodge cargo van in the summer of 1979. Really. Dug a big hole, a bulldozer pushed in the van and buried it. Not out of frustration, though it had completely broken down. Out of pragmatism.

Let's step back and learn about these TACF volunteers first.

Mike is an environmental scientist. “I am on the science committee of the PA/NJ TACF Chapter. I also coordinate about 20 owners and operators of F_2 hybrid chestnut plantings that are part of what’s now called the Pennsylvania Chestnut Timber Tree Program. I also oversee several plantings of chestnuts at parks, preserved lands, and elementary schools in central New Jersey.”

Louise discovered TACF because of Mike. “I found ways I could help out with our Pennsylvania/New Jersey Growers meetings and orchard activities. I was on the local board of directors for a while.”



The Aucotts have moved around the two states. “We’re in semi-rural New Jersey now,” says Louise, “There really is a semi-rural New Jersey, believe or not.” Their orchard is in north central Pennsylvania where they used to live, and where they planted their van.

Planting and nurturing are part of Louise’s character. She is a midwife with more than 1,000 babies to her name. Louise is writing a memoir about her midwife experience.

Mike is a self-proclaimed “data guy.” He earned a Ph.D. in environmental science from Rutgers. He now teaches chemistry part-time at the College of New Jersey. “Spreadsheets make him happy,” says Louise, and proves how data-driven Mike is. Check Mike’s field notes (pictured) from years ago about planting on their land.

Mike and Louise have a stellar blend of skills for the chestnut cause.

About 1 $^1/_2$ acres of their 37-acre plot is planted in chestnuts. TACF can use this land long-term. The deed has a rider that gives TACF permission for entry and research no matter who owns it.

First planted in 2013, the Aucotts’ orchard is starting to yield data on phenotype, blight resistance, and other qualities of F_2 hybrids and how they compare with pure American chestnuts.

Mike is a fan of the genetically engineered (GE) Darling 58 chestnut, now under review by the USDA for non-regulated status. “It’s 100% American except for a gene from wheat inserted in its genome,” says Mike. “The gene appears to give the tree a strong blight tolerance, because it causes the production of an enzyme that denatures oxalic acid. Oxalic acid is secreted by the blight fungus, and it kills the tissues of the tree, enabling the fungus to feed on the dead tissue.”

Genetic engineering is critical, according to Mike. “There is a danger that the transgenic American chestnut could become a casualty of a larger battle between opponents and proponents of genetic engineering. But the transgenic chestnut’s arrival in the fray could also catalyze the growth of a better understanding of the potential of GE to protect and enhance forest diversity and environmental health in general.” That is from Mike’s recent essay, “Medical biotechnology as a paradigm for forest restoration and introduction of the transgenic American chestnut.”[1]

The Aucotts worked their land, back to their flower child days. “Bought the land in 1974,” says Mike. “Went back to the land with a pick-your-own strawberries business and sold cauliflower wholesale. We’d load our pickup truck with the crop and go to markets in Buffalo or Sunbury.”

The Aucotts learned. “We didn’t have enough land to make a go of it in farming,” says Louise, “Though we wouldn’t trade those years.”

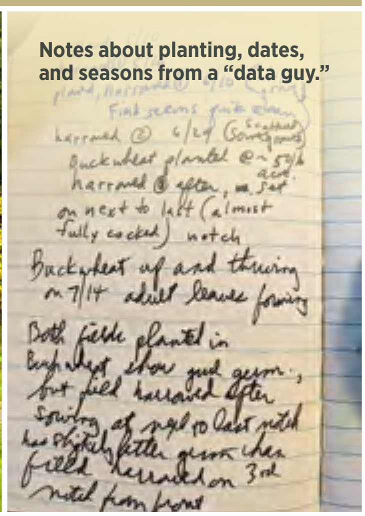
Neither would their children. The oldest two (of four) have the most memories. “We had a free-range childhood, literally playing in the dirt. I felt and still feel like a part of nature, rather than separate from it,” says Aimee, the oldest. Jasmine, says, “I remember being free to wander through the fields and woods, and used to make up games and stories from the things I found.”

Fun was a byproduct; values were strengthened in the children: “At mealtimes we would celebrate which menu items contain ingredients we’d grown,” says Aimee. Then she reflects, “It wasn’t buying a house or getting married that made me feel like an adult. It was having my own garden. I take pride in fixing things myself and take inspiration from my parents to write letters to, and call, the powers that be when concerns need to be addressed.”

> "I see farming and forestry increasingly moving to permaculture and with that, human civilization becoming more in harmony with nature."
>
> – Mike Aucott

Students get hands-on experience by planting a tree with Mike's help.

TACF volunteer, dedicated to the forest, and happy about it.



Notes about planting, dates, and seasons from a "data guy."

Mike and Louise posing in front of a vehicle that stayed above ground.

Mike and Louise with their children in the earlier days.

Mother Nature is the children's elixir. "I'm still soothed by nature. I feel at home anywhere there are lots of trees. I love growing a vegetable garden. There's something special about growing your own food'" says Jasmine. "And between my husband and daughters, I'm the only one not afraid of bugs. I'm the designated person to catch a spider and let it outside."

Mike and Louise learned a lot on that farm that helps their chestnut work. "Being aware," says Mike. "Dealing with plants living in the wide, wide world, you are at the mercy of the flows of nature."

"Self-discipline," says Louise. "You have to be process-oriented." (Maybe like attending to the birth of babies as a midwife?) "You have to keep your eye on all that's going on. That goes back to our foray into farming." Louise pauses, then, "We were so young."

Young, and mighty inventive. For instance, their long driveway always got muddy. A patch of rubbly, rocky soil was just off the driveway. They hired someone with a backhoe to pull out 500 tons of the rock and spread it on the driveway.

That stone left a big hole. "That was also when our van quit. Beyond repair," says Mike. "So we drained the oil and gas, took the tires off, and had a dozer push it in the hole. Buried the van; made an amazingly effective cold cellar."

"We had hay bales in front and could keep our potatoes, carrots and rutabagas stored well over the winter," says Louise.

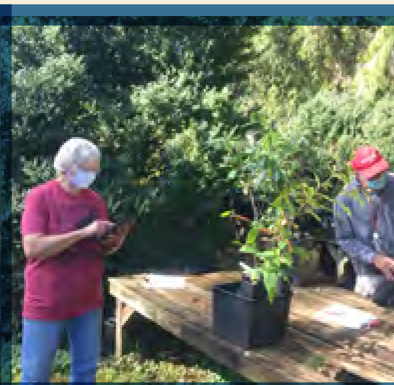
The Aucotts want to be team members with the world. Mike says, "Farming has helped me realize the tremendous legacy of thousands of years of domestication of plants and animals. I see farming and forestry increasingly moving to permaculture and with that, human civilization becoming more in harmony with nature."

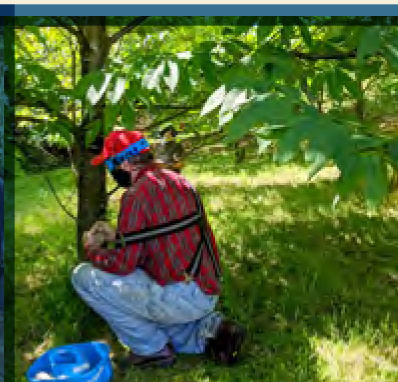
FOOTNOTES

[1]Aucott, M. and Rex Parker, 2020, Conservation Biology, https://doi.org/10.1111/cobi.13566

OUR HEARTFELT THANKS

For most, it is customary to welcome a new year as we reflect on the old. However, 2020 has become the year we long to forget or, more importantly, the year we leave behind without reluctance as we look toward a brighter future.





As an invaluable member of The American Chestnut Foundation, we wish to take this opportunity to sincerely thank you for staying with us during a national pandemic that has left no one untouched. Your unceasing commitment and faith in our efforts have allowed us to continue, without losing ground, our singular grand mission to rescue the American chestnut tree.

You are fundamental to the success of our mission and we are truly grateful for your dedication. Together, we enter a new year with confidence, hope, and the promise of one tree's future.

THE AMERICAN CHESTNUT FOUNDATION®

FOR ONE TREE'S FUTURE

Flippo Gravatt views a large American chestnut at Scientists Cliffs, MD in 1959.

A Notable American Chestnut

IN CONNECTICUT

By Richard A. Jaynes, CT-TACF Chapter and TACF Honorary Director

In January 1959, I visited Flippo Gravatt, a plant pathologist with the USDA. He took me to Scientists Cliffs, Maryland, to show me some remarkably large American chestnut trees that were surviving in the presence of chestnut blight. Scientists Cliffs is an unincorporated community in Calvert County that Flippo and his wife established in 1935 for retired scientists. They had a home there, Chestnut Cabin, which was constructed entirely of chestnut. It is now a museum.

We collected scion wood (cuttings) from one of the trees and I grafted them on Chinese chestnut seedlings, a combination we subsequently learned was often incompatible. However, a few survived and one was planted at the Lockwood Farm of the Connecticut Agricultural Experiment Station in Hamden, CT. This grafted survivor is likely the largest surviving American chestnut in Connecticut. It is 32″ in diameter at breast height (dbh) and about 45′ tall. A secondary stem growing from the base is 16″ dbh. The grafted Scientists Cliffs' tree is next to several American chestnut seedlings planted in the 1970's and inoculated with hypovirulent (attenuated) strains of the chestnut blight (bottom right). In general, these seedlings are surviving to a larger size than the typical American chestnut seedling or native sprout.

There definitely is chestnut blight in the trunk and crown of the grafted tree. Some branches have died from encircling cankers and thus limited the tree's height. To my knowledge no controlled crosses have been made with this tree, nor have nuts been collected to grow seedlings.

I have contacted the American Chestnut Land Trust and the Scientists Cliffs Association in Maryland. Apparently, none of the large trees seen in 1959 remain. Since none of the trees seen with Flippo have survived, except possibly as sprouts, it leaves open to question their actual blight resistance. Certainly, their existence in 1959, when other chestnuts in the area had died suggests some resistance, as does the survival of the large grafted tree in Connecticut.

Dieback in crown of the tree was likely from the presence of the chestnut blight fungus.

American chestnuts seen in 1959 at Scientists Cliffs with Flippo Gravatt (foreground) and Fred Berry, forester.

Scientists Cliffs graft in 1972 (33 years old) with Martin Gent of the CT Agricultural Experiment Station.

A 1959 graft of one of the American chestnuts from Scientists Cliffs, MD, and growing at Lockwood Farm of the Connecticut Agricultural Experiment Station, Hamden, CT in March 2020 (61 years old). Sally Jaynes holding yard stick.

BIOGRAPHY

Richard Jaynes received his Ph.D. from Yale in 1961. His dissertation was on the genetics and cytology of chestnut. He was a plant breeder at the Connecticut Agricultural Experiment Station for over 25 years and worked on chestnut and laurel until 1984 when he left to start Broken Arrow Nursery and expand his Christmas tree business. He has been an honorary director of The American Chestnut Foundation for many years.

All photos taken by Richard Jaynes.

SITE SELECTION FOR A

Germplasm Conservation Orchard

By Kendra Collins, New England Regional Science Coordinator

Germplasm conservation is an important component of TACF's 3BUR science strategy, and state chapters across our organization are being asked to locate more wild American chestnut trees that can be incorporated into our programs. These trees may produce nuts, provide scion for grafting, or even sprouts that can be transplanted, and all can be used to populate germplasm conservation orchards (GCOs). But what makes a good chestnut orchard site? As more and more material is available for planting in GCOs, selecting the best sites for these collections is an important factor in their success.

Former TACF intern Dan Hale teaches students how to plant chestnuts during a stewardship work day organized by the orchard manager, a partnering nature center, the local junior high school and TACF. Photo by Annie Card.

The first consideration for any new orchard site is always viability – is this a good site for chestnut? From there we can explore whether the site is also suitable as an orchard. Another consideration is visibility. While not critical, a visible site allows for additional outreach or public relations opportunities in addition to meeting our science program needs. Partnerships can also come into play – either honoring existing, positive relationships or establishing new, promising ones. Financial considerations are also important, in terms of any support available for the project. And finally, are there research needs this orchard would help TACF meet? In the case of a good GCO site, the answer is a solid 'YES.'

A look at Web Soil Survey shows one predominant soil type for the planting site in question, which happens to be appropriate for growing chestnut (top). A site visit further confirms good drainage and acid-loving vegetation in the form of blueberries (high and low-bush) and white pine (bottom). The Soil Test Results show a good soil pH for chestnut (right).

SOIL TEST RESULTS

Avail. Phosphate (ppm P)	1.1
Potash (ppm K)	78
Magnesium (ppm Mg)	41
pH	5.2
Calcium (ppm Ca)	405

What Makes a Good Planting Site?

There are two ways to look at site viability when assessing a prospective orchard site. Is the site viable for chestnut growth and is it viable for orchard management? First, a good chestnut planting site starts with well-drained, slightly acidic soil (pH 4.5-6). A good first assessment of a site is to find it on the Natural Resources Conservation Service's (NRCS) online Web Soil Survey. This resource will identify the soil types, allow you to read the soil series descriptions, and look at a few parameters related to drainage (drainage class, KSat, permeability, percent clay), depth to any restrictive layer, and soil pH. While the soil survey is no substitute for a site visit and soil test, it is a good way to determine if the site is one you want to pursue. If all looks good, follow up with a site visit to collect a soil sample to test soil pH and basic nutrition, and do a percolation test (or "perc test") to look at on-site drainage. A look at the existing vegetation can also offer clues to drainage and site conditions.

If the site appears promising for chestnut growth, consider how an orchard might work. Is there full sun on the site? Chestnut requires a lot of light to flower and reproduce, which is important in a GCO. How would water be accessed, at least while the trees are small? Is it relatively easy for the orchard manager to get to for basic maintenance and inventory activities? An orchard that is tucked away can fall into the "out of sight, out of mind" pitfall of orchard management, and sites that are difficult to access or work in often receive less care. Can equipment be transported and used on-site for mowing or to address other vegetation management tasks? Would it be possible to bring a bucket truck on site for pollination or harvest? If not, could a ladder be safely used? Steep slopes may be fine for chestnut growth and some types of plantings but for a GCO, more level or accessible ground is preferred. Finally, what cultural practices are needed to manage competing vegetation and protect from wildlife?

What Makes a Good Cooperator?

Most chestnut orchards in TACF's network are hosted by volunteer cooperators. These partners are often private individuals, local, state, or federal agencies, conservation organizations, or educational institutions. There are pros and cons to working with any type of landowner and one of the first considerations is often the expected longevity of the planting. The timeline for a GCO is at least 15 years to get it fully planted and growing flowering trees that can be used for making new crosses. A host for this orchard type should be able to reasonably commit to the site remaining a chestnut orchard for at least that 15-year time frame. In some situations, a handshake agreement may be appropriate; however, it is often beneficial to develop a more formal agreement (orchard host agreement, Memorandum of Understanding, etc.) that outlines the expected timeframe of the orchard and the dedicated use of the land.

In addition to the security and longevity of the planting site, what is the future host's level of interest in helping to manage the orchard? Ideally, a new orchard site provides a local orchard manager, or ties into a local source of volunteer labor where one might be identified. It is a good practice to have new growers take on a small test planting of 10-20 trees in their first year, to test out the site, cultural practices, and the grower. If the trees struggle, or the host is difficult to communicate with, it is often best to re-assess and likely move on.

While there is no magic formula for the perfect orchard site or host, picking a site that is appropriate for growing chestnut, will make for a good orchard, is secure for the duration of the project, and has a dedicated host is a great place to start! As always, TACF's regional science coordinators are available to help evaluate new sites or hosts.

After a successful test planting (right), the trees have grown well on this site (below), thanks to good site selection and proper management. The orchard has also become a great teaching tool for the local junior high and nature center programs (bottom right). Photo by Annie Card. All photos taken at an orchard in south-western New Hampshire.

MEADOWVIEW

Oxalate Oxidase Inheritance Assay:

LOOKING INSIDE A TRANSGENIC SEED

By Lily Kingsolver, Meadowview Nursery Manager

MEADOWVIEW

We are living in the future, and with TACF's trademark commitment to sound science and thorough research, we are embracing cutting-edge technology to peer inside the American chestnut genome. Our great hope is that the information within can outwit that nasty chestnut blight. We can learn all there is to know about the genome, but we cannot outsmart genetics, and not every seed produced from breeding a transgenic tree with a non-transgenic tree will contain the inserted gene. After breeding comes the less than glamorous part: testing each chestnut from each cross to see which nuts inherited the transgene. This process is long and tedious, but a bit magical to watch, as each seed reveals what is hiding inside its shiny shell.

The transgene, a gene found in wheat, works by breaking down the harmful oxalic acid produced by *Cryphonectria parasitica* (chestnut blight) into hydrogen peroxide and carbon dioxide, two completely tree-safe byproducts. To determine which seeds contain the transgene, we must test to determine which ones break down this acid. We create two solutions: one to act as a control, and one containing oxalic acid which will interact with the transgene to turn the tissue sample and surrounding solution a dark blue-black color. A small amount of each solution is pipetted into two tubes marked to indicate the staining solution versus the control. Each set of tubes also displays a unique code: a letter representing the parental cross and a number representing the individual nut. This code allows us to keep track of seeds that contain the transgene.

The hard outer shell of the chestnut and the papery seed coat within it are integral to the

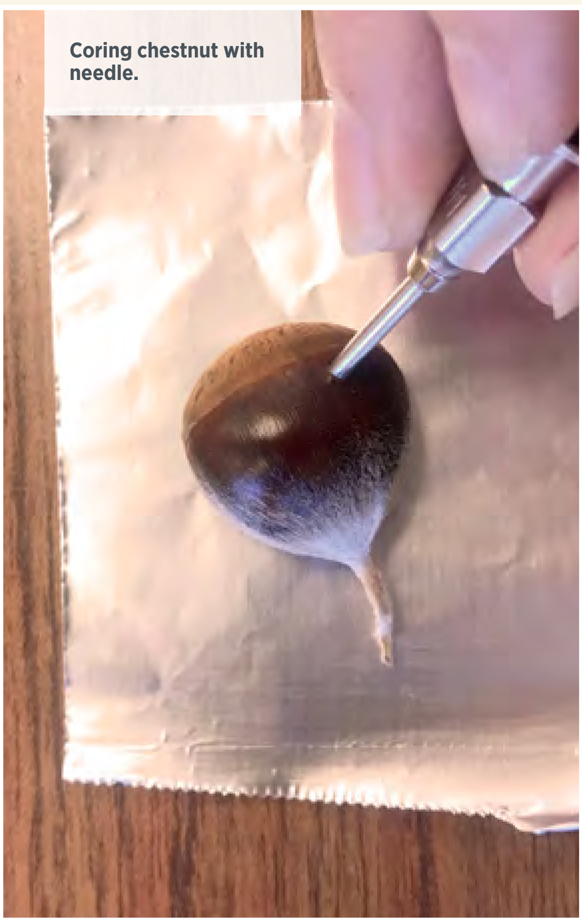
Staining solution displaying positive result on left, control solution on right.

Preparing to pipette staining and control solutions into microcentrifuge tubes.

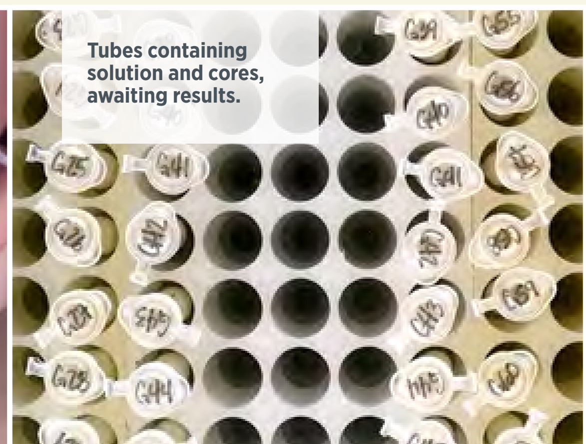
survival of the organism, protecting the seed until its germination. Because of this, it is very important not to damage the structure of the shell and seed coat as samples are collected. What we want is the inside: the soft, tan cotyledons, the part that you might have eaten before or, if uneaten, will become those first two fleshy leaves of the plant. This nutrient-rich tissue will tell us whether our seed has inherited the transgene. To collect it we use a veterinary-grade bone marrow needle, an excellent way to take a small core from a sensitive seed. After carefully piercing the seed near its base, avoiding the fragile embryo near its apex, the core is pushed out with a straightened paperclip – we improvise in this lab! Then, the shell and seed coat are separated from the cotyledon tissue before the core is divided into two pieces: one for the tube filled with staining solution and one for the tube containing control solution. The tubes are capped and given a gentle shake to distribute the plant tissue, and the hole we punched is sealed with silicone. Then begins the waiting game, with each sample taking anywhere from 15 minutes to an hour to reveal its true colors: blue if it contains the transgene, clear if it does not.

Next we need to store our chestnuts. Seeds are terrible at wearing nametags, so each one goes into a small bag with its ID code written on the outside. We use teabags, which are strong enough to hold together when stored in potting medium but porous enough to prevent the seeds from becoming desiccated during storage. Now the seeds get a temperature-controlled rest until early spring, when they will be sowed.

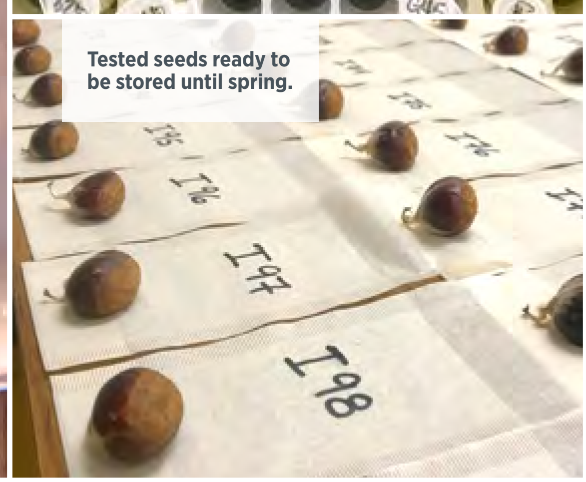
This assay is practical, but it also offers a window into the genetic code of each potential tree. It is a reminder of the power of nature, the continuation of a millennia of evolution through breeding, and how exciting it is to be involved in that process in some small way. For all the work of the brilliant scientists who inserted the transgene, these magnificent trees are still overwhelmingly in control. I am hopeful that with a little help from gene insertion, these trees will one day dot the mountains around Meadowview again, and I will have helped by asking each tiny seed to tell me its secrets.



Coring chestnut with needle.



Tubes containing solution and cores, awaiting results.



Tested seeds ready to be stored until spring.

Small Stem Assays

SHOW PROMISE AS A FAST AND HIGH-THROUGHPUT RESISTANCE SCREENING METHOD

By Bruce Levine, MD-TACF Chapter President and Tom Saielli, Mid-Atlantic and Southern Regional Science Coordinator

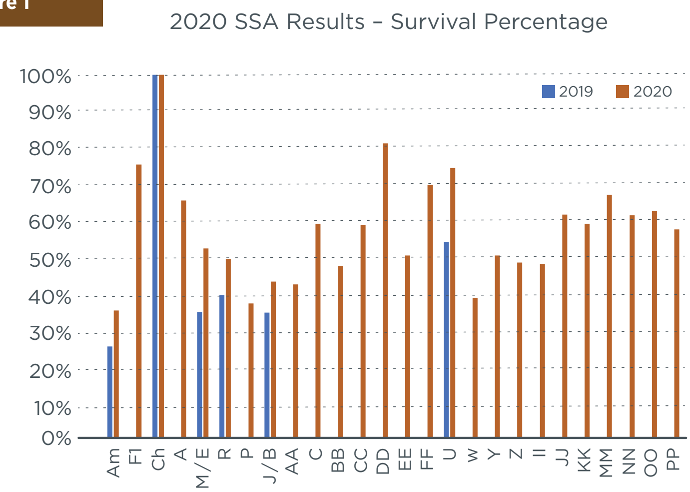
The Maryland Chapter of TACF (MD-TACF), along with other TACF state chapters, have been experimenting with Small Stem Assays (SSAs) as a way to screen seedlings for blight resistance, earlier, more quickly, and in greater numbers than under TACF's standard inoculation assay. The method was described in the winter 2019 issue of *Chestnut*[1].

It is worth recalling the rationale for SSAs. Over the past several years, TACF has learned that the B_3F_3 generation (the fifth generation of TACF's backcross breeding program) has not shown the levels of reliably high resistance to chestnut blight we hoped for. While B_3F_3 trees do show higher resistance on average than wild-type American chestnuts, resistance varies considerably from individual to individual and from family to family, between what we would expect from American chestnuts to what we would expect from Chinese-American F_1 (50 - 50) crosses. Few if any B_3F_3s have shown resistance comparable to Chinese chestnut.

MD-TACF Vice President Karl Mech surveys seedlings in the greenhouse, spring 2020. Photo by Bruce Levine.

The reason for this disappointing performance appears to be that the genetics of chestnut blight resistance is far more complex – involving far more genes – than originally understood. Now that we know this, we also know that we will have to screen more seedlings than we originally expected in order capture the rare combinations of genes that can confer robust resistance through cross-breeding. This potentially involves more labor, land and effort than we can manage using traditional methods. SSAs are a means to screen trees more quickly, but it will take several years of experimentation to confirm whether it is accurate or sensitive enough to predict the long-term resistance of individual trees.

Figure 1

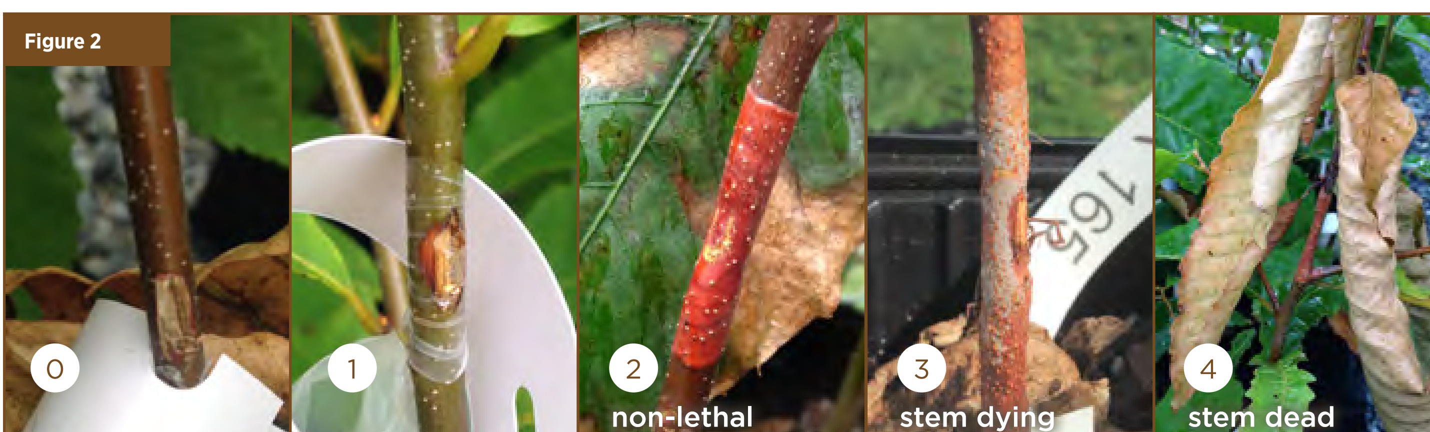


MD-TACF's SSA results in 2020, as measured by survival percentage. The blue bars represent results form 2019 for seedlots that were screened in both years.

The MD-TACF just completed its second year of SSAs, and results are promising. We were pleased to see that SSA survivor seedlings we planted in November 2019 suffered no transplant shock. Other than losses to voles, our only losses were to chestnut blight, which is essentially a continuation of the SSA through the winter. Results so far from Maryland's SSA program are entirely consistent with what TACF has seen in its seed orchards in Meadowview, VA. Specifically, for two years, after comparing average performance by seedlot (seeds taken from a single mother tree), we have seen Chinese controls perform best, American controls perform worst, and our backcrosses (MD-TACF's backcross program produces B_2F_2s and B_4F_2s) showing resistance between American and F_1 controls (**Figure 1**). In 2020, the chapter included B_2F_2 seedlings from the Musick source of resistance, alongside the B_4F_2 Clapper trees that make up the majority of our breeding lines. As expected, the B_2F_2s outperformed the B_4F_2s as a group. In both years, there was considerable variability within and between seedlots.

The chapter has also made progress in terms of increasing the efficiency of SSAs, potentially increasing the number of trees we are able to screen. We learned in 2019 that the application of solid inoculum from Petri dishes and the use of parafilm to wrap the inoculation site were stumbling blocks for our volunteer workers in the inoculation process. On top of this, COVID-19 forced us to rethink how we could perform inoculations without people working close together. To address these two problems, we switched to using a slurry-type inoculum that could be squeezed out of an

Figure 2



Examples of cankers rated 0-4. Score 0 means there are no fungal signs. Score 1 means fungal signs are confined to the immediate vicinity of the inoculation site. In Score 2 cankers, the fungus is expanding through the tree tissue well beyond the inoculation site, but not causing harm to the stem beyond the inoculation site. Score 3 cankers show evidence that the cambium layer is dying – in this picture there are fungal fruiting bodies, which is an indication that the fungus is anchored in dead tissue underneath. A score of 4 means the stem has died from the inoculation.

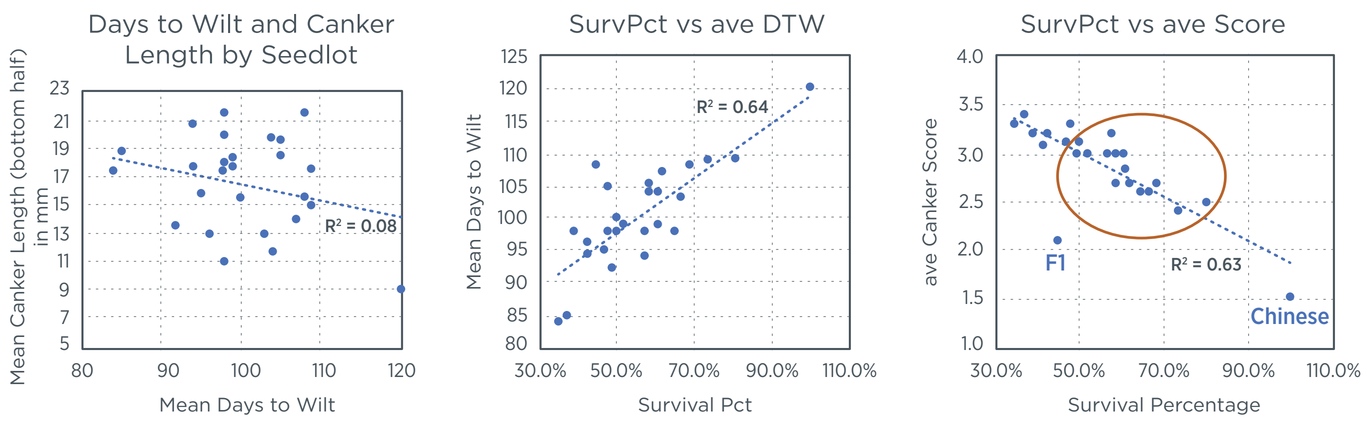
oral syringe, and replaced parafilm with masking tape. The result was about a four-fold increase in efficiency – we inoculated more trees, in less time and with fewer people in 2020 than in 2019. We will continue to refine our methods to make SSAs as efficient and reliable as possible.

One area that is the focus of our attention now is the question of how you measure resistance in a seedling. In our first limited experiments with SSAs in 2017, we measured resistance by measuring the length of the inoculation canker at a specified point in time (e.g. 90 days post inoculation). We found, however, that there is significant variability in this measurement method, which is likely related to the physiology of each individual tree, and exacerbated by the fact that it can be difficult to clearly identify where a canker begins and ends. In 2019, we added new measures, such as "days-to-wilt" (the number of days between inoculation and when the part of the stem past the inoculation site dies), and "survival percentage" (the percentage of seedlings that had surviving stems at a specific point in time). We also scored each canker on a 0 (least severe) to 4 (most severe) scale based on specific observable canker features (**Figure 2**), that was previously described[1].

Plotting correlations between these various measures (**Figure 3**) supported our view that canker length is far more variable and difficult to use than either days-to-wilt or survival percentage. It also suggests that scoring captures aspects of resistance that we cannot detect by measuring whether and when the seedlings wilt. Note that in **Figure 3**, two points lie below the trend line. These are the Chinese and F_1 controls. Measuring by survival percentage (or by days-to-wilt) would lead us to think that several hybrid seedlots outperformed F_1s. Measuring by score, however, shows F_1s second only to Chinese chestnut, a result which far more consistent with our observation that all of the F_1s still alive at the time of measurement had superficial, healing cankers, while cankers on most hybrids were still expanding.

In the spring of 2021, the MD-TACF will do field inoculations of the first SSA seedlings we produced, which were planted in our seed orchards in 2017. By the end of 2021, we should have our first indication of whether and how well SSA results correlate with standard inoculation assay results on the same trees. TACF will get more results from similar tests from other chapters over the next several years. Until we see evidence of a good correlation, we will not be able to say confidently that SSAs are truly effective for screening. We do know that we can see significantly different results between Chinese, F_1 and American controls, but whether SSAs are sensitive enough to choose the best seedlings within a moderately resistant population, and whether they can be used to draw conclusions about mother trees through SSA testing of their progeny still remains to be confirmed. The chapter looks forward to working with other parts of the TACF community to answer these questions.

Figure 3



Comparison of the chapter's 2020 SSA data using three forms of measurement. LEFT: Canker length is the poorest measure, correlating poorly with days-to-wilt (shown) and survival percentage (not shown) data for the same seedlots. MIDDLE: There is a strong correlation between days-to-wilt and survival percentage for the same seedlots. Analysis from other SSA experiments by TACF (not shown) suggests that survival percentage is a more repeatable measure. RIGHT: Canker scores correlate well with survival percentage, except for F_1 and Chinese controls. The red circle represents hybrid lines that outperformed F_1 controls by survival percentage, while the scoring system set F_1s and Chinese apart from and superior to all the hybrids, suggesting that scoring captures aspects of canker development that mere length of survival does not.

REFERENCE

[1]Saielli, Tom, and Levine, Bruce, Small Stem Assays may be a Reliable Screening Tool for Testing American Chestnut Resistance to *Cryphonectria Parasitica*, *Chestnut*, Issue 1 Vol. 33, Winter 2019, pp. 24-26

Chemical Fingerprinting:

A POTENTIAL TOOL FOR IDENTIFYING DISEASE RESISTANT CHESTNUT TREES

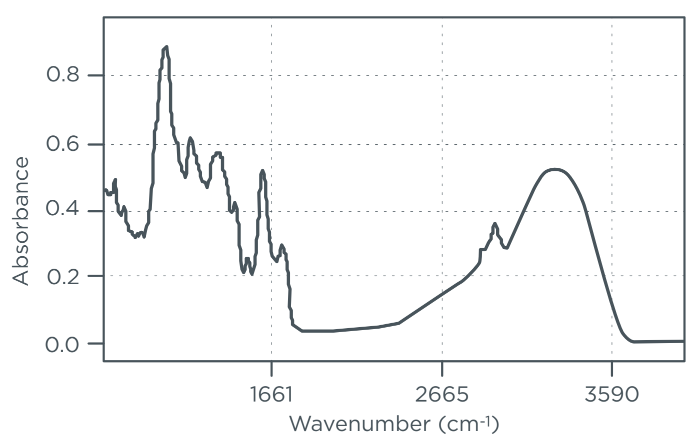
By Anna O. Conrad, Research Plant Pathologist, U.S. Forest Service, Northern Research Station, Hardwood Tree Improvement and Regeneration Center

Resistant trees are one important strategy for managing diseases, such as chestnut blight and Phytophthora root rot (PRR). However, waiting for disease symptoms to develop in order to identify resistant trees can be a lengthy process. So, alternative, more rapid approaches for identifying disease resistant trees are needed.

One such approach is chemical fingerprinting. Chemical fingerprinting provides a snapshot of the chemical composition of a plant tissue or extract at a given time. Infrared (IR) spectroscopy is one method of chemical fingerprinting. IR spectroscopy measures changes in the absorption of IR light by different chemicals over specific windows of the electromagnetic spectrum (e.g. the mid-IR spectrum runs from 400 - 4000 cm^{-1} or 25000 - 2500 nm). When analyzing complex plant samples like extracts from chestnut trees, individual or specific chemicals present within a sample cannot be identified. What this approach can do is pick up on differences in the types of chemicals and their concentrations. By measuring how different chemical groups respond after being hit with IR light, a unique spectrum or chemical fingerprint for each sample is generated (**Figure 1**).

Chemical fingerprinting may be useful for identifying disease-resistant trees, because plant-produced chemicals are known to be important components of how plants defend themselves against pathogens. Moreover, genetics and environmental factors can impact the levels of chemicals present within a tree. The levels and types of chemicals can change over time, including in response to pathogen infection. So, chemical fingerprint data can be combined with disease phenotype data (e.g. whether a tree is resistant or susceptible) to develop models for predicting if a tree is likely to be resistant or susceptible based on its chemical fingerprint.

Figure 1

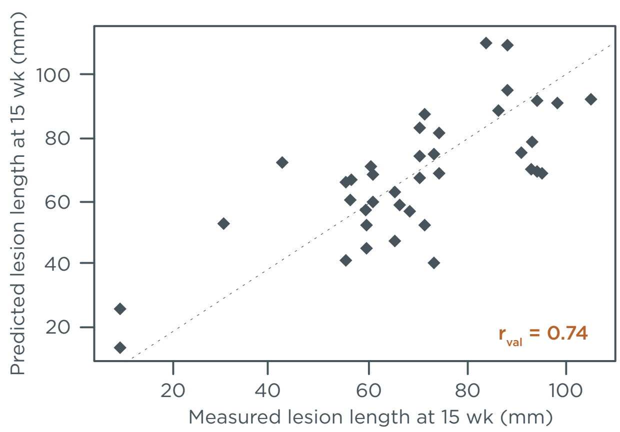


A representative chestnut chemical fingerprint from 700 – 4000 cm^{-1}.

Evaluating chemical fingerprinting as a tool to screen hybrid chestnut for disease resistance

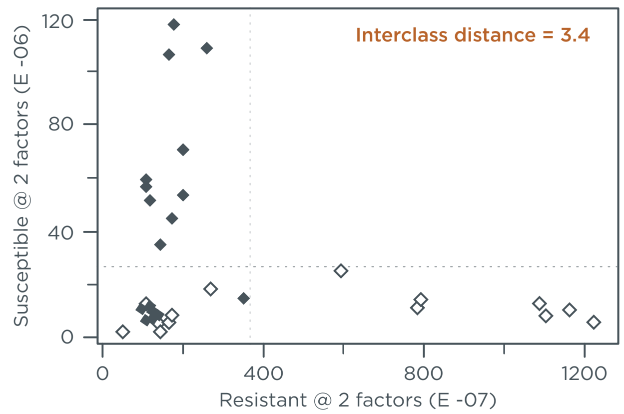
With funding from The American Chestnut Foundation (TACF), the use of chemical fingerprinting for screening hybrid chestnut for disease resistance and susceptibility was evaluated. Albert Abbott (University of Kentucky), C. Dana Nelson (U.S. Forest Service), Pierluigi (Enrico) Bonello (Ohio State University), and Luis Rodriguez-Saona (Ohio State University) were co-principal investigators on this project. To test this approach, non-infected stem tissue from BC_3F_2 and BC_3F_3 hybrid families which had been traditionally screened for resistance to blight and/or PRR were analyzed. Tissue and phenotypic data were provided by Jared Westbrook (TACF), Tetyana Zhebentyayeva (The Pennsylvania State University), and Stephen Jeffers (Clemson University).

Figure 2



Correlation plot from 7-factor PLSR model showing the relationship between predicted and measured blight lesion lengths for 'Clapper' BC_3F_3 hybrids (N = 40 excluding samples removed based on preliminary analysis). rval: correlation coefficient of cross validation. The closer rval is to one the stronger the correlation is between predicted and measured values.

Figure 3



Two factor SIMCA can distinguish between samples from PRR-resistant (closed) and susceptible (open) trees (N = 31 excluding samples removed based on preliminary analysis). The interclass distance between resistant and susceptible trees is 3.4 (the higher the value the less likely the model is to classify samples as both resistant and susceptible).

To collect chemical fingerprints, two undergraduate researchers at Ohio State University, Lauren Schnitkey and Caleb Mathias, finely ground stem tissue and extracted it with methanol. Then they concentrated extracts and analyzed them using a Fourier-transform infrared (FT-IR) spectrometer over the range of 700 – 4000 cm^{-1}. Chemometrics (statistical analysis of chemical data) was performed to evaluate whether chemical fingerprints can be used to predict hybrid chestnut susceptibility to blight or PRR. Two different statistical methods were tested: soft independent modeling of class analogy (SIMCA) and partial least squares regression (PLSR). SIMCA develops models for classifying samples into different groups (e.g. resistant or susceptible), while PLSR allows for the prediction of quantitative traits, like lesion length, another measure of susceptibility.

Initially, chemical fingerprints from different resistance sources – 'Clapper' and 'Graves' – were grouped together for statistical analysis. However, preliminary tests revealed that the accuracy of chemical fingerprint-based predictions improved when samples from different sources were analyzed separately. For 'Clapper' derived BC_3F_3 hybrids, two spectral regions from 1072 – 1618 and 744 – 1001 cm^{-1} were useful for predicting variation in the length of blight lesions. Moreover, there was a strong positive correlation between measured lesion lengths and predicted lesion lengths based on chemical fingerprint data (**Figure 2**). Whereas for 'Graves' BC_3F_2 hybrids, the region from 1001 – 1029 cm^{-1} was important for discriminating between hybrids classified as resistant or susceptible to PRR (**Figure 3**).

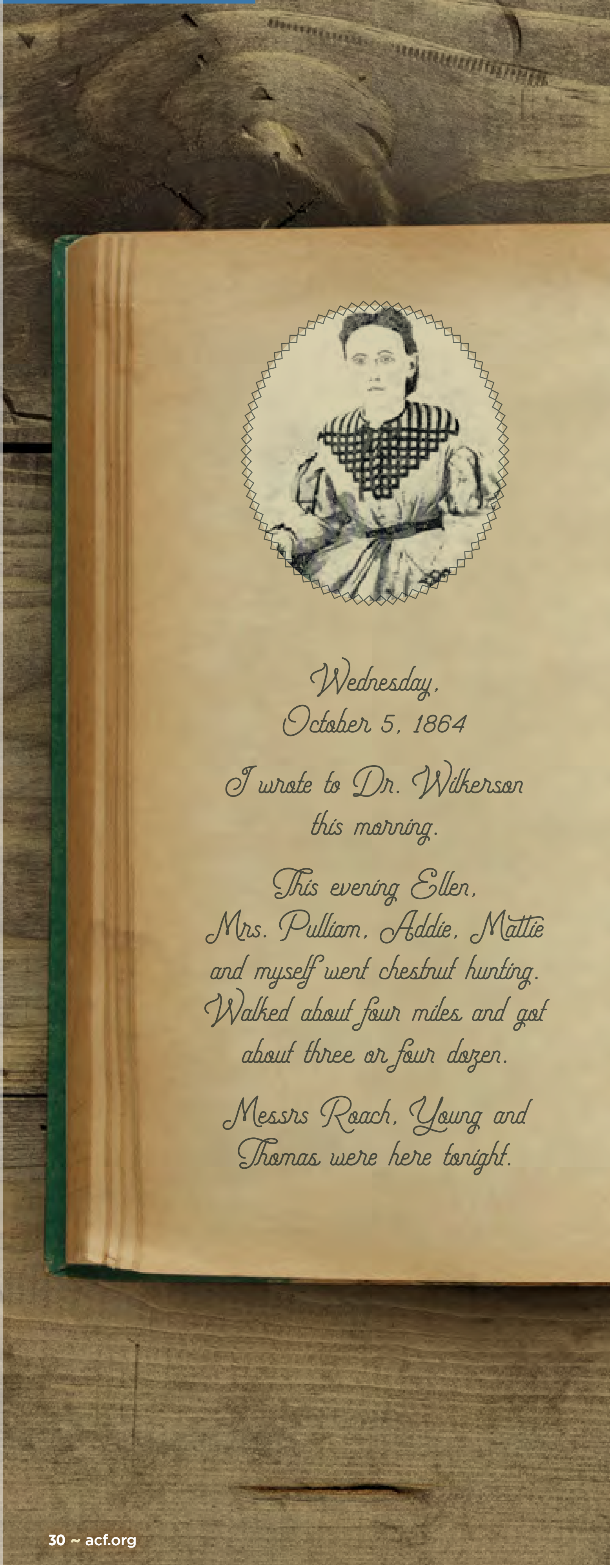
Figure 4

Field collection of a chemical fingerprint directly from an intact leaf using a handheld near-IR spectrometer. Photo by Enrico Bonello.

While these results are encouraging, further testing and validation are needed before the method is ready to be deployed as a tool for reliable identification of susceptible or resistant hybrid chestnuts. The accuracy of chemical fingerprint-based predictions may also be improved by using other types of predictive modeling, such as machine learning. Furthermore, handheld spectrometers are available and may be useful not only for identifying disease resistant trees but also for identifying diseased plants. In the latter case, near-IR spectroscopy shows great promise, as it is relatively non-invasive, requires minimal to no sample preparation, and chemical fingerprints can be collected in a matter of seconds (**Figure 4**).

For greater detail on these experiments, search for Anna's report from her 2015-2016 External Grant funded by TACF, available here: acf.org/resources/external-grants/

The use of trade names is for the information and convenience of the reader and does not imply official endorsement or approval by the USDA or the U.S. Forest Service of any product to the exclusion of others that may be suitable.



The Diary of Hattie Fraser, 1864

By David Smith
TN-TACF Chapter

I always read with great interest stories in *Chestnut* magazine about chestnut hunting in the olden days. Eight years ago, while conducting research in the history room of my local library, I discovered a brief documented report of chestnut hunting in the extreme southwestern portion of the tree's native range.

It comes from the diary of Harriet Elizabeth "Hattie" Fraser of Somerville, TN. She was a young unmarried woman, 23 years of age at the time she wrote this relevant entry in October 1864. She lived in Somerville with her parents and several kinfolk. Hattie kept the diary throughout the Civil War years and made several entries about gathering everything the woods could provide, including hickory nuts, walnuts, muscadines, and persimmons.

Hattie later married, lived in Memphis, and died in 1911. While her chestnut entry is brief, it documents the prominence of chestnuts around Somerville prior to chestnut blight. There are surviving stump sprouts and saplings still in the vicinity today. It's possible Hattie and her family may have even visited the parent trees of these now stump sprouts to gather their nuts.

Photo by Freepik.com

Roasted Chestnuts and Brussels Sprouts

By Jules Smith, TACF Media and Communications Manager
Recipe from Food Network

I love a quiet snow fall. I love breathing in cold air through my nose. I love winter. Over the years, I have learned that being a lover of frigid weather is not very popular, which made me ponder. Maybe my adoration of this season goes beyond the weather and it has just as much to do with the food I consume during colder months? For example, I savor root vegetables, squashes and, yes, Brussels sprouts. Of course, I also enjoy chestnuts, and recently learned that combining these two cool weather delicacies is a flavor sensation! It is a simple recipe but takes time if you choose to roast the chestnuts. For me, it was worth the experience. I hope you enjoy this dish as much as I do.

Ingredients

2 pounds chestnuts
1 $^{1}/_{2}$ pounds Brussels sprouts, trimmed
4 tablespoons unsalted butter
2 tablespoons minced shallots
$^{1}/_{4}$ cup minced fresh rosemary and/or other herbs
Salt and freshly ground pepper to taste

Method

Preheat oven to 400 degrees.

With a sharp knife, score each chestnut 1/4-inch deep all around. Arrange them in one layer in a baking pan and roast for 20 minutes, or until shells have just opened. Peel off both layers of skin with a knife while the nuts are still warm. In a large saucepan of boiling, salted water, cook the Brussels sprouts for 12 to 15 minutes, or until tender. Drain and refresh with cold water. Then thinly slice the cooled Brussels sprouts.

In a large skillet set over moderately high heat, melt the butter. Add shallots and cook, stirring, two minutes. Add the chestnuts, Brussels sprouts, herbs, and salt and pepper. Sauté for two to three minutes, or until heated through.

REMEMBERING CATHY MAYES

By Lisa Thomson, President & CEO

Cathy Mayes was a fighter…for nature, for justice and for those she loved. For years, she fought against the most daunting odds of an insidious disease and finally succumbed to it on October 30, 2020 with her devoted husband Randy by her side. Her grace and determination during her illness were an inspiration to all of us. “Cathy and I became good friends when we served together as board members of TACF. She always gave us wise professional advice. Later I learned she was also a talented pianist, and we often talked about pieces we were working on together. It was a gift to share that aspect of her life,” said Science and Technology chair and Executive Committee member Steve Barilovits.

Cathy was the President of the Virginia Chapter for 10 years, and engaged hundreds of new supporters and important plantings at prominent Virginia landmarks. She was a national leader as well, serving on the board of directors as Audit, Governance and Promotion and Outreach chairs, as well as Secretary. A retired attorney, she also served on the Executive Committee, where she and general counsel Donald Willeke engaged in many lively debates on behalf of the organization. “She was a steady rock of quiet, unassuming competence and I admired her greatly,” said TACF’s senior science advisor Kim Steiner, who served on the Executive Committee with Cathy for more than 10 years.

There are not enough words to fully describe Cathy’s immense value to TACF. To many of us she was a dear friend, tireless worker and wise mentor. She personally took me under her wing early in my tenure, introducing me to many important contacts for TACF. She was the master event planner behind some of the organization’s most festive gatherings and galas.

When Cathy stepped down from the board at the 2019 annual meeting at Gettysburg, she was given a rousing send-off by Don and a research orchard at the Smithsonian Biological Conservation Institute was dedicated in her honor. She will be sorely missed by so many in her wide and diverse circle of friends and family, and never forgotten.

IN HONOR
OF OUR TACF MEMBERS
July 30, 2020 – December 14, 2020

Sir David Attenborough
From:
Karen Osgood

Mike Aucott
From:
Karen E. Linder

Sarah C. Baldwin
From:
Steven B. Bloomfield

Douglas A. Batt
From:
John Batt

Ramona Beshear
From:
Mary Jane Owen

Michael A. Coleman
From:
Susan Coleman

In honor of our Earth Mother
From:
Caryn MacLuan

Davis Ferguson
From:
Meg Ferguson

Henry French
From:
Cary Gramsch

Kaylen Fry
From:
Roy M. Hall

Peter Gale
From:
Allison Gale

Keith Gilland
From:
Ryan Benjamin Homsher

Jaime Grinch
From:
Rob P. Grinch

Samuel I’on Hagerman
From:
Kara S. Moore

Hillary Hendryk
From:
Karen Hendryk

Buz Holland
From:
Anonymous

Raymond T. Hollenbaugh
From:
Holly Fogle

Laurel Hoskins
From:
Bonnie C. Hoskins

Ingrid Johnson
From:
Deborah Nelson

Karen and Jonathan Kays
From:
Laurel Kays

Ron Levine
From:
Kathryn Rorke

Rex Mann
From:
Susan and Gerard Cormier

John Marsala
From:
John and Patti Trotsky

Catherine Mayes
From:
Barbara Bodson
Elizabeth and Mark Merz
John P. Moliere

Ray Partin
From:
Gary Partin

Brad Price
From:
Glenda Price

Ian Runnels
From:
Joseph Runnels

Chris Schlegel, David Schlegel, Eric Schlegel, Jane Schlegel, and William Schlegel
From:
Howard J. Schlegel

Russell Schmidt
From:
Cynthia Schmidt

Mr. Douglas A. Scott
From:
Thomas J. O’Connell III

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

IN MEMORY
OF OUR TACF MEMBERS
July 30, 2020 – December 14, 2020

Akiva Silver
From:
Barbara Silver

Jules Smith
From:
Jennie Smith-Pariola

Jo Han Soon
From:
Kyu Jin Im

Charles Spurgeon, MD
From:
Pamela and Dallas Peak

Marjorie Stansel
From:
Dorothy B. Padgett

Walker and A.J. Stone
From:
Steven C. Stone

Pete Sutherland
From:
Matthew Peter Sutherland

Robert Sypolt
From:
Mark and Mindy Double

TACF's Tennessee Chapter
From:
Rob and Liz Waggener

Lisa Thomson
From:
Guilford Garden Club

Walter M. Thomson
From:
Alyce T. Fritz

Barry Towers
From:
Frederick J. Heagy

Ottis G. Turner
From:
John W. Turner

Phyllis Vance
From:
Martha Vaughan

Tatum, Jack, and Sierra Walker
From:
Dianne M. Walker

Sue Woodson
From:
Bonnie C. Hoskins

Adolph Zlabis
From:
John and Patti Trotsky

West Abrashkin
From:
Diane Abrashkin

All American Veterans
From:
Jeanine Jacoby

Randy Anderson
From:
Susan Stuebing Anderson

David M. Anderson
From:
Ruth Anderson

Dave Armstrong
From:
Mary Ayres and Jack Kerr

Mr. and Mrs. Russell D. Aylor
From:
Delmer D. Aylor, C.F.

Michael Babyak, Jr.
From:
Michael Babyak, Jr. II

Carlene T. Blankenship
From:
Delia and John Olson

James Ely Bradfield
From:
John G. and Amy Bradfield

Allen G. Brailey, MD
From:
Rachel Gundersen

John Boyers Breinig
From:
Mary L. Breinig

I. D'Arcy Brent II
From:
Duffy Brent III

Charles Briening
From:
Mary Delurey

George Brooks
From:
Marian Post and Paul Eisenhauer

Fitzhugh L. Brown
From:
Mary Florence Brown

Charlotte Brunson
From:
Danny and Cathy Brunson

Essie Burnworth
From:
Victoria H. Jaycox

James Edward Craddock
From:
Thelma S. Fenster

Emilie Crown
From:
Ronald Kuipers

Dr. Jay F. Davidson
From:
Frank W. Davidson

Jim Dionis
From:
Deb and Michel Ridgeway

Julius Dziak
From:
Michelle Dziak

Sheila Norbury Grainger Edee
From:
Darel Preble

Alexander Federowicz
From:
Yvonne M. Federowicz

Jay A. Felty
From:
Dana and Joyce Felty

Ralph M. Forbes
From:
Dorothy F. Morse

Augustine P. Gallo
From:
Richard Gallo

Lauren Catuzzi Grandcolas
From:
Larry and Barbara Catuzzi

Alan Hagstrom
From:
Alan Kyle Hagstrom

John and Bernice Hoffman
From:
Steve and Catherine Palmateer

Patricia Howell
From:
Hugh B. Howell

Franz Hugel
From:
Antone Hugel

Robert Keeley
From:
David Glick

Jack Kindschy
From:
Corrine Kindschy

Vince and Sidonia LaMonica
From:
John LaMonica

Ben M. Laursen
From:
John Laursen

Lowell Edwin Lingo
From:
Deborah W. Loverd

Deb Lord
From:
Dianne M. Walker

Lloyd Lupfer
From:
Daphne Neveras-Lupfer

Cornelius A. Mahoney
From:
Daniel A. Mahoney

Charles Martin
From:
Cindy Westley

Catherine Mayes
From:
Phillip Brown
Richard and Candace Faulk
Laurie Flynn
Richard Johnson
Old Rag Virginia
Lisa and Walt Thomson
Master Naturalist
Michael and Susan Wilson
Lisa and Walter Thomson

Polly Wyan Moore
From:
William R. Moore

Grant and Barbara Mortenson
From:
Kevin Mortenson

Reverend James Mussman
From:
Dr. Sarah Ruden

William S. Payne
From:
Jacqueline Payne

Rebecca Pernice
From:
Ralph L. Pernice

Dennis L. Perry
From:
Hoosick Falls Kiwanis Club

Jim Reed
From:
Robert A. Berger

Cindy Eaves Reynolds
From:
Larry G. Johnson

Jerry Sawma
From:
Christine Pinney
Maggy Sawma

Herman Schoeb
From:
Maureen Binetti

Karen Simicsak
From:
Carol Nancy Geck

Choi Hak Song
From:
Kyu Jin Im

Alice Sweeney
From:
Lawrence Lee

Walter G. Thomson
From:
Alyce T. Fritz

Barrett D. Transue
From:
Elise H. Transue

Bernie Turanchik
From:
Joan Campbell

Rebecca Harlow Watkins
From:
Wayne H. Harlow

Jaqueline White
From:
Michael Hall

Cleo M. Williams
From:
Cynthia Latty
Maudine B. Williams

Larry Wright
From:
John H. Russell

Willis B. Wright
From:
William Visnic

Catherine Yandell
From:
Hope Yandell

THE AMERICAN CHESTNUT FOUNDATION™
50 N. Merrimon Avenue
Suite 115
Asheville, NC 28804

TACF'S WILD-TYPE AMERICAN PROGRAM

Beginning this March, The American Chestnut Foundation will once again be selling wild-type American chestnut bareroot seedlings in bundles of 5, 10, 25, and 50.

TACF members will receive an email Sunday, March 7 to give advance notice of the sale, including a link to the order form, which will be live March 9. Those without email may place their order by calling the national office at (828) 281-0047. The sale will open to the public on Monday, March 22 (while supplies last).

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

This is a very popular program and the seedlings sell out quickly. Due to the limited supply this year and high demand, customers are restricted to a max order of 50 seedlings. Distribution range is only available to states east of the Mississippi (no exceptions). Orders will be mailed by mid-April.

PRICING FOR WILD-TYPE AMERICAN SEEDLINGS:

Only sold in quantities of 5, 10, 25, 50 – includes shipping

5 seedlings – $35.00	10 seedlings – $65.00
25 seedlings – $150.00	50 seedlings – $250.00

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