Chestnut of the American Chestnut Foundation

A BENEFIT TO MEMBERS

> THE AMERICAN CHESTNUT

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

PRESIDENT'S MESSAGE from Lisa Thomson

NEWS FROM TACF Mined Land Reforestation Ed Hutchison Jules Smith Welcome Charity of the Week

VOLUNTEER SPOTLIGHT Brad Stanback

12

CHAPTER HIGHLIGHTS Date of Emergence Nut Production Orchards Deer Ticks & Lyme Disease Dixmont Community Forest Seed, Soil, Water

18

EDUCATION Future Generations & A Reforestation Project Undergraduate Education Chestnut Connection 27 SCIENCE Merging Genomics and Biotechnology OxO Blight Resistance

36 PIONEERS Rufin Van Bossuyt 39 REFLECTIONS By Stan Messick

RECIPE Cranberry & Chestnut Gingerbread Scones

4(

IN MEMORY AND IN HONOR







Lisa Thomson President and CEO

DEAR CHESTNUT ENTHUSIASTS,

One of the most impressive characteristics of our members, volunteers and donors is their passionate loyalty and commitment. If I could describe the collective "personality" of The American Chestnut Foundation, I'd use descriptors like scrappy, determined, and patient.

Science *requires* patience. We are asking big, complicated questions to save a species once thought lost, and solving such complex problems can be a long, slow slog of trial and error, wins and disappointments. Our citizen scientists, board of directors, and science staff have been working tirelessly since 1983 to seek the best path forward. In those 33 years, we have seen vast changes in technology, especially in the field of genomics. Thanks to efforts within the organization to embrace change, we are implementing new methods and innovations to further enhance the backcross breeding program conceived by visionary Dr. Charles Burnham, and carried out by Dr. Fred Hebard for more than 2 decades. To document this journey, the history and state of our breeding program was recently chronicled in a manuscript which was lead authored by TACF Chief Science Advisor Dr. Kim Steiner.

Currently, the staff science leadership team of Dr. Jared Westbrook and Ms. Sara Fitzsimmons are tackling breeding innovations through their collaborations and analyses. Along with our citizen scientists, Meadowview staff and regional science coordinators, we continue to plant and cull in more than 500 breeding and seed orchards from Maine to Alabama. We have an enormous cache of data with which to analyze and make educated decisions about the "best of the best" progeny of these field experiments, all while ensuring we have captured genetic diversity that is essential for the American chestnut to flourish in forest environments.

Concurrently, a team of committed scientists is participating in an unprecedented effort to collaborate using the best tools we have to recover the American chestnut. Encouraged by Board Chairman Mr. Michael Doochin, and chaired by Dr. William Powell at SUNY's College of Environmental Science and Forestry, the "3BUR" Committee (Breeding, Biotech and Biocontrol United for Restoration) is proposing new ways for chestnut researchers to work more closely across organizational boundaries. Along with the 3BUR Committee, the Strategic Planning Committee has made terrific progress with a team of hardworking volunteer leaders to propose overall goals and actions in our four areas of focus: science, restoration, fundraising and marketing, and organizational advancement. I am deeply grateful for everyone's commitment to move our mission forward through the next pivotal decade, and I will be reporting on their recommendations in the near future.

Despite the fact that we are working with one of the fastest growing hardwoods known, TACF's mission will not be achieved overnight. Our mission will span 5, 10 and even 25+ years, but we are patient, and we will achieve success, one planting at a time. Thank you for everything you do as part of this grand experiment, one which future generations will look back and say, "Well done, American Chestnut Foundation. Well done!"

Wishing you all a bountiful harvest season,

Lisa Thomson, President and CEO The American Chestnut Foundation



Follow me on Twitter (@MadameChestnut).

American Chestnut Bur

"Brandon's Favorite Tree" by Chris Dover. This picture of an open bur was taken at Wayah Bald, NC at the base of a 62" circumference American chestnut tree.





WHAT WE DO

The mission of The American Chestnut Foundation is to restore the American chestnut tree to our eastern woodlands to benefit our environment, our wildlife, and our society.

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TACF's Mined Land Reforestation Efforts

CONTINUE IN 2016

By Michael French

Volunteers from Sierra Club, Boy Scouts of America, and Berea College helped reforest the Daniel Boone National Forest project on April 2.

In 2008, TACF and the U.S. Department of the Interior's Office of Surface Mining Reclamation and Enforcement signed a Memorandum of Understanding to promote the use of improved mined land reforestation practices developed by the Appalachian Regional Reforestation Initiative (ARRI), known as the Forestry Reclamation Approach (FRA) and to promote chestnut restoration by using mined lands as locations for chestnut re-introduction. Since then, TACF has worked in cooperation with ARRI and later, Green Forests Work (GFW) and numerous other partners to conduct mined land reforestation projects across eight states. Since 2009, this partnership has led to the planting of more than 1.8 million trees on nearly 3,000 acres using both volunteer and professional tree planters. NEWS FROM TACF



A Restoration Chestnut leafs out on the Daniel Boone National Forest a ew weeks after planting.

A view of the site after clearing and ripping.

A D-6 bulldozer pushes autumn olive into piles for wildlife after the herbicide had killed the unwanted vegetation.

The spring of 2016 was another successful year for this partnership. In 2016, ARRI, GFW, TACF and our other partners hosted or participated in 42 tree planting events, which led to the reforestation of 386 acres and resulted in the planting of more than 239,000 seedlings. Although all of these projects were mixed hardwood plantings that included native high-value hardwoods and wildlife shrubs and trees, many of the reforestation projects had a chestnut component. Additionally, as has been done in previous years, partners conducted extensive outreach and engaged a diverse array of volunteer groups. Before the planting events, project organizers taught volunteers about American chestnut's history, ecology, importance, and restoration. Volunteers also learned about proper mined land reclamation for reforestation, how forests benefit society, other threats that our forests are facing, as well as how to properly plant tree seedlings. This past spring, ARRI, GFW, TACF engaged 2,140 volunteers. 1.586 of which were under the age of 24.

One project highlight of this planting season occurred in Laurel County, Kentucky on the Daniel Boone National Forest. The property was acquired by the US Forest Service after the reclamation had been completed, but the land was returned to hay/pastureland as the post mining land use and was not being maintained by grazing or harvesting hay. The 29-acre area created an opening in what was otherwise a largely contiguous forest. As such, the area was quickly becoming colonized by autumn olive, an invasive exotic species. ARRI, GFW, and TACF staff worked with USFS staff to develop a plan for reforesting the area back to the forest type that was present prior to mining. Herbicide was prohibited, so the autumn olive, fescue, sericea lespedeza, and other vegetation was pushed to the periphery to expose the soil. The ground was then ripped with a large bulldozer to loosen the compacted soil. After more than 100 volunteers and several professionals were contracted, more than 20,000 seedlings had been planted, including nearly 300 Restoration Chestnuts.

TACF was proud to partner with the National Park Service for the fifth consecutive year to assist with their reforestation efforts at the Flight 93 National Memorial. Thanks in part to funding from the R.K. Mellon Foundation, this year, more than 350 volunteers planted 16,000 seedlings on 23 acres in two days. During the planting events, 1,500 Restoration Chestnuts were planted along with pines, oaks, maples, and other native seedlings. Every volunteer had the opportunity to plant a chestnut seedling.

Another highlight was a project done in cooperation with the US Forest Service – State and Private Forestry as part of the Appalachian Forest Renewal Initiative that is occurring in multiple states. In a collaborative effort between ARRI, GFW, TACF, private donors, and the Ohio Department of Natural Resources – Division of Wildlife, a total of 86 acres were reforested with more than 60,000 seedlings, including 500 Restoration Chestnuts. Approximately thirty of the eighty-six acre area had become overrun with autumn olive. The shrubs were killed through a helicopter application of herbicides and the brush was then pushed into piles for wildlife habitat. The grasses in the remaining area were also sprayed before the area was ripped and planted with seedlings.

ARRI, GFW, and TACF would like to thank all of our volunteers, donors, and partners who made these projects successful. Without their support, this work would not have been possible.

A Passion That Lives

ON THROUGH MULTIPLE GENERATIONS

By Emily Nowels



Ed Hutchison turns 93 in September. He spent his childhood in Pennsylvania before moving to the West Virginia mountains. At 17 years old, Ed joined the Navy to fight in WWII. There he served as an LCD landing craft operator and helped to land American troops on the beaches of Normandy. When he returned home after the war, he married and raised five children in the town of Elkins, West Virginia. Now on any given day, you might find Ed tending to his bees along the North Carolina coast, or perhaps vacationing in the Appalachian Mountains with his son, far away from busy roads, internet and cell phones.

E d is a true outdoorsman, and has spent much of his life dedicated to preserving and exploring the natural world. He has witnessed several environmental tragedies in his lifetime, not the least of which was the destruction caused by the American chestnut blight. Ed is able to remember a time when the tree once dominated the Pennsylvania mountains and served as an important part of animals' and people's diets.

"I was just a youngster-I was maybe 8 years old, when I would gather chestnuts off of the forest floor with my dad," Ed said. "I remember eating them raw and cooking them up at home. I don't remember all we did with them, but I know they were good. They were really good." He was still quite young when the blight spread through the forest. The disease eventually rendered the chestnut tree, nicknamed the "redwood of the East," functionally extinct and resulted in a marked change in the appearance of the forest.

"Before the blight hit, the trees were prevalent in the forest, so people didn't think anything of them. But when they started dying they realized how important it was," Ed said. "The chestnut tree was a mighty big tree.... It made a big difference in the Pennsylvania forests. There on the hills where the chestnut trees grew there was no vegetation left. They were all gone, and it was over and done with just like that." After the war when Ed was starting a family and living in West Virginia, he became friends with a man by the name of Mr. Oliver, who had a similar appreciation for nature. The two men did all they could to save the tree. They would smear tar on the cankers of infected chestnuts with the hopes of stopping the blight from spreading, and distributed chestnut seedlings to anyone who would plant them. They also tried grafting American chestnuts with the blight resistant Chinese chestnut. Six of those trees still stand today in a field in Elkins, WV.

Ed's nephew Steve Antoline, a member of The American Chestnut Foundation, recognized his uncle's early attempts to save the chestnut tree by sponsoring a legacy tree



at the TACF Meadowview Farms in Ed's honor. This past April, Ed was also able to attend a chestnut restoration planting at Antoline's farm in southern West Virginia, where a roughly one-acre site has been cleared to support the planting of 100 American chestnut seedlings.

Ed says his passion for the chestnut tree is simply a natural outcome of his lifestyle. "Well I liked the outdoors to begin with, and I just got interested," he said. However, his son David explained that his father often spoke of how important the chestnut tree was in regards to one of Ed's other passions-beekeeping.

"He's had honey bees for 50 or 60 years and (the tree) was one of the best blooms for the honey bees to gather pollen off," David said. It was a very important tree for the bees." The honey that the bees made from the flowers of the American chestnut tree was dark, rich and slow to crystallize.

Ed's effort to save the chestnut tree is only one small piece of the work he does to save the environment. He is also a founding member of his local chapter of the Izaak Walton League, an organization dedicated to conserving and restoring natural resources. He and Mr. Oliver, who were both advocates for the conservation of American songbirds, would build bird boxes for safe nesting on the Izaak Walton property. Ed said that he has built over a thousand bird boxes in his lifetime.

Ed's five children often helped their father and Mr. Oliver in their restoration and preservation efforts. His daughter, Nancy Fahey said, "When we were young [Mr. Oliver] would come over on Saturday mornings, and my little brother and I would help him go around and get the little birds out of the nests and hold them why he would band them and identify them. I love birds to this day. I'm still an avid bird watcher, bird enthusiast...I have bluebirds that nest here in my yard."

Ed's environmentalism inspired each of his children in different ways. Fahey continues her father's passion for birds by volunteering with the North Carolina Audubon Society. She also serves as the director and permit holder for the Sea Turtle Project at Wrightsville Beach.

"I do attribute my interest - my avid devotion to conservation and environmental preservation to all the many things I was taught and exposed to as a kid growing up in the mountains with my dad and Mr. Oliver. And even though the boys were maybe more involved..., I think just having the exposure to those kinds of passions and interests, it spurred my own passion for the very same kind of thing. It led me to get involved in issues that are specific here to the coast of North Carolina."

David feels very much the same about the impact his father has had on his life. "He's been an outdoorsman his whole life and he's taught us the same values," David said. "My brother is a forester and works in the woods everyday; I'm in the hardwood business. Both of those passions have evolved from what he did while we were growing up and all that he taught us."

The Gift of Membership

LOOKING FOR GIFT IDEAS FOR THOSE HARD-TO-PLEASE PEOPLE, THOSE RELATIVES WHO CAN NEVER TELL YOU WHAT THEY WANT, OR THOSE FOLKS WHO SEEM TO HAVE IT ALL? THIS HOLIDAY SEASON, CONSIDER AN ALTERNATIVE GIFT THAT GIVES BACK – A GIFT MEMBERSHIP TO TACF!



"I like to gift TACF memberships to family members as a symbolic toast to shared environmental values. I think that these values have the potential to cross generations and become firmly rooted in the cultures of future societies. Bringing back the American chestnut is a success story poised to be lived. I want my family to help live that story. And to any of my nieces or nephews who are reading this and rolling their eyes, remember, it's better than a sweater!" -Penny Firth



"Even though I'm very involved with TACF, I wanted to also help educate my family about the work there, eager to inspire them to learn more about the American chestnut. Gifting them with a TACF membership made the most sense because it benefits TACF and my state chapter (Alabama), all while providing a learning opportunity for family members, hoping that in turn they'll share their commitment to TACF with others, causing a cascading benefit." ~Jack Agricola



"I got involved with TACF a number of years ago when I stumbled upon an American chestnut during an autumn walk. TACF confirmed its identity and since then, I've been passionate about the tree and my desire to help educate others. Last year, I gifted a seed-level membership to my former school and assisted them in planting/growing four seedlings. It gives me great pleasure knowing that young people are learning about the importance of this majestic tree and its restoration back into our forests." ~Sally Quale

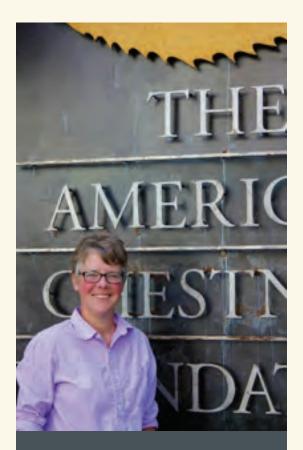
Members receive a year's subscription to our magazine, *Chestnut*, our monthly electronic newsletter, *eSprout*, a TACF car decal, membership in one of our state chapters, opportunities to participate in local breeding and research activities, and more.

There are many levels of giving to choose from and several ways to give. It's easy and safe to purchase a membership gift online by visiting our website (www.acf.org). Simply click on the "Membership and Support" menu to view all of the options available. Gift memberships are also available through the national office. Call 828-281-0047.

TACF depends primarily upon its members to support the research in the development of a blight-resistant tree, so every new member makes a difference. Giving the gift of a TACF membership not only benefits the financial need of the foundation, but helps to educate and potentially inspire others to learn about and get involved in the restoration of this mighty tree.

THANK YOU FOR SHARING YOUR LOVE OF OUR MISSION WITH OTHERS!

TACF WELCOMES Jules Smith EDITOR AND SOCIAL MEDIA SPECIALIST



"It thrills me to be working for an organization with this kind of longevity and tireless commitment to restoring the American chestnut."

Chestnut: What initially attracted you to TACF?

Jules: I have always cared deeply about environmental conservation and the impact each of us have in regards to the well-being of this planet. When I learned that The American Chestnut Foundation was founded in 1983 and has been working diligently ever since to restore the American chestnut tree into its native eastern forests, I knew I wanted to be a part of that kind of dedication and commitment.

Chestnut: What is your role at TACF? What are your duties?

Jules: As TACF's editor and social media specialist, I am responsible for maintaining and updating our social media platforms such as Facebook, Twitter, and YouTube. I also gather and edit content for our monthly electronic newsletter, *eSprout*, and our print journal, *Chestnut*. I have a background in television news & freelance videography and am excited about utilizing those skillsets to help further promote TACF's mission through videos to help tell our story.

Chestnut: Can you give us a quick bio? Where did you grow up and go to school?

Jules: I was born and raised in the hills of East Tennessee – Grainger County, a small farming community known for its homegrown tomatoes! Growing up, my siblings and I spent more time outside than inside, exploring the massive amount of woods that was our back yard, fishing in small ponds, and riding our bikes through the neighborhood. I received my Bachelor's degree in Communication Arts with an emphasis in Television Production from Carson-Newman University, a small liberal arts school in Jefferson City, TN. I have lived in Knoxville, TN, Birmingham, AL, and Cambridge, MA. I moved to Asheville in 2010 from Cambridge and am delighted to be living in this beautiful, progressive city.

Chestnut: What is your favorite part of working for TACF?

Jules: I was honored to be offered this position at TACF. It thrills me to be working for an organization with this kind of longevity and tireless commitment to restoring the American chestnut. It feels good to know that I am part of this vital effort in helping to promote and support such a worthy undertaking.

Chestnut: Any unique facts that you would like to share?

Jules: When I worked in television news I had some unique experiences and met a number of famous people. For example, I covered Al Gore's camp in Nashville on election night in 2000 – it was quite the adrenaline rush. Remember the "hanging chads?" It was very exciting to be involved in that historic election. My favorite famous person encounter was meeting Dolly Parton on several occasions. I was the "official" videographer of all news stories that involved Dollywood so I had the privilege of shooting and interviewing her about the theme park.

Chestnut: Favorite activity to do in your free time?

Jules: I love hiking, biking, gardening, and yard work. In fact, I enjoy most any outdoor activity. Being out in nature and digging my hands into the dirt is therapy for my mind, body, and soul.



the AMERICAN CHESTNUT FOUNDATION EARNS Charity of the Week

This summer, TACF was recently published in *THE WEEK* magazine as its "Charity of the Week." This is quite an honor as *THE WEEK* has a global readership in the hundreds of thousands. The magazine contains perspectives of the week's news and editorial commentary from global media to provide readers with multiple political viewpoints.

TACF member and woodworker, Gary Carver nominated the Foundation and the write-up was published in the June, 2016 issue. Gary has been an active member of TACF's Maryland chapter since 1999, and his chestnut carvings are available at the online store on our website. We would like to thank Gary for all the time, support, and talent he has given TACF over the years.

THE WEEK only publishes not-for-profit organizations that have a four-star rating with Charity Navigator, the nation's largest and most-utilized evaluator of charities. Four stars is its highest rating. It examines two broad areas of a charity's performance: Financial Health and Accountability & Transparency. TACF has had this rating with Charity Navigator for more than a decade, and we were honored to be recognized in *THE WEEK*!

National Boy Scout Jamboree

VOLUNTEERS NEEDED



JULY 19-28, 2017 GLEN JEAN, WV

TACF's West Virginia chapter is sponsoring a booth at the upcoming 2017 National Boy Scout Jamboree in Glen Jean, WV. Volunteers are needed to work the booth throughout the course of the event. Would you like to help? The chapter is designing the booth but they need help staffing it so they are appealing to TACF members nationwide to make this event a HUGE success!

If you are ready to volunteer, contact Sam Muncy at sam.muncy@msesinc.com.

DECADES LONG EXPERIMENTAL FOREST By Lisa Thomson

Brad Stanback has a long history with the chestnut tree and with The American Chestnut Foundation (TACF). He has always been an outdoor enthusiast, learning to hunt and fish as a teenager and taking up a special interest in wildlife. Later, after moving from the Piedmont to the mountains of Western North Carolina, Stanback's love of nature and natural history expanded to include the ecology of his mountain home.

CAROLINAS CHAPTER

"Just by living in the mountains, I started learning more about the local ecology and realized that the American chestnut was one of the biggest pieces of it. I think of it "He had the material and it was more of an early test of the breeding program. What we hoped to see in the B_2F_2 planting was what we could expect from the B_4F_2 seed

as the ecological equivalent of the bison on the Great Plains or the salmon in the Pacific Northwest. That's what the Appalachian ecosystem really needed was a key player like that, like the chestnut was. So I thought what a shame that it's gone and what could be done to get it back surely there must be something. After a couple of vears, I learned about TACF. The Foundation formed in 1983; I became a member in 1985, and I have been involved ever since." said Stanback.

On his off-the-grid farm in Western North Carolina, Brad has planted pure Americans, pure Chinese, and every hybrid ever bred by TACF, including the B_3F_3s , the latest generation tree.



"Just by living in the mountains, I started learning more about the local ecology and realized that the American chestnut was one of the biggest pieces of it." orchard that would be producing the $B_{z}F_{z}s$; the same levels of blight resistance and the same percentage of trees with Chineselevel resistance, except that the B_zF_as would be 15/16 American compared to the B₂F₂s which are 7/8 American. Out of about 200 nuts that were planted, we've got about 10 that have healed up any blight cankers. They still have live intact crowns growing straight and tall. It looks like an interesting test case of what our breeding program could produce at a more advanced level. I think there may be some genetic complexities that we weren't aware of in the early days. but this still is an interesting example of what can be done with breeding."

When asked about

"I started out planting every chestnut I could get my hands on, from commercial sources: Chinese or various hybrids that people made different claims about. I used to say I was invoking the powers of mongrelization for restoring the chestnut on my land."

In 1998 he was approached by Fred Hebard, retired chief scientist for TACF's breeding program, who had the idea of planting B_2F_2s on Stanback's farm. Meadowview Research Farms had produced many of these nuts, but they weren't part of the breeding program just yet.

the future of the American chestnut, Stanback had some encouraging words. "There are so many different ways that we are working on it now, from our breeding program to transgenic work and a few other things that can be tried. I think we'll get something back that will replace this lost component of the Appalachian ecosystem – doing what it needs to do, what the American chestnut once did."

Date of Emergence

AND MULTI-STEMS

By Bruce Levine, Maryland Chapter and TACF Board of Directors

The Maryland Chapter of the American Chestnut Foundation (MDTACF) planted a large number of B₂F₂ seeds at its Washington Suburban Sanitary Commission (WSSC) seed orchard this spring that never germinated. As a result, we had to replant many plots very late in the season (May and June) with seeds left over in storage.

Many of these seeds had long radicals, dead radicals, or were starting to rot in their bags. We were not surprised by the very low germination rate, which was about 20 percent, compared to over 90 percent for seeds planted in March-April, but another noticeable phenomenon, was that a significant number of these seedlings produced multiple stems.

Most chestnuts, especially American chestnuts and predominantly American hybrids send up a single plumule (the thing that turns into the stem and leaves) when they first break through the soil. We estimate that only about 5% of seedlings start with more than one stem. But a large number of the B₃F₂s trees that MDTACF planted late, or which emerged late in 2016, had multiple stems. The key factor seems to be when the seedlings emerge from the ground, not when they were planted.

Plot 36 in our WSSC seed orchard provided an excellent accidental experiment. In early June, a time when we would expect almost all viable seedlings to have emerged, we removed all the seedlings from plot 36 to transplant them elsewhere. We considered plot 36 empty. Over the next two months, however, 26 more trees came up. And of these, 13 (exactly 50%) had multiple stems. We observed the same multiplestem phenomenon in the trees we planted late in the season in pots.

We suspect that both the late emergence and the multiple stems arise from the nut's efforts to resprout after earlier roots and shoots have rotted. When the first radical that emerges from the nut dies, for example, we see that chestnuts don't just replace the radical, but instead grow multiple new roots.

The same thing appears to happen when plumules die. A single root can give rise to multiple plumules. Figure 3 is the same tree shown in figure 1. Three plumules have emerged from damaged but still living dominant radical, and these new stems tend to branch out into additional stems underground in a manner that is not typical of healthy chestnuts.

Does any of this matter? Perhaps not. In the case of multi-stemmed seedlings, one stem often comes to dominate, and the tree usually ends up with a single stem. However, it may matter in seed orchards where the seedlings are closely packed and in intense competition with each other. Here, time lost and energy expended growing multiple stems can set a tree back. As we enter the late part of the growing season, trees that emerged on schedule with single stems in our seed orchards are already poking out of their tubes, while their multi-stemmed competitors are much shorter.

If these multi-stemmed trees don't catch up, they may be at a great disadvantage to their siblings at inoculation time, regardless of which resistance genes they have inherited, and that could be a confounding variable in our tree selection process. Although our hypothesis is anecdotal, MDTACF plans to avoid having to deal with the potential setbacks of late-planted multi-stemmedness in the future by growing spare trees in pots for every B3F2 plot we plant in our seed orchards, to provide potential replacements for trees that die, or that never emerge.



Nut Production Orchards

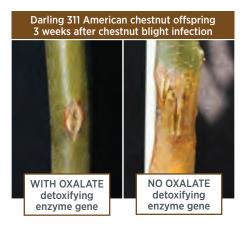
BEING ESTABLISHED FOR THE 'DARLING' AMERICAN CHESTNUT TREES

By William Powell, Ph.D.; Director, Council on Biotechnology in Forestry; Co-Director of the American Chestnut Research & Restoration Project; Roosevelt Wild Life Station Scientist-in-Residence; Professor, State University of New York College of Environmental Science and Forestry; New York Chapter Member



While we are working toward regulatory approval from the three federal agencies (EPA, FDA, and USDA) of the Darling 54 and Darling 58 varieties of the transgenic American chestnut developed at SUNY-ESF, we are already planning on how to produce enough trees to meet all the requests that continue to come from the public. We don't want to get to the point of receiving approval and not have the trees to distribute.

Pictured is one of two new nut production orchards established to produce trees for the public once we have regulatory approval. These orchards are specifically design to maximize inheritance of the OxO blight resistance gene by only planting two clonal lines, one transgenic and one wild-type. Since chestnut very rarely self-pollinate, this design encourages outcrossing, the first step in increasing genetic diversity. We expect that half the offspring from this orchard will be fully blight resistant. An example is shown in the F1/T1 full siblings inoculated with the chestnut



blight fungus. These trees have both the same mother and same father. The sibling inheriting the OxO gene is resistant and the one not inheriting the gene is susceptible to blight. We will use our simple OxO leaf assay to confirm which trees inherit the gene (see video on this webpage: http://www.esf.edu/chestnut/genes. htm). People can do similar breeding on a small scale by planting two-tree orchards and get similar results. In addition to nut production, this orchard can provide pollen for controlled, hand pollinations in diversity orchards or to surviving American chestnut trees to help rescue their genotype and the genetic diversity of the surviving population. The pollen could also be shipped to locations throughout the American chestnut range, once we have federal regulatory approval.

Another type of orchard than the one pictured and described above. is also being planted. These are conservation orchards containing trees found throughout the American chestnut range. These trees will be hand pollinated instead of open pollinated, since these are in a mixed orchard. The purpose of this orchard is different than the nut production orchard described above and will help to ensure local adaptability and further genetic diversity enhancement. From these offspring, more nut production orchards can be planted throughout the chestnut range providing trees to meet the public demand.

Deer Ticks, Lyme and What You Can Do

ABOUT THEM AROUND THE HOME

By Clark Bebee, PA/NJ Chapter

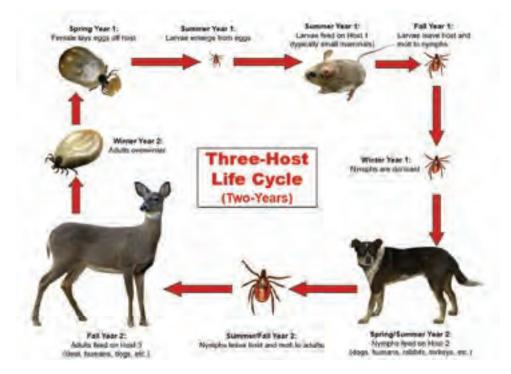
Almost everyone is aware of Lyme disease and the deer ticks that carry it. You've learned to wear light colored clothing (so ticks are easier to spot); wear long pants rather than shorts in the woods and grass (so they have less of a chance to grab on); and to thoroughly inspect your entire body after a trip outside (to ensure you did not pick up any unwanted hitch hikers during your outing). You've been told that if you find a tick on your person and it has been there less than 24 hours, you can pull it off and not worry about getting Lyme. You know that the deer tick is small, not like the much larger dog ticks. You know the long term effects of untreated Lyme disease can include fatigue, joint or muscle aches, and cognitive dysfunction. Symptoms might continue for weeks, months, or even years. The disease may affect many parts of the body including the skin, nervous system, heart, joints and eyes. You've learned that Lyme disease is serious!

> TO PULL A TICK OFF, grab it with tweezers by the head and pull it straight out. DO NOT SQUEEZE THE BODY as that may force the stomach contents of the tick – which carries the infection – into your body.)

Deer ticks are not found out in the middle of your lawn, they live where yards border wooded areas, ornamental plantings and gardens, or anywhere it is shaded and there are leaves with high humidity. By trimming shrubs and low branches, as well as raking and removing leaves, you can remove conditions that allow ticks to survive in your yard. Pay special attention to frequented border areas, tall grass, wood piles, stonewalls, and sheds. Tick nymphs begin to emerge from the leaf litter around mid-April. Once they find a blood meal in May, June or July they again retreat to the forest floor, where they molt and re-emerge as adults in the fall.

Deer, for which the tick is named, is not the key host in the fight against Lyme; the ubiquitous white-footed mouse is. The ticks attach to the mouse in both nymph and adult stages and both infect the mouse and receive the infection back from the bacteria residing in the mouse's blood. So a key to interrupting the infectious cycle is to cure the mice! But don't call your vet--the mice probably won't show up for the examination.

The answer is to buy a spray can of generic (all pharmacies usually carry their own brand) permethrin. It is sold over-the-counter for lice but works on deer ticks as well. Then spray it on a bunch of cotton balls; don't saturate them but cover them. Put the cotton balls in a piece of tubing (PVC, metal, a tennis ball can, whatever as long as it is maybe a foot long and will not dissolve in the rain. Now put the tube outside your house. The mice will come and take the cotton balls for their nests. The permethrin will protect the mice from the ticks. Monitor how many cotton balls are taken. More balls gone means more mice in your area (which is not necessarily a bad thing-they are prey for owls and hawks among other species and they are a major predator of Gypsy moth pupae; more mice means fewer Gypsy moths). If the balls disappear quickly, put out more to ensure all the mice in your area are protected.





Deer, for which the tick is named, is not the key host in the fight against Lyme; the ubiquitous white-footed mouse is.

If you want to measure the effectiveness of your efforts, do a tick population survey before and after you put out the treated cotton balls. Take a white pillowcase or tee shirt and drag it through the high grass and bushes around your house. Do this after maybe 10:00 AM so all the dew is gone. Wear gloves. The ticks and nymphs will attach to the cloth. Because it is white, you will be able to see them easily. Count them (before you kill them). A few weeks after the cotton balls are put out, run the tick population survey again. You should see a decrease in the count. Note that an adult deer tick is about the size of a sesame seed. The nymph however is only the size of a poppy seed! Nymphs can latch on, drink their fill of blood and drop off without you ever being aware. For this reason, they are more dangerous than the larger adults. Only females drink blood because it stimulates egg production. As a result, adult females can enlarge up to 1,350 times their size, if left intact to drink their fill of blood!

For more information you can check out the website http:// quantum.esu.edu/dna/ which is the Northeast Wildlife DNA Laboratory at East Stroudburg University.

Lyme disease is caused by a bacterium called Borrelia burgdorferi, a member of the family of spirochetes.

Planting Chestnuts IN DIXMONT COMMUNITY FOREST

By Judy Dann, Co-Writer, Project Canopy Grant





Volunteers are planting in rows by each red flag. In this area, brush was removed and small trees were girdled so they would eventually die, opening up the site to more sunlight and reduce the competition for water and nutrients. Every other planting site has a tree protector on it.

Judy is a former first Selectperson of Dixmont and the co-writer for a Project Canopy grant. The grant provided the opportunity to write a management plan on a 100-acre piece of property that had been deeded to the town from the federal government. Knowing that The American Chestnut Foundation was interested in expanding its research, the grant was written to include chestnut research plots as part of the grant application.



What a great day we had earlier this year getting about 75 chestnut seeds and 75 seedlings into the ground in Dixmont, Maine.

Rob Nelson did a lot of the prep work by putting little red flags in rows where he wanted the plantings to go. He has a map so we can keep track of how well each one is doing. Holes were dug, seeds or seedlings inserted, and tree protectors were put around half of them. Small trees were cut to open the areas to sunlight and brush was piled up in "bunny bunkers" for around nesters. Larger trees for which there is no market were girdled and will eventually die. Woodpeckers will drill holes in them and they will become homes for cavity nesters.

Thank goodness for a good turnout of volunteers. The prize for coming the farthest were Barbara Meyers and Bill Dowling, who live on Cranberry Island, Rob's whole family showed up to help and we had several folks from town who had never been to the town forest before. Thank you all. Many hands make light work.

We had so much gear to bring chestnut seedlings, protectors, stakes, shovels, chain saws, signs, and snacks. Thanks to Clark Staples, Dana Ivers, and Rob Nelson who were able to drive their trucks at least partway. We would have made it in okav without vehicles but I'm not sure we would have had the energy to walk back out!

Thanks again to everyone, including Brian Roth who negotiated getting the chestnuts for us. We are officially opened for business.

Seed, Soil, Water, AND A LOT OF HELP

By David Gill, Maryland Chapter President

What does it take to plant more than 4,400 chestnut seeds?

635 man-hours, 45 bales of peat moss, perlite and vermiculite, 100 cubic yards of mulch, and a whole lot of tree shelters and stakes!

This spring, dedicated volunteers from TACF's Maryland chapter took on the herculean task of increasing the average spring plantings by about 900%! Over a 10-day period, the crew managed to plant 1,350 seeds at the Central Maryland Research & Education Center (CMREC) orchard, 2,700 seeds at the Washington Suburban Sanitary Commission (WSSC) orchard, and 370 seeds at the Beltsville Agricultural Research Center. This task could not have been accomplished without the help of others; there was participation from groups such as the Master Gardeners of Howard County; the WSSC staff; Montgomery College; Prince George's Community College; The University of Maryland, College Park; and the Beltsville Agricultural Research Center staff. The WSSC was particularly helpful in our efforts. They provided mulch, a pizza lunch, a water truck and a port-a-john which made our task so much easier to accomplish. They also coordinated the student volunteers from the three colleges through the Hillman Entrepreneurs Program. Other contributors were Meadtree & Landscape, LLC who provided mulch for the CMREC orchard, the University of Maryland Agricultural and Natural Resources Department which provided a water truck for the CMREC planting and the Beltsville Agricultural Research Center for providing the mulch and staff to assist in the construction of the orchard fence. It was a heroic effort by everyone involved. Thanks to all who participated in such a successful spring planting.



future generations & the american chestnut: A Reforestation Project IN APPALACHIA

By Alice L. Tackett, National Board Certified Teacher Southside Elementary School Pike County, Kentucky

Michael French of TACF talks to 98 students from Southside Elementary School and Belfry High School about American chestnut's history, ecology, importance, and restoration as part of a grant received by Coal Country Beeworks on 5/18/2016. As an elementary teacher in eastern Kentucky, the heart of the Appalachian Mountains, I was awarded a project-learning grant from the Appalachian Renaissance Initiative for students to solve real-world problems. I chose to focus on reforestation in Appalachia, specifically the majestic American chestnut. I wanted my students to learn the amazing legacy of this native tree and how this vital resource is being restored.

Since students in the eastern Kentucky coalfields are already familiar with various mountaintop removal reclamation projects, I asked my second graders to develop and share creative ways to transform abandoned mine land into something beneficial to the region. Once they discovered that the natural range of the American chestnut tree was in the areas that are now barren, they concluded that planting American chestnut seedlings on previously mined they had learned and explained their project to the high school students who later helped them plant the trees.

In May of 2015, the students were able to plant thirty-two blight-resistant American chestnut trees. This wonderful opportunity came through the generosity of the organizations and agencies that helped us, including the Kentucky Chapter of The American Chestnut Foundation, Green Forest Works, Appalachian Regional Reforestation

land would improve their community.

To launch this rigorous unit of study, students examined nuts, burs, and leaves from the American and Chinese chestnut trees from a "Learning Box" developed by The American Chestnut Foundation. They inspected chestnut tree rings in order to find the age of the tree. determine years of



Initiative. Office of Surface Mining Reclamation and Enforcement. **United States** Department of the Interior (USDI), and the Kentucky Department for Natural Resources. It was wonderful to watch second graders make a positive contribution to the local environment. At the end of this unit, each student was provided with a tree to take

drought, and identify damage from fire, insects or disease. In addition, they observed photos and examined wood samples of the American chestnut tree before, during, and after the blight, documenting the changes they observed. The class recorded data and other relevant information and worked in small groups to do specialized tasks. In order to understand the significance of revitalizing the forests, they utilized technology to access the webcam at Robinson Forest in eastern Kentucky and saw examples of local reforestation. They did virtual field observations using live webcams available from six different locations in the southern and eastern regions of the Appalachians. Students generated slide shows to share the information with other classes in the school. The second graders visited the Belfry High School Biology Honors class where they shared what home and plant. On May 20, 2016, students will be planting trees once again. Only this time we will have a new class of youngsters and a new location. This project and reforestation of the American chestnut tree has been shared with regional, state, national, and international educators through the Action Research Summit for the last two years.

Restoring the American chestnut tree to its rightful place in the eastern woodlands is going to be a century long endeavor. Therefore, someone has to carry on this important task. Through this project, students developed an understanding and passion for replenishing trees of the forest that mankind has inadvertently destroyed. This unforgettable experience not only gave hope for a brighter future for our forests, but also empowered students to be agents of change in their generation.



2016 End-of-Year Appeal

The mission of The American Chestnut Foundation (TACF) is one of hope. Hope for the restoration of an iconic species. Hope for healthy forests filled with American chestnuts in our future. Together, we stand at the cusp of restoration.

TACF's bold mission cannot be accomplished without the dedication and commitment of our chapters, members, volunteers, and staff. We are at very a critical time in TACF's history, and we need your help.

Let's turn hope into action. Let's accomplish this bold mission together.

(828) 281-0047 ~ chestnut@acf.org



Undergraduate Education

AND AMERICAN CHESTNUT RESTORATION RESEARCH IN MAINE

By Brian E. Roth, Ph.D., Associate Director, Cooperative Forestry Research Unit School of Forest Resources, University of Maine TACF Maine Chapter



There will be a time in the near future when The American Chestnut Foundation (TACF) will be producing and distributing large numbers of potentially blight resistant B₃F₃ nuts from chapter seed orchards including those in Maine at the northern limit of the species native range. For a reintroduction program to be successful, information will be needed about what the best forest conditions and planting techniques are. For the past several years, the Maine Chapter of the TACF (METACF), led by board member Dr. Brian Roth at the University of Maine, has been establishing various field trials on suitable sites around Maine aimed at addressing several relevant issues ahead of the reintroduction phase.

Nuts and seedlings that are planted into the forests of Maine will need to compete for light, water and nutrients with established trees, stump sprouts, brush, shrubs and other plants. One question relates to how much overstory should be harvested before establishing a planting of restoration chestnuts? Another question is what type of seedling should be planted (direct seed, container seedling or bareroot stock)? Also, how best to protect these young seedlings from animal and plant competition (shelters, herbicides, fencing)? A large ongoing research program is underway in Maine aimed at providing information to help answer these questions, and this offers a unique opportunity to engage undergraduate students in education initiatives.

The METACF has connected with several universities and colleges in Maine to collaborate on education initiatives that incorporate chestnut research and conservation. What follows is a brief description of their programs and how the students have been helping with research to inform the upcoming chestnut reintroduction phase in Maine.

UNIVERSITY OF MAINE

Dr. Amber Roth is an assistant professor of Forest Wildlife Management at the University of Maine and teaches an undergraduate course titled "Introduction to Wildlife Conservation." One of the course requirements is a student service learning project in partnership with a local conservation organization. "There is considerable value in having some hands on experiential learning built into the curriculum" states Dr. Roth. Of the many projects to choose, over a dozen students wanted to work on projects with METACF. These

students grew potentially blight resistant hybrid seedlings from seed in greenhouses at the University of Maine and then planted them in a series of designed field experiments. The experiments will test the performance of these seedlings in comparison with bareroot seedlings and seeds, both with and without browse protection. There were two locations where the students worked: the Black Forest Demonstration Area owned by Peter Kliem in Greenville, ME and a forest estate in Eastbrook, ME owned by Anders and Christina Höglund and managed by LandVest. Sue Aygarn is a forester with LandVest who managed the project. "The opportunity for undergraduate students to participate in applied research is a win-win situation for both the landowner and the students" stated Sue.



1. Landowner, Peter Kliem with volunteers planting container B_3F_3 stock at the Greenville, ME study site on June 11th, 2016. Photo: Peter Kliem

2. B₃F₃ seedlings with and without protection from browsing moose and deer in reclaimed gravel pit in Eastbrook, Maine. Photo: Sue Aygarn (LandVest)
 3. University of Maine students, (left to right) Nikole Robbins, Ryan Cole, and James Zuhlke at the entrance to Peter Kliem's forest estate near Greenville, ME; one of several reintroduction study locations planted in Maine in the spring of 2016. Photo: Brian Roth

UNIVERSITY OF NEW ENGLAND

Dr. Thomas Klak is a Professor in the Department of Environmental Studies at the University of New England (UNE). He teaches an ecological restoration class and contributes to a capstone course which is a requirement for graduating seniors. This year four students in the capstone course chose to work with American chestnuts. One of the students, Sarah Fleischmann, explained her involvement this way: "We are trying to leave a legacy at UNE involving students, faculty and the campus community. This project is a true culmination of what I've learned on this campus -- the restoration of an important species, and working with important individuals from a variety of different fields and disciplines." Using the restoration questions identified by TACF, the students designed the field trials, selected the planting locations on campus, organized a media event for the planting on Earth Day and followed up with data collection and study documentation. The chestnut research plantings will be available for future cohorts of students to work with as they develop. The students also designed a beautiful sign that describes the history of the American chestnut, efforts to restore the species and the research effort at UNE.



- 1. Maine Chapter President Emeritus, Glen Rea, introduces the restoration effort to University of New England students on campus during Earth Day 2016 at Biddeford, ME.
- 2. University of New England students plant $B_{g}F_{g}$ seeds and seedlings on campus during Earth Day 2016 as part of their senior capstone project. 3. Six week old B,F, seedlings at the University of New England greenhouse grown by UNE students as part of their senior capstone class.
 - Photos: Thomas Klak

UNITY COLLEGE

Dr. Matthew Chatfield is an associate professor of conservation biology within the School of Biodiversity Conservation at Unity College in Unity, ME. He teaches two conservation biology classes which includes lab work. Chatfield has been engaging "...Unity students about reintroduction and restoration, showing them that what they learn in the classroom can be applied in the real world." In addition to a lecture about the chestnut restoration effort, the students participated in sowing chestnut seeds in the greenhouse at the McKay Farm and Research Station for a cold tolerance/blight resistance field experiment. The seeds came from across the native American chestnut range and included three levels of blight resistance (pure American, B3F2 and B3F3), across two provenances (southern vs. northern sources). Chinese seed was included as a control. These were outplanted in two field experiments; one on land owned by the New England Forestry Foundation in Knox, ME and the other on the Small Woodland Owners Association of Maine in Vienna, ME. Feedback from students was overwhelmingly positive, with many stating that "this was among their favorite parts of the course." The collaboration between METACF and Unity College was featured on a local news station, thus furthering the mission of TACF by garnering additional community support. Chatfield has plans to incorporate the field experiments



into the senior capstone course this fall and in future courses. "The partnership that we've formed holds enormous promise to continue engaging students that are eager for hands-on, authentic experiences. By monitoring these young trees in coming years, students will be able to contribute to a scientifically meaningful project while helping to restore an iconic American tree." In conclusion, there is an opportunity to further chestnut silviculture research ahead of the reintroduction phase by combining undergraduate education and research. The chestnut reintroduction program has always relied on the long-term vision of many dedicated scientists and citizens. Involving undergraduates at this key stage will ensure the success of the program by creating environmental citizens that are aware of the program's mission and objectives. Perhaps some of these young scientists will, themselves, become the next generation of chestnut stewards. Additionally, the experimental plots are a legacy for future cohorts of students, as they will continue to be a source of study for many years to come. Increasingly, employers require students to have the analytical abilities necessary to solve all types of conservation issues. The chestnut reintroduction program is easily accessible by students, is cost effective, and provides the perfect training ground for our future conservation scientists. We hope that our experiences in Maine will serve as encouragement for other chapters to build partnerships with local institutions on similar efforts in their states.

- 1. Unity College student, Adrianna Bessenaire, works with Maine Chapter President Emeritus, Glen Rea, to plant chestnut seeds in the McKay Farm and Research Station greenhouse.
- 2. Unity College Conservation Biology class students fill containers with potting soil specific for chestnuts in the McKay Farm and Research Station greenhouse.

LOUISVILLE, KENTUCKY · FRIDAY, NOVEMBER 11, 2016 TACF'S 33rd Annual Meeting

Join TACF for a full day of activity at the Embassy Suites Louisville Downtown. This year's event features prominent speakers throughout the morning presenting on various areas of research. The afternoon schedule is left open to enjoy all Louisville has to offer! The Louisville Visitors & Convention Bureau is located inside the hotel to help participants plan their own afternoon of entertainment.

Our evening event features welcoming remarks by leaders at the Brown-Foreman Corporation. Campbell Brown, President and Managing Director of Old Forester and member of the Board of Directors, along with Rob Frederick, Vice President and Director of Corporate Responsibility, will outline their company's commitment to sustainability and the environment since its founding in 1870.

To register for the 2016 Annual Meeting, please call the National office at 828-281-0047. The full agenda is available online: http://www.acf.org/AnnualMeeting2016.php



Chestnut Connection in the Classroom

By Tom Paris, PA/NJ Chapter Member



Tom Paris, a volunteer with the PA/NJ chapter of TACF, shares his experience as a guest presenter at the Brunswick Acres School in New Jersey. Tom lives in Kendall Park, NJ and has been a TACF member since 2005. These types of classroom experiences help us connect future generations to our beloved chestnut.



In early May of this year, my daughter Kristin, who teaches 4th grade in the Brunswick Acres School in Kendall Park NJ, asked me to do a talk on trees for Earth Day. I accepted as I have a great love and respect for trees, especially the American chestnut.

The PA/NJ chapter provided materials including: educational hand-outs and TACF branded pencils and stickers for all of the students. Additionally, I cut a chestnut log into round sections and gave each student a chestnut cookie (cross-section) for them to keep and examine.

I prepared a one-hour presentation about trees in general and then focused on the American chestnut. I enjoyed this experience as much as the kids and hope to do it again next year. When talking about the chestnut tree I explained the history of the tree and how valuable it had been for the early Americans and how valuable it is now. I talked about the current efforts of TACF to restore the American chestnut. The students were amazed at how much damage the blight caused; killing so many trees in the Eastern forest. At the end of my talk the students each planted an American chestnut seed in a container and placed it on the window sill of their classroom. The seedlings went home with them at the end of the school year with directions on how to replant them into a bigger pot and eventually into their yard. It is my wish that this experience will allow them to feel connected to the story of American chestnut.

Merging Genomics and Biotechnology with Breeding TO RESTORE THE AMERICAN CHESTNUT

By Jared Westbrook, Ph.D. Director of Science Edited by Sara Fitzsimmons Director of Restoration

The Journal of The American Chestnut Foundation ~ 27

Restoration of the American chestnut depends on producing a founder population of trees that (1) are sufficiently blight-resistant to reproduce as self-sustaining populations; (2) have the timber-type growth form of American chestnut to compete in the forest; and (3) have ample genetic diversity to be adaptable in a changing world. While The American Chestnut Foundation (TACF) has focused on traditional breeding to accomplish this task, parallel research efforts in genomics and molecular biology offer tools that can improve both the efficiency and efficacy of creating blight-resistant American chestnut populations.

Opening up the "black box" of blight resistance with genomics

In TACF's backcross breeding program, the major bottleneck has been in screening the thousands of trees necessary to combine the right mixture of blight-resistance and American chestnut traits. Ideally, we could discover the genes or DNA sequence variants from Chinese chestnut that contribute to the blight resistance of American chestnut backcross hybrids. Then, by sequencing DNA from regions of the genome where resistance genes reside, we could diagnose which genes for resistance a tree inherited. Those individual trees that inherited the DNA variants with the largest effect on resistance could be rapidly and accurately selected with a genetic test.

Understanding the genetic basis for blight resistance at the DNA level is a scientific odyssey on which we have just left shore. On this odyssey, reference genome sequences for Chinese chestnut sources of resistance and American chestnut are the world maps and the parts list. Chestnut genomes contain - 800 million nucleotides of DNA divided among 12 chromosomes. Together these chromosomes contain about 38,000 genes, which encode for proteins. A small fraction of these proteins are likely to be involved in sensing the invasion of Cryphonectria parasitica, the fungus that causes chestnut blight. These signaling proteins in turn regulate the cascade of cellular responses involved in defending cells from fungal invasion. Assembling

chestnut reference genome sequences is a first step in discovering the DNA building blocks for blight resistance.

TACF is seeking funding to sequence and assemble reference genomes for the Mahogany, Nanking, and Clapper trees. These three sources of resistance contributed resistance genes to the vast majority of backcrosses produced by TACF's breeding programs. Comparing reference genomes of blight-resistant parents to susceptible American chestnuts will provide clues about the genetic basis of blight resistance.

The genetic variants that confer resistance to C. parasitica are expected to be located on segments of the American chestnut backcross genomes that were inherited from Chinese chestnut parents. Chinese chestnut genomic segments may be identified by sequencing DNA in American chestnut third backcross trees and by aligning those sequences to the reference genomes of their Chinese chestnut source of resistance. Regions of the genome inherited from Chinese chestnut will be identified where backcrosses are heterozygous for Chinese chestnut specific alleles. This means that DNA inherited from one parent is identical to the Chinese chestnut source parent while the DNA from the other parent differs from Chinese chestnut and was presumably inherited from American chestnut.

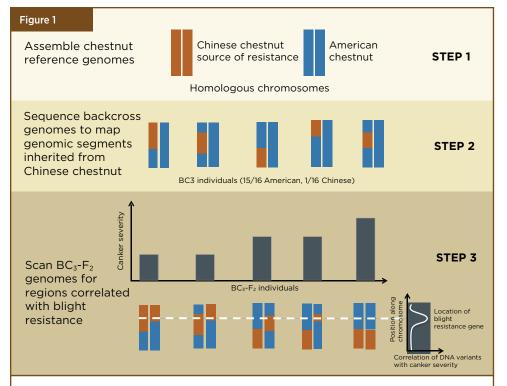
Not all of the genomic regions inherited from Chinese chestnut are expected to contribute to pathogen resistance. Furthermore, genomic regions inherited from Chinese chestnut may span several million DNA nucleotides and may possibly contain hundreds to thousands of genes. To more finely map the regions of the genome where resistance genes reside, TACF is collaborating with scientists at Virginia Tech to scan the genomes of advanced generation American chestnut backcross trees for DNA sequence variants that are correlated with blight resistance.

The ultimate objective of this research is to identify the regions of backcross genomes that underlie blightresistance (Figure 1). Results will be used to develop targeted genetic tests for blight-resistance genes. Genetic testing has the potential to partially circumvent the time-consuming and laborious process of field inoculations to infer the relative disease resistance of a given tree's parents. Genetic sequencing will also inform future breeding between trees that inherited different subsets of resistance genes in order to further enhance blight-resistance in their progeny.

New horizons in breeding

Backcross lines in aggregate are likely to have inherited all of the resistance genes from their Chinese chestnut parents. Blight resistance may be enhanced by intercrossing backcross trees that inherited different subsets of resistance genes and selecting for blight resistance after each generation. This breeding strategy called *recurrent selection* is used to enhance traits, such as growth, that are controlled by many genes.

It may not be necessary for individual backcross trees to be as blightresistant as Chinese chestnut to



A strategy to map the genetic variants underlying blight resistance in American chestnut backcross hybrids. A first step is to sequence reference genomes for Chinese chestnut sources of resistance and American chestnut. These reference genomes map chestnut's 38,000 genes onto 12 chromosome. Reference genomes are important for understanding the DNA sequence differences between Chinese and American chestnut that account for differences in blight resistance. Considering that American chestnut backcross populations inherited their resistance from Chinese chestnut, the genes for blight resistance are expected to be located on segments of the backcross genome that were inherited from Chinese chestnut. The second step in discovering the genes for blight resistance is to sequence BC, genomes to identify regions of the genome that are heterozygous for Chinese chestnut specific alleles. The regions inherited from Chinese chestnut may span hundreds of genes and not all of these genes contribute to blight resistance. To more finely map resistance genes, the third step is to scan genomes of BC,-F, trees for correlation between DNA sequence variants and variation in chestnut blight canker severity. Once DNA markers that are highly correlated with variation in canker severity are discovered, reference genome for Chinese chestnut sources of resistance will be useful for identifying candidate blight resistance genes in the vicinity of DNA markers associated with resistance. Comparison of the Chinese chestnut and American chestnut reference genomes in the regions of the genome associated with resistance may reveal why American chestnut is susceptible to the chestnut blight.

have sufficient blight-resistance to reproduce in the forest. The exact level of blight-resistance required remains to be demonstrated. This year at TACF's Meadowview Research Farms, we conducted controlled pollination between the most blight resistant trees in our seed orchards. The progeny from these crosses will be screened for resistance to chestnut blight and all but those individuals with the least severe chestnut blight cankers will be culled. As the remaining trees reach reproductive maturity in the next 5 - 7 years, they will be become a source of seed with

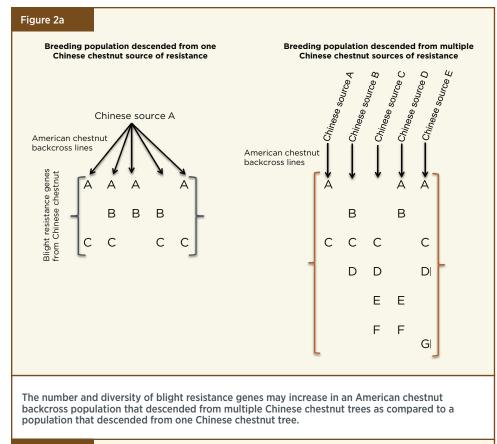
improved blight resistance. This seed will be planted in demonstration plantings and the populations will be monitored over time for growth, survival, and reproduction relative to plots of pure American chestnuts.

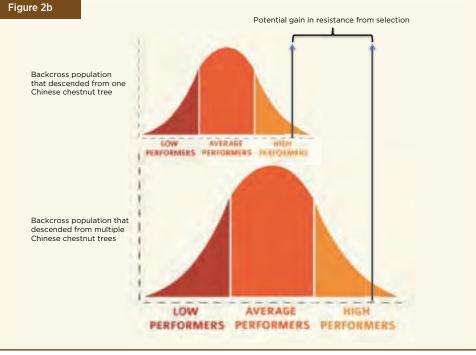
Intercrossing American chestnut backcross hybrids that descended from different Chinese chestnut trees may enhance the number and diversity of blight resistance genes present in the population. With a greater diversity of blight resistance genes, greater resistance gains from selection may be possible as compared with gains in backcross populations that descended from a single Chinese chestnut tree (Figure 2). With more genes for resistance present in the population, larger numbers of progeny may need to be screened to realize these gains. As long as the founder population has enough resistance to survive in the forest, natural selection may enhance resistance in future generations.

Representing genetic diversity from American chestnut in the breeding program

A genetically diverse breeding population is essential for maintaining the capacity for adaptation to the diverse habitats in which American chestnut grows and for avoiding inbreeding depression when blightresistant populations are restored to the forest. TACF's chapter breeding programs are crucial for representing the genetic diversity remaining in American chestnut sprout populations and for developing regionally adapted blight-resistant seed. In total, state chapters have developed 196 backcross lines for Clapper and 165 lines for Graves (Mahogany), and 34 Nanking lines. Chapter breeding programs are also conserving American chestnut genetic diversity by collecting seed from wild American chestnuts in the forest and planting them in germplasm conservation orchards (GCOs) where they can readily be used for future breeding.

Genomics may have as an important a role in conserving genetic diversity as it does in characterizing the genetic basis of blight resistance. Using DNA sequencing to characterize genetic diversity will enable comparison of the diversity represented in TACF's backcross breeding populations and GCOs to the total genetic diversity remaining in C. dentata sprout populations in the forest. These comparisons will shed light on how much of the remaining genetic diversity of C. dentata that TACF has captured. It will also help us to target populations that are underrepresented for future germplasm conservation efforts.





Potential gain in blight resistance from selection may increases in a population derived from multiple Chinese chestnut trees with different genes and greater genetic variation in blight resistance.

Potential integration of backcross and transgenic blight resistance

In parallel with the backcross program, scientists from the State University of New York's College of **Environmental Science and Forestry** (SUNY-ESF) have enhanced the blight resistance of American chestnut through transgenic insertion of the oxalate oxidase (OxO) gene from wheat. Some American chestnut trees with the OxO gene have smaller cankers than Chinese chestnut when infected with the chestnut blight fungus. Led by Drs. William Powell and Chuck Maynard, the SUNY-ESF research team has started breeding the original transgenic trees with susceptible American chestnuts. With a single gene that confers resistance, half of the progeny are expected to inherit the OxO gene. The latest field resistance results from SUNY show that American chestnut siblings which inherited the OxO gene are highly resistant to chestnut blight, whereas those that did not inherit the gene are susceptible. The implication is that the OxO gene may be rapidly bred into diverse American chestnut backgrounds, pending its approval for release by the government agencies that regulate transgenic plants. Much research is required prior to release of transgenic American chestnuts in the forest. This research includes testing whether the insertion of the OxO gene affects growth, metabolism, and ecological relationships with beneficial fungi and pollinators. If this research demonstrates that insertion of OxO has no negative side effects (early results suggest that it does not), then American chestnut with OxO may be approved for use in breeding in 3 to 5 years.

Pending approval for the release of the OxO transgenic tree, TACF is considering three routes to species restoration (**Figure 3**):

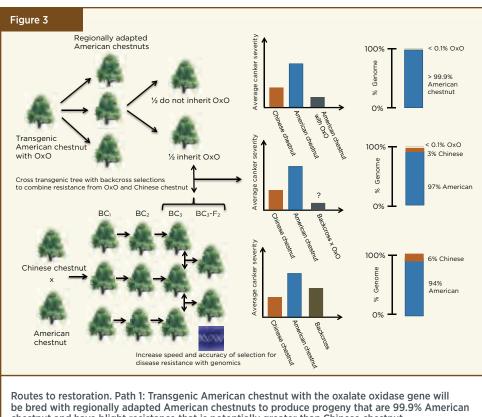
(1) We will continue with backcrossing and recurrent selection to incorporate blight resistance from Chinese chestnut into American chestnut genetic backgrounds. Genomic testing will be incorporated to increase the speed and accuracy of selecting the most blight resistant hybrids.

(2) The OxO tree will be bred with pure American chestnut trees to produce a diverse blightresistant transgenic population that is > 99.9% American chestnut in its genomic composition.

(3) The OxO transgenic tree will be bred with advanced generation backcross trees to produce progeny that are ~ 97% American chestnut with the disease resistance conferred by OxO and genes from Chinese chestnut. We are testing whether combining resistance from OxO and Chinese resistance genes enhances blight resistance beyond what has been observed in independent OxOtransgenic or backcross populations.

In addition, we are also testing whether resistance to chestnut blight and *Phytophthora* root rot (PRR) can be combined by crossing OxO trees with backcross trees that are resistant to PRR. PRR eradicated American chestnut from low elevations forests in the southern U.S. prior to the introduction of chestnut blight. Currently, there is no transgenic American chestnut that is resistant to PRR.

Ultimately, there is much is to be gained through merger of the backcross breeding and transgenic methods to restoration. While the blight resistance conferred by the OxO gene is potentially superior to Chinese chestnut and the most blightresistant backcross selections, TACF's backcross populations and germplasm conservation efforts will be critical for incorporating the blight resistance conferred by OxO into a genetically diverse population that can continue to evolve in the forest. Combining the blight resistance conferred by OxO with resistance to PRR in the breeding program potentially saves decades of research and millions of dollars in regulatory review costs that would be required to pursue a purely transgenic route to combining resistance to both pathogens.



be bred with regionally adapted American chestnuts to produce progeny that are 99.9% American chestnut and have blight resistance that is potentially greater than Chinese chestnut. Path 2: Increase the speed and accuracy of the selection of the most disease resistant 15/16ths backcross trees with genomics. Path 3: Cross the most disease resistant backcross trees with transgenic American chestnut to combine resistance from OxO and Chinese chestnut.

How you can help

When the backcross method was proposed in the 1980s, there was limited knowledge about the genetic basis for blight-resistance. Today genomic sequencing technology has progressed and decreased in cost to such a degree that discovering the DNA sequence variants that underlie blight-resistance is within reach. Understanding the genetic basis for blight resistance will help us to develop targeted genetic tests to rapidly and accurately select the most blight resistant trees. Genomics has the potential to save an enormous amount of time, labor, and monetary resources in artificially inoculating progeny of trees to infer the parent's relative genetic resistance. Genomics will also inform our efforts to conserve the genetic diversity that remains in chestnut sprout populations.

Through sustained efforts to develop blight-resistant backcross populations and transgenic American chestnut, TACF and our collaborators are on the cusp of species restoration, and we need your help. To accomplish this bold mission, please consider simple efforts such as a TACF membership, volunteering, increasing awareness of our programs within your community, or making a donation to our End of Year Appeal. **Your contributions allow us to continue this critical work and we are very grateful for your support. Thank you.**

OxO Blight Resistance Sustainability

By John R. French, Ph.D., President, TACF Georgia Chapter and Plant Pathologist



This article addresses the oxalic acid secretion mechanism as it pertains to Cryphonectria parasitica, the fungal pathogen that causes chestnut blight, and the oxalic acid oxidase (OxO) blight resistance trait that has been developed by our colleagues at the State University of New York College of Environmental Science and Forestry (ESF). For readers who may not yet appreciate the fine points of how chestnut blight kills its host trees, namely American chestnut (*Castanea dentata*) and chinkapin (*Castanea pumila*), I would recommend some excellent synopses presented by Drs. William Powell and Charles Maynard which can be easily accessed at these websites: http://www.esf.edu/chestnut/genes.htm and http://www.esf.edu/chestnut/resistance.htm

A salate (oxalic acid) is well known as a toxin in mammalian biochemistry, as well as in plants. In chestnut tree tissues, oxalate can lower the pH at the margin of the chestnut blight canker from a normal pH level of approximately 5.5, to a toxic level of pH 2.8¹. Oxalate also chelates calcium needed by plants for the formation of pectin (one of the main substances that binds plant cells together), and suppresses the oxidative burst used by the plant in defense against invading microorganisms². In some plant systems oxalate has been shown to induce apoptosis (programmed cell death)³. The naturally occurring OxO enzyme functions to degrade and remove the oxalate so it can no longer exert adverse effects on the host plant.

Transgenic insertion and over-expression of the OxO gene from wheat in American chestnut enhances blight resistance to levels greater than observed with Chinese chestnut. Breeding the transgenic trees with genetically diverse locally adapted American chestnut trees holds promise as a complementary approach to hybrid backcross breeding to restore American chestnut and chinkapin to their former prominence in the eastern forest ecosystem. In contrast to hybrid backcross breeding, in which an unknown number of resistance alleles from Chinese chestnut are being introduced into American chestnut, breeding in a single transgene with a large effect on resistance is less complicated. Whereas large numbers of hybrid backcross trees must be screened to identify few offspring with blight resistance, one-half of the offspring of an OxO transgenic American chestnut crossed with a non-transgenic tree are expected to inherit the OxO gene. With OxO trees, only one copy of the gene is required for resistance because it is a dominant trait, and seedlings that inherited the OxO resistance trait may be rapidly and easily identified through an enzymatic assay of OxO activity.

Some folks have expressed concern that the chestnut blight fungus could evolve to overcome resistance conferred by the OxO gene. This article addresses why resistance conferred by OxO is unlikely to break down.

Throughout my 40 year career as a plant pathologist, most of my time has been devoted to the study of plant diseases affecting agriculture. I have worked with my colleagues on several fungal pathogens with a virulence mechanism similar to that exhibited by the chestnut blight fungus, including for instance Sclerotinia minor and Sclerotium rolfsii. These organisms live primarily on dead plant residues in the soil, and to move from the soil environment and infect their host plants they secrete oxalic acid to kill and invade plant cells. Importantly these types of fungi are incapable of invading living tissue, just as the chestnut blight pathogen. These fungi are termed 'necrotrophic' pathogens, meaning that they only live on dead plant tissues, killed bark and vascular cambium in the case of chestnut. Necrotrophic fungi are mainly saprophytes, meaning they are quite "happy" living in

¹McCarroll, D.R., and Thor, E. The role of oxalic acid in the pathogenesis of Endothia parasitica, in: F.C. MacDonald, J. Luchok, H.C. Smith (Eds.), Proceedings of the American Chestnut Symposium, West Virginia University Books, Morgantown, VA, 1978, p. 122.

³Kim, K. S., Min, J.-Y., & Dickman, M. B. (2008). Oxalic acid is an elicitor of plant programmed cell death during Sclerotinia sclerotiorum disease development. Molecular Plant-Microbe Interactions, 21(5), 605–612. http://doi.org/10.1094/MPMI-21-5-0605

²Cessna, S. G., Sears, V. E., Dickman, M. B., & Low, P. S. (2000). Oxalic acid, a pathogenicity factor for Sclerotinia sclerotiorum, suppresses the oxidative burst of the host plant. The Plant Cell, 12(11), 2191–2199. http://doi.org/10.1105/tpc.12.11.2191



the soil environment on decaying plant residues. The chestnut blight pathogen, *C. parasitica*, does colonize dead plant tissues throughout the forest ecosystem, and even becomes a very weak pathogen at times on some species of oak. Thirty years ago an excellent article was published on that topic, herewith referenced , and which can be provided to interested readers at any time. Drs. Nash and Stambaugh have done a great service by documenting the fact that *C. parasitica* does attack oaks, because it provides us chestnut folks with a clear insight on distinguishing the chestnut blight fungus from other plant pathogens which are not necrotrophic.

To people who are not plant pathologists, and who do not understand the basics of how plant diseases differ from animal or human diseases, perhaps it is important to distinguish necrotrophic, saprophytic, opportunistic pathogens such as the chestnut blight fungus from those which are obligate parasites. Several types of plant pathogens, as well as most animal and human pathogens, are obligate parasites. They absolutely must find living host tissue to invade, otherwise they cannot survive. Examples of plant pathogens which are obligate parasites are those which cause "rusts" (wheat leaf rust, etc.), powdery mildews, and downy mildews. With obligate parasites, there is extreme selection pressure to maintain virulence. If a gene for resistance exists in the host, by definition there is a reciprocal gene for avirulence (inability to cause disease) in the pathogen. This is known as the gene-for-gene relationship of plant disease, which was well documented by Dr. H. H. Flor more than 70 years ago. Thus throughout the history of wheat breeding for resistance to rust diseases, every time a resistance gene is found and bred into the wheat population, selection pressure is immediately placed on the rust pathogen to evolve a corresponding gene for virulence to circumvent the host resistance gene. As a result, genetic resistance to obligate parasites is an ephemeral (non-lasting) solution, and this has been the most frustrating thing for plant breeders to deal with throughout the history of agriculture. To non-plant pathologists, this concept gets easily extrapolated, though, to all diseases, which is entirely inappropriate. Importantly, since non-obligate parasites like the chestnut blight fungus can and do live and reproduce as saprophytes, there is virtually no selection pressure on them to devise alternative virulence mechanisms.

When the American chestnuts died, the blight fungus continued to live ubiquitously in the forest litter and on tree surfaces (such as oaks) as a saprophyte. Even if *C. parasitica* were to now encounter the oxalate oxidase enzyme in a chestnut tree, it wouldn't be killed (Figure 1, Small stem resistance assay and *C. parasitica* isolated from superficial cankers on 'Darling 54' American chestnut). It would simply move elsewhere and find something else dead to thrive on. Quite simply, there is no selection pressure on the fungus to do anything different than it always has done as a saprophyte in order to survive.

To those who may be concerned about *C. parasitica* suddenly producing "more" oxalic acid if faced with an OxO transgenic American chestnut tree, it is useful to consider that if such an effect were likely to occur, it probably would have happened already, not only in North America over the past 110 years, but also in Asia over the past 50 million years, where there is an abundance of plants that *C. parasitica* might potentially invade, if only it could do so. Our colleagues at ESF who developed the transgenic American chestnut obtained their OxO gene from wheat, which is a grass. Grasses, and even several species of dicotyledonous plants, probably have evolved

the ability to produce OxO as a resistance mechanism against necrotrophic fungi that secrete oxalic acid. In agriculture, in order to take advantage of the disease suppressive effect from the oxalate detoxification mechanism in grasses it is always recommended to rotate Sclerotinia susceptible broadleaf crops (soybean, sunflower, canola, peanuts) with grass crops (wheat, barley, rye, etc.) every second or third year in order to minimize survival and proliferation of necrotrophic Sclerotinia fungi which utilize the same oxalic acid secretion mechanism as the Chestnut blight pathogen.

In some agricultural situations fungicides are routinely applied to the soil surface in order to minimize the potential for Sclerotinia and other necrotrophic fungi to infect broadleaf crops. A more rational idea would be to insert the OxO gene into soybean, sunflower, canola, or peanut, so that fungicide applications for preventing Sclerotinia would not be needed, or in situations where rotations with grass crops might not be feasible. That concept has been proven to be highly effective in experimental trials, but the plant pathologists who are working on those concepts have virtually given up the effort due to market pressure against GMOs. Fear against deploying biotechnology to resolve critical issues involving plant diseases has done a great disservice to the environment and the sustainability of agriculture, because the net result has been continued use of fungicides to control Sclerotinia and other diseases caused by certain necrotrophic fungi.

In a philosophical sense anyone can usually come up with myriad reasons not to progress technology which may be poorly understood, or which exhibits high probability of conferring adverse effects upon human health or environmental integrity. No one is advocating to be injudicious in how we approach the prime objective of our Foundation. Using the oxalate oxidase enzyme to enhance the blight resistance of American chestnut holds great potential to restore this important species to its former prominence in the forest ecosystem. The transgenic OxO technology is therefore intrinsically sustainable, and moreover holds the potential to significantly augment the on-going breeding program at TACF.

⁴Nash, B. L., and Stambaugh, W. J. 1987. Lack of host specificity in North Carolina isolates of Endothia parasitica. Phytopathology 77: 414-417.

Enduring Enthusiasm

RUFIN VAN BOSSUYT

The American chestnut tree once dominated forests spanning from Maine to as far south as Alabama. The nuts from the tree served as an important source of food for wildlife ranging from mice to bear, from turkeys to the now extinct passenger pigeon. The lumber from these giant trees is extremely straight and rot-resistant, making it an excellent choice to frame early homes and barns, as well as construct railroad ties and utility poles. Some of those utility poles still stand today, including a few in Massachusetts, where The American Chestnut Foundation (TACF) board emeritus member Rufin Van Bossuyt lives. Van Bossuvt is a retired forester previously employed by the New England Power Company as a systems arborist and is now dedicated to saving and restoring the American chestnut tree. Given his past career and lifelong passion for the American chestnut, it's almost impossible not to imagine Van Bossuyt scaling a chestnut utility pole, trimming trees to prevent power outages, and foreshadowing the type of work he would eventually do to restore the magnificent tree.

"When I was quite young I had a lot of interest in trees. And one summer when I was about 12 years old I went to a summer camp. One of the things they had was an environmental class, and the person teaching... talked about the American chestnut and the chestnut blight," Van Bossuyt said, remembering his first introduction to the American chestnut tree, an experience which sparked an enduring enthusiasm.

When Van Bossuyt speaks about the American chestnut's unique history, he tells the stories with a weight and warmth similar to a person recounting their own family biography. Interestingly, Van Bossuyt's family name means "from the forest."

In order to impress the importance and once prevalence of the tree, Van Bossuyt recounted a short chestnut anecdote. He frequently chuckled as he told the history: "I read some of the historical documents on the town where I live in Massachusetts, and in about 1790 they voted at a town meeting to allow the swine to run free. The reason being that they fattened them on the chestnuts and acorns that were in the forests. So if "...when I was about 12 years old I went to a summer camp. One of the things they had was an environmental class, and the person teaching... talked about the American chestnut and the chestnut blight," Van Bossuyt said.

you had a garden, it was up to you to keep the pigs out of your garden; it wasn't up to the owner of the pigs."

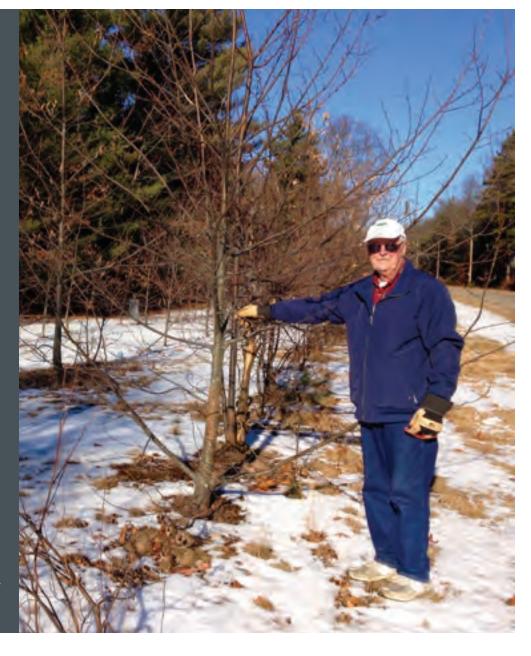
Years after graduating from Rutgers with a degree in Forestry, working for the power company, and becoming a member of TACF, Van Bossuyt responded to a letter from members of TACF's Connecticut chapter who were interested in starting a Massachusetts chapter. He soon became a key player in getting that branch started. The founding group came from diverse backgrounds and included a librarian, an electrical engineer, a lawyer, a physician, and a Latin teacher. "People from all different walks of life were involved, and they all had a passion for the chestnut tree," Van Bossuyt said. "I'm the only one who was forming the agency that had a background in forestry. That always interested me."

The Massachusetts chapter promptly found land, planted orchards, and began an extensive pollination program. The pollination process begins by pruning the male flowers of the chestnut, called catkins, and placing bags over the female flowers. Ten to 12 days later, bags are removed from the females, pollen is applied from the male catkin to the females, and the flowers are re-bagged. In order to protect the flowers from becoming contaminated by other species' pollen, the trees remain bagged throughout the summer.

This pollination program necessitates reaching the blossoms at the very top of the chestnut tree. In order to make this possible, Van Bossuyt drew on the contacts he developed as a forester and while serving as the president of the Utility Arborist Association to acquire bucket trucks to aide in the pollination process. These large bucket trucks made it possible to easily reach the flowers, which are located at the top of the chestnut tree.

"It didn't take much convincing. They were eager to help. I was dealing with foresters or arborists who naturally had an interest in trees,... and most of them had learned in college about the chestnut." Van Bossuyt said. "The tree work that's done to provide continuous electric service is usually done by companies that specialize in that work... under contract to the utilities. So the equipment we were using was actually owned by these tree service companies, but their time was paid for by the electric utilities."

"I think there's a good chance for survival or reestablishment of the chestnut as a major part of the forest. There's a good chance but it's certainly not a sure thing.... There are a lot of miles and a lot of acres between Maine and Georgia.... We won't be able to do it all ourselves.." Van Bossuyt said.



In the fall, Van Bossuyt and other TACF members go back up in the bucket trucks to collect the pollinated flowers, from the bags. The chestnut seeds are removed from their casing, and are then stored in volunteer refrigerators over the winter. In the spring the nuts are planted in orchards, where they are then evaluated for resistance to the blight.

Through the years, Van Bossuyt has had a hand in every step of this complicated procedure: "I've done planting, weeding, irrigating, actually injecting them with the fungus and then checking to see what the resistance is."

The amount of personal time and energy Van Bossuyt has contributed to saving the American chestnut is both impressive and has undoubtedly made an impact on the future of this tree. However, the collaboration and partnerships he coordinated and inspired, exemplify the type of collaboration that will be necessary to reinstate the chestnut to the forests where it once flourished.

"I think there's a good chance for survival or reestablishment of the chestnut as a major part of the forest. There's a good chance but it's certainly not a sure thing.... There are a lot of miles and a lot of acres between Maine and Georgia. And to get the chestnut back there we're going to need cooperation from others. We won't be able to do it all ourselves. It's just too much," Van Bossuyt said. "We'll need private landowners, various environmental organizations, state organizations, universities, and land trusts, who are interested in the environment, and who can help get the trees restored in the forest." Back in the 1950's my mother and father would take me on the trip up the mountains from Hickory, NC to visit my dad's brother who had a cabin in Blowing Rock behind the Green Park Inn.

My dad had a small business making sofa and chair frames. As we started up the mountains he would often point out the many fallen trees in the forests along the road and tell the story about the chestnut blight that was killing all of the chestnut trees.

Being a young boy I hadn't much interest in the trees but my dad was passionate about them and in his telling of the story he imbued me with his passion.



Some sixty years later I retired and set up my woodworking shop in my home in the mountains. As I talked to people I met about my interest I discovered that there was American chestnut to be acquired locally from old barns and houses that had been demolished. I was fortunate to acquire a store of chestnut which I have used in making my woodworking projects, being especially careful not to waste the smallest scrap.

I often think of my late father as I go about selecting and shaping chestnut, about him telling the story of the chestnut blight. I feel very fortunate in being able to make things out of the chestnut that I have. I feel like I'm keeping the wood alive to be appreciated by other folks, and it's a labor of love.

> By Stan Messick TACF member

Cranberry & Chestnut Gingerbread Scones

By Natalie Ward, Cook Eat Live Vegetarian blog

foodblogandthedog.wordpress.com/2011/12/30/fresh-cranberry-and-chestnut-gingerbread-scones/



Makes about 12 scones Prep time: 10 mins Cooking time: 25 mins

Ingredients

2 cups (300 gr) flour 1/2 cup (85 gr) brown sugar 1 tbsp baking powder 1 tsp cinnamon 1 tsp powdered ginger 1/4 tsp freshly grated nutmeg
a pinch of salt
8 tbsp (120 gr) cold butter, cut into cubes
1/4 cup (55 ml) milk, I used soy milk
4 tbsp molasses

1 egg the zest of 1 orange 200 gr fresh cranberries 75 gr peeled chestnuts, roughly chopped

Instructions

- Preheat the oven to 355 F (180 C) and line a baking tray with baking paper.
- 2 Add the cold butter cubes and the flour mixture to a food processor and pulse until the butter is the size of peas.
- 6 In a large bowl whisk together the milk, molasses, egg and orange zest until well combined.
- **4** Add the flour mix to the wet ingredients along with the cranberries and chestnuts. Mix together until just combined and a ball of dough is formed. Do not overmix or your scones will be tough.
- Flour your work surface and tip the dough out. Gently shape into a circle about 1 inch thick. Cut out circles using a 3-inch cookie cutter or chefs ring, rubbing the ring in the flour each time to stop them sticking. You will have to reshape the off cuts to get the last couple.
- **9** Put them directly on the baking paper on the tray, do not push them down to flatten them. Gently brush the tops with a little milk and bake for 20 25 minutes until the tops are golden and cracked and a toothpick inserted comes out clean.

Serve warm or at room temperature, if you can wait. Store in an airtight container, not in the fridge.

IN MEMORY AND IN HONOR OF OUR TACF MEMBERS

MARCH 1, 2016 - AUGUST 25, 2016

IN MEMORY

Essie Burnworth from: Victoria Jaycox

John Calvert from: Virginia Calvert Merle Melvin

Edwin Farrar Camp, Jr. from: Dr. John Agricola Dr. Billie Holladay Pam Kotis Mary McDonald David Morris Thomas Clinton Neel Martin Schulman Rhonda Tinsley Warren Averett Companies Beth Young Cynthia Zakrzewski

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Bryan Burhans from: John and Ann Chalk

> Kaylen Fry From: Roy Hall

Ruth Goodridge from: French Broad River Garden Club

Highland Rim Branch from: Alabama Wildflower Society

Curtis Laffin from: Bedford Historical Society, Inc.

> William Lord from: Chestnut Ridge Park

KO Summerville from: Henderson Forestry Consultants

/e regret any errors or omissions and hope you will bring them to our attention



50 N. Merrimon Avenue Suite 115 Asheville, NC 28804





TURNING HOPE INTO ACTION

2016 End-of-Year Appeal

The mission of The American Chestnut Foundation (TACF) is one of hope.

Hope for the restoration of an iconic species. Hope for healthy forests filled with American chestnuts in our future. Discovery is within our reach, but TACF's bold mission cannot be accomplished without the dedication and commitment of our chapters, members, volunteers, and staff.

LET'S ACCOMPLISH THIS BOLD MISSION TOGETHER.

(828) 281-0047 ~ chestnut@acf.org