WINTER 2015 ~ ISSUE 1 VOL. 29

Chestnut

THE NEW JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

A BENEFIT TO MEMBERS

THE AMERICAN CHESTNUT FOUNDATION®

Genes for Resistance ~ Meadowview Farm 25th Anniversary ~ Chestnut Blight in Turkey ~ Pure American Program

Chestnut

THE NEW JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

Thank you for all that you do for the American chestnut within the community, as critical partners in our mission.

Chestnut is a new, quarterly publication of The American Chestnut Foundation (TACF) that is designed to reach a broad audience in order to educate members and the greater public about the research and initiatives of the Foundation and its chapters.

As the NEW Journal of The American Chestnut Foundation, ***Chestnut*** is one of the benefits of TACF membership and we are very interested in your feedback. Our goal is to provide members with timely information about the Foundation's restoration efforts including science and research, events and activities, as well as the dedicated volunteers and partners who play such an essential role in our mission.

TACF depends upon your support. For more than 30 years, we have worked with members, like you, to combat one of the worst ecological disasters of the 20th century. Our work is far from complete, but neither is our commitment. Thank you for helping TACF in this important journey to restore the Mighty Giant.

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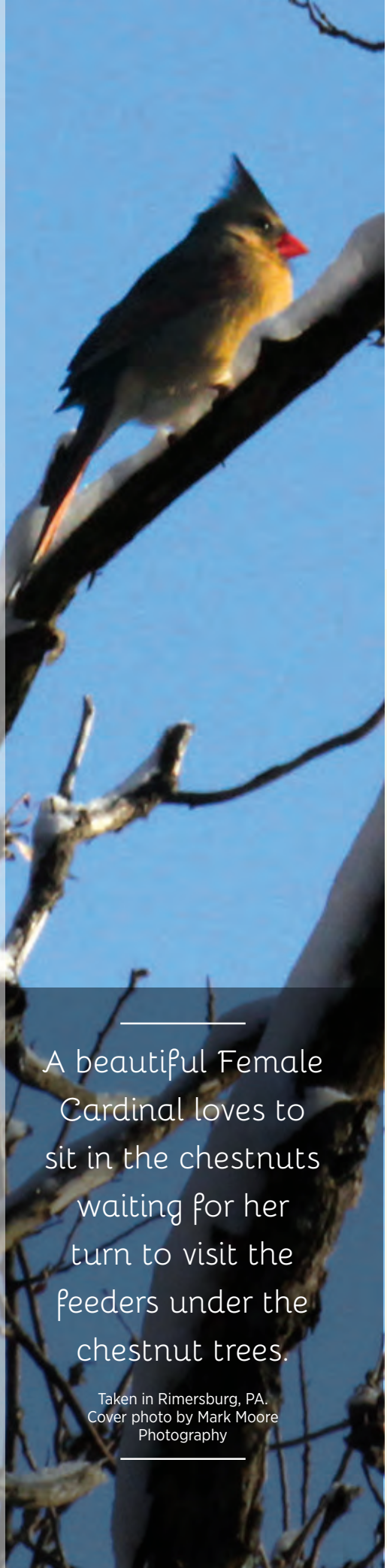

A beautiful Female Cardinal loves to sit in the chestnuts waiting for her turn to visit the feeders under the chestnut trees.

Taken in Rimersburg, PA.
Cover photo by Mark Moore Photography

THE AMERICAN CHESTNUT FOUNDATION®

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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Lisa Thomson
President and CEO

DEAR MEMBERS,

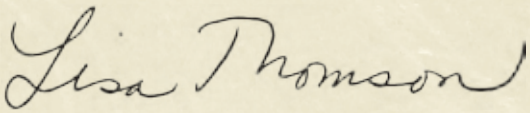
Spring is often a time of renewal and it is in that spirit that I write my first welcome letter to you: the dedicated and diverse constituency that makes up The American Chestnut Foundation. I am honored and thrilled to serve as your new President and CEO. I began my duties on January 12, 2015 at the national office in Asheville.

When I received a phone call from Search Executive Chip McGee encouraging me to apply to this prestigious position, my first reaction was curiosity. I began to research the history and scope of TACF's incredible mission. I was aware of the terrible demise of the American chestnut, but not of the scientific successes that were bringing it back from the brink of extinction. The more I read, the more I wanted to tell everyone on the street about it. "Did you know, that in just 30 years, talented scientists and researchers actually figured out a way to create a potentially blight-resistant chestnut? Isn't that remarkable?" The more I read about the organization, with its cadre of energetic volunteers planting chestnuts, year after year, the more excited I became to join its ranks. After 28 years with The Nature Conservancy, and 4 years at Rollins College, coming to TACF felt like a perfect professional home.

So often in conservation, it is about fighting against, or reacting to, new and seemingly insurmountable threats. It seems that we are constantly on the defensive. I was reminded of this when I introduced myself to a TACF board member who remarked, "what attracted me to this organization was that, for once, we are on the offensive." Although we are still fighting the blight fungus that can destroy this tree, our scientists are working tirelessly on methods to prevent infection, to ensure chestnuts can grow to their former glory.

As fellow chestnut enthusiasts, we must realize that scientific conservation messages are not easily grasped by the general public. Now that we are poised to take the organization to the next level, I am committed to ensuring that the message of our mission is embraced by all sectors of society. This may take some organizational change, such as increased use of social media; increased membership; and new and innovative partnerships. But I cannot do it without all of you, so please know I will be reaching out for advice, introductions, and ideas. We must honor the past but embrace the future.

I would like to thank Bryan Burhans for his five years of dedicated service as my predecessor; Betsy Gamber for graciously serving as Interim President; and the members of the board's executive search committee for their confidence in my candidacy. I look forward to meeting each of you and celebrating this conservation success story together.



Lisa Thomson
President and CEO
The American Chestnut Foundation

Speaking of social media, feel free to follow me on Twitter (@MadameChestnut).

TACF'S FEARLESS, NEW LEADER

Q&A with Lisa Thomson

Thomson poses with the new TACF office sign (made of reclaimed American chestnut) in Asheville, NC.

After only three months as president and CEO of The American Chestnut Foundation (TACF), Lisa Thomson is making tremendous strides. She hit the ground running in January of this year and has not looked back. In fact, her dedication to TACF is undeniable, and her enthusiasm for it is simply contagious. She recently talked with Chestnut about her vision for this "vibrant organization," her commitment to building relationships with its stakeholders, and her passion to create a lasting impact through her work.

Chestnut: What are your main objectives for TACF within your first year?

Thomson: I joined a vibrant organization, with a solid foundation built by my leadership predecessors. I've had the opportunity to visit the incredible work of our staff at Meadowview Research Farm and spend a day at Dr. Joe James' Chestnut Return Farm in Seneca, SC. To fully appreciate the hard work of our Regional Science Coordinators and network of dedicated volunteers, I also hope to get to as many Chapter plantings and events as possible.

That being said, my first and continuing priority will be to grow membership and reach new audiences. One of our most important constituencies has been the science community, but I hope to broaden our visibility and share the compelling story of the chestnut recovery farther and wider. I have already been "on the road" quite a bit, meeting key stakeholders: Board members, Chapter presidents, financial supporters, and agency partners. I will continue this "listening tour" to ensure that I hear the ideas, hopes, and concerns of those most important to our mission.

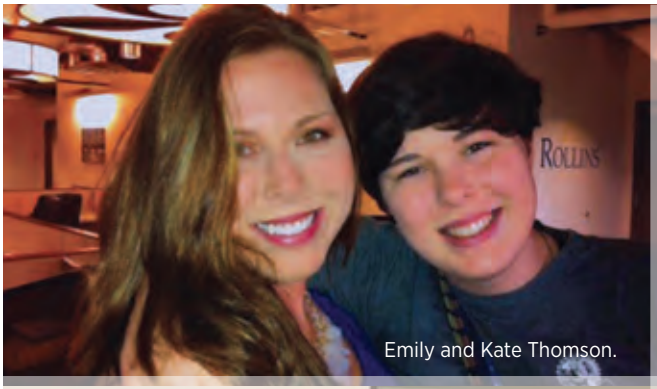
I have been impressed by the hard work and collective professionalism of our talented staff. I have promised them support, regular feedback, and opportunities for professional development. Beginning this summer, we will undertake a strategic planning process with our Board. I have already initiated some detailed assessments of the organization: a review of policies and procedures, assuring that our fundraising, marketing, and communications reflect current best practices. Since our staff is not centrally located (from Asheville to Burlington, VT), we will gather early May for a retreat. All full-time, permanent staff will participate in workshops led by outside experts; define the future vision of the organization; and have a rare opportunity to unwind and get to know each other better. We are grateful this retreat was co-sponsored by new TACF donors Genevieve Lykes Dimmitt and Margaret Pennington.

Chestnut: What initially attracted you to TACF / sealed the deal for you in accepting your position?

Thomson: After 28 years with The Nature Conservancy (TNC), in a variety of increasingly senior positions, and my most recent post of Associate Vice President at Rollins College, I was not initially looking for a new position. However, when Chip Magee, the search professional contracted by TACF, called me in August, 2014, about the President & CEO position, I was immediately intrigued (not to mention flattered). I greatly enjoyed my work with TNC but during my time in higher education, I found that I missed my conservation work. The more I read about the chestnut story, the more I wanted to be a part of it. TACF reminded me of the very early days of TNC, where we had a large cadre of dedicated volunteers in the field and committed, long-tenured Board members. This grass-roots, hands-on structure represents a sincerity of purpose that is inspiring and effective.

Chestnut: What are your visions for the future of TACF?

Thomson: We will be developing a strategic plan as the guiding document for the next stage of the organization's development. Within this strategic plan will be the Vision, Mission, and Guiding Principles which will shape our future. Board leadership will be deeply involved at this early stage of the planning:


Emily and Kate Thomson.


Doug Gillis and Lisa Thomson with an American chestnut garden rake. Photo by Tom Saielli.


Tom Saielli and Lisa Thomson at the Southern Pines Garden Club in Pinehurst, NC.


Walt Thomson.

- restoration and breeding plans
- a marketing and communications plan
- education and outreach
- chapter activities
- fundraising

I envision an organization true to its roots and founders, with a great deal of name recognition, which will build a stronger membership and donor base, and a reputation for continued integrity within its scientific discovery and research. The ultimate goal is that our children and grandchildren will be able to find, within a day's drive, a cathedral stand of American chestnut and learn the story of how these trees were brought back from near extinction, thanks to scientists, supporters, and donors who would not give up on a dream.

"One of our most important constituencies has been the science community, but I hope to broaden our visibility and share the compelling story of the chestnut recovery farther and wider."

PRESIDENT & CEO
LISA THOMSON

Chestnut: Is there anything you would like our members/readers to know about you?

Thomson: I strive to be a transparent and self-aware leader. I enjoy building relationships and making connections between people who want to leave the world a better place, whether through volunteering, giving, or acting as strong ambassadors for the organization and its mission. Since I will be seeking counsel throughout my tenure, I hope you will take time to visit with me. No one can do this ambitious mission alone. I am here to serve. Since I'm absolutely committed to the mission and the positive story of the chestnut, this will come naturally!

As for my personal background, I moved to Asheville in January to begin my post at TACF at its national headquarters, after living in Central Florida for nearly 45 years. Like so many other Floridians, we vacationed in Western NC often and have longed to live here permanently. My husband Walt and 18 year-old daughter Kate will be joining me this summer after she graduates from high school. I've been married to Walt for 34 years; we met in botany class at Stetson University, where we both received our undergraduate degrees. I later received my graduate degree in art from Florida State University, and Walt has his master's in botany from the University of Central Florida. He also pursued a career in conservation, having spent 15 years with the Florida Park Service and 18 years with TNC as a fire ecologist and land management specialist. Now an environmental consultant, he also looks forward to life in the mountains so he can ride his motorcycle and, hopefully volunteer for TACF. My oldest daughter, Emily, lives in St. Augustine, FL, where she is a lab technician at the Anastasia Mosquito Control District. We all love to hike, listen to music and read. I hope to set up a new studio in our home to rekindle my pottery skills and in my spare time, volunteer for local organizations such as historic preservation, pet rescue, and the farm to table food movement.

AN AMERICAN CHESTNUT SANCTUARY Grows in Greenwich

In mid-November, the Greenwich Land Trust in Greenwich, CT planted a 1.5 acre American Chestnut Sanctuary. TACF donated and planted more than 350 Restoration Chestnut 1.0 seedlings for this test planting on a part of their 14 acre meadow/forest preserve.

This new sanctuary is the first approved test planting site in Fairfield County for growing the American chestnut seedlings, Ginny Gwynn, executive director of the Greenwich Land Trust, explained.

She described the acre-and-a-half planting area on Burning Tree Road as having "ideal conditions for American chestnut trees," Gwynn said. "They will be protected from deer and voles with a perimeter fence and tree tubes, with water, nutrient and pest management to be performed by GLT staff and volunteers."

The Greenwich planting represents a great new partnership for the Connecticut Chapter of TACF.

Prior to this, there was not a huge TACF presence in this community. So when the out pour of enthusiastic volunteers came to help with planting, it demonstrated the anticipation of the community for this collaboration.

"It was a darn cold day and we weren't sure how that would impact turn-out, but we had a lot of people join us to get their hands dirty. I wasn't quite sure what to expect, but I was so pleased with the outcome," New England Regional Science Coordinator Kendra Gurney said.

An estimated 75 volunteers helped plant these trees in a modified test protocol with experimental treatments that focused on different weed management tactics. Kendra led instruction on this. "This is the first opportunity we've had to plant bare root B_3F_3s in Connecticut," Kendra explained. This allows TACF to follow the long-term performance of the trees with the hopes that they will naturally spread into the surrounding forest.

This collaboration has been a year in the making after Steve Conway, conservation & outreach manager of the Greenwich Land Trust, reached out to Regional Science Coordinators Sara Fitzsimmons and Kendra Gurney. Kendra then took lead on this project and spent a few visits surveying the site and fleshing out details with the land trust.

"The GLT staff was very well-organized, they had all the supplies we would need on-hand, the planting design was clearly laid-out. They did a great job of making sure they were ready to keep anyone who showed up busy with meaningful work and also provided training to new volunteers as they arrived to help ensure quality work," Kendra remarked.

Steve Conaway said that: "with a few quality control adjustments and wood chip spreading this week the orchard should be buttoned up and ready for the winter."

It also opened up the opportunity for the Greenwich Land Trust staff to engage with local students that can, in turn, help with data collection and maintenance in the future. The planting was designed to assess the effectiveness and effort required for a few common methods of vegetation management, but it also received the added benefit of engaging the community and providing visibility to the project.

Pure American Program

CREATES LEARNING OPPORTUNITY FOR CHESTNUT ENTHUSIASTS

Pure American chestnut trees will eventually succumb to the blight fungus. However, the process of growing these trees is a wonderful learning experience, especially for those interested in American hybrid chestnuts. Pure Americans can also survive up to 10 years. Learn more about the history of this unique program.

In 1997, Pathologist Fred Hebard approached Ohio chapter member Greg Miller to assist with the distribution of pure American seed packets to TACF members. Hebard said it provided a good way for members to learn about this important species. Miller liked the idea and wanted to take it a step further by sprouting some of the seeds.

"TACF published a notice in the 1997 fall newsletter asking people to collect and send me pure American seeds. A few people responded to the notice, but I actually got most of the seeds from the Pennsylvania, New York, and Maine chapters," Miller explained.

In 1998, Miller started selling around 50 seed packets and 2,000 seedlings each year, and the TACF national office processed all of the orders and payments. Unfortunately, it came to a drastic halt in 2010 when gall wasps invaded Miller's supply.

In the wake of this set-back, Maryland chapter President Gary Carver and Forester Michael French stepped in to maintain what had become a very popular program. The Maryland chapter has three pure American groves (WMREC, Sugarloaf, and Scrivener), and more than 11,000 chestnuts were harvested in 2014. Carver attributes the plentiful harvest to the cruddy bark syndrome that keeps the blight from girdling these trees.

"I think it's really important for people to get involved with pure Americans," said Carver. "It's a way for individuals to learn how to grow and care for these precious chestnuts without having to waste them. It's a stepping stone that allows you to figure out if you're ready for the B_3F_3s."

The Kentucky Division of Forestry's nursery in Morgan County, KY, holds about 2,000 pure American seedlings, donated to TACF by the Maryland chapter. They will be lifted as one year old bare root seedlings for the pure American program and mined land rehabilitation come spring of 2015.

After the seeds are harvested, French maintains a portion of them in cool storage for distribution in the spring. He has also expanded the seedling program through partnerships with Rick Williams of Native Forest Nursery in Chatsworth, Georgia (2013) and the Kentucky Division of Forestry Morgan County Nursery (2014). These facilities sprout, care for and distribute the pure American seedlings for TACF. Rick Williams and his associates have already committed to managing the seedling project next year.

The smaller seedlings are pulled from distribution and donated to the Appalachian Regional Reforestation Initiative (AARI). The remaining seeds are used for various Maryland chapter outreach activities, such as the Maryland Correctional Training Center. Carver stated, "Growing pure American seeds is a very popular activity and participants look forward to the seed donation each year. I enjoy talking about the American chestnut and its history, answering questions and providing detailed instructions about how to plant and care for these rare pure American seeds."

TACF's pure American program is offered to members only when seeds and seedlings are available. It provides a fun opportunity to learn about the American chestnut.

B_3F_3 Seedlings

TEST PLANTING IN WAYNE NATIONAL FOREST, OHIO

By: Bruce Willis and Brian McCarthy, Ohio chapter members

Photo by Dr. Brian McCarthy

Months of planning came to fruition when the first TACF Ohio progeny test was installed on the Wayne National Forest in Athens County. On October 25th, nearly 90 volunteers gathered to plant 700 seedlings (25 replicates of 28 families). The primary goal is to provide feedback to Meadowview about which families provided the highest level of resistance to the blight, as well as, survival and growth. This information allows Meadowview to cull out poor performing families and identify those families that do well under southeastern Ohio growing conditions. Added benefits will be using this as a field site for additional chestnut research, a demonstration site for those interested in American chestnut restoration, and providing mast in the future for local wildlife.

The success of this planting was largely a function of the strong partnership between the Ohio Chapter of TACF, TACF National, USDA Forest Service, Athens Ranger District (Jarel Bartig, Ecologist; Steve Blatt, Biologist; Gary Chancey, Public Affairs Officer; Todd Dempsey, Silviculturalist; and Gary Willison, Watershed Group Leader), USDA Forest Service, Northern Research Station (Todd Hutchinson and Leila Pinchot, Research Ecologists), and the National Wild Turkey Federation (Lee Crocker, Ohio Regional Biologist). Each partner played an integral role in the success of this project.

In addition to the mentioned partners, dozens of eager volunteers showed up on the planting day from all parts of the State. In addition to Ohio chapter volunteers (including officers Keith Gilland, Carolyn Keiffer, and Bruce Willis), many helpers from regional colleges, organizations, and agencies showed up to assist. Some of these included: Hocking College, Miami University, Ohio University, ODNR-Forestry, and USGS, not to mention a broad array of energetic community volunteers. After a sign-in at the Wayne National Forest HQ in Nelsonville, and a welcome and instructions by Dr. Brian McCarthy, former President of the Ohio Chapter of TACF, personnel were shuttled by buses and vans to the nearby planting site.

Justin Lee, one Meadowview's seasonal employees, transported the 700 containerized seedlings from the Meadowview Farm in Virginia the night before, and showed up at the planting site bright and early. Jeff Donahue had all the seedlings pre-arranged and pre-labeled. Bill Scripp, USDA FS Technician, used a Marooka Rubber Track Carrier to haul supplies and the seedlings the quarter mile up the hill to the planting site. To facilitate the planting process, all of the holes were pre-dug via tractor auger. This allowed the volunteers to focus on doing a careful job in planting the seedlings, often the primary determinant of early success.

Ohio chapter leadership anticipates that this will be the first of a series of planting events on the Wayne National Forest using B_3F_3 seedlings and looks forward to future partnerships. If the excitement and energy surrounding this project was any indication, there is a bright future for chestnut restoration in southeastern Ohio.

Above photos by Alexandria Polanosky

25th Anniversary

CELEBRATION AT MEADOWVIEW

By Dick Olson, Southwest Virginia Restoration Branch (SWVA)

Despite the rain and cold temperatures, people showed up in droves to celebrate the momentous 25th anniversary of Meadowview Research Farm on October 11th. Hundreds of guests participated in the event and didn't seem to mind the inclement weather at all.

When the sun peeked out from behind the dark clouds, guests didn't hesitate climbing into the wagons for an orchard tour. Unfortunately, the rain wasn't done for the day, but most guests had their umbrellas ready. I think they would have gladly accepted hot coffee, but instead, they savored chestnut beer and fresh apple cider. The freshly roasted chestnuts also provided a bit of warmth, as well as a new taste for many. By any measure, the 25th Anniversary Celebration of the American chestnut restoration program at Meadowview Research Farm was a huge success.

"It was amazing to have folks continue to come throughout the afternoon and to have so many ask about the progress of chestnut restoration and endorse TACF's work. It was very satisfying to see the payoff from the hours that the Branch volunteers and TACF staff put in to preparing for the event," Interim President & CEO Betsy Gamber said.

As the anniversary date approached, the weather forecast changed daily and members of the Southwest Virginia Restoration Branch debated whether to move the event under cover. Caution won out, luckily, and the farm staff took on the task of cleaning the barn along with its machinery and equipment. Staff hung tarps across the large entry doors, strung lights all around, and gave the barn its best cleaning in years.

Photos by Ruth Goodridge

"It was amazing to have folks continue to come throughout the afternoon and to have so many ask about the progress of chestnut restoration and endorse TACF's work."

INTERIM PRESIDENT & CEO BETSY GAMBER

"The celebration was awesome. So many people came out despite pouring rain. The rain was so bad that the one quick wagon tour we sent out came back very wet. Yet people kept coming, and they stayed until the end! Jeff Donahue working with the SWVA Branch members magically transformed our upper barn into a wonderful event space," Pathologist Fred Hebard explained.

Since the event was taking place during harvest, the barn was actually still being used to shuck and count this season's crop of chestnuts. Many of the red bags of burs were moved, but those hanging on the south wall were left as a colorful backdrop for the bluegrass trio that played throughout the day. Guests shared their own chestnut stories, talked about their own restoration chestnuts, and inquired about updates within the Meadowview program.

More than 250 guests attended the celebration, in addition to the Branch members, staff, and volunteers who stood out in their colorful 25th Anniversary t-shirts. Special guests included Jennifer and Cheri Wagner whose generosity and farsightedness is the reason the breeding program is situated in Meadowview. In all, the enthusiastic crowd was certainly confirmation of the continued support and interest in the Meadowview program.

TACF WELCOMES

Jared Westbrook

"I think the cause is noble," states Jared Westbrook, quantitative geneticist with TACF. Jared joined the ranks of TACF's national office in January of this year after completing his doctoral thesis in plant genetics. "I'll get to apply all that I've learned in my Ph.D. work, such as gene mapping and predicting blight resistance from DNA sequence," he continued.

Jared first discovered TACF through his Ph.D. advisor, Dr. John Davis. Davis served on the advisory board for the deregulation of blight resistant transgenic chestnut trees developed by the scientists at SUNY. He encouraged Jared to apply and sent him the job announcement for the position last year.

Jared grew up in Grand Rapids, Michigan, his dad a dentist and his mom an operations manager for a brokerage firm. Jared cites his parents as being "a good set of parents that have been supportive throughout." His mom pushed him to continue playing the violin "even when it wasn't cool in middle school." He eventually grew to love playing and joined youth symphonies, string quartets, and chamber music ensembles in high school. He has one brother living in San Francisco, who also works in the non-profit world designing graphics and animations.

He majored in environmental science at the University of Michigan. Post graduation he took three years off from school but stayed immersed in plant life and biology. During his first year, he worked for AmeriCorps in Longview, WA teaching forestry education.

Next, he went to Harvard University, where he spent a year working in a biophysics lab, studying how ferns disperse their spores. This experience whetted his appetite for scientific

Westbrook visited Chestnut Return in Sencea, SC which is home to Dr. Joe James, member of TACF's Science Oversight and Restoration committees. Photo by Lisa Thomson.

"Inserting candidate genes into American chestnut trees that are susceptible to blight, and testing for an increase in blight resistance is the most powerful evidence that a particular gene contributes to blight resistance," he explained.

research, and solidified his desire to work in a meaningful and applied scientific field that would benefit the environment.

Prior to earning his MS in botany from the University of Florida, Jared mapped out a plan to hike the Appalachian Trail in its entirety. It took him five months to trek 2,160 miles, after several shorter "test" hikes around the New England area in preparation.

"I think there is a healing power to being in the wilderness for an extended period of time. There is a quiet you can have in yourself," Jared remarked. "And it's an adventure, it's physically strenuous, and you are constantly adapting to whatever the weather is throwing at you."

As a graduate student, Jared spent most of his time in a lab. His master's work examined how the variation of the toughness of leaves relates to growth and survival of tropical woody plants growing in shady environments in a tropical forest in Panama. He continued at the University of Florida where he earned his Ph.D. in plant molecular and cellular biology. His dissertation research studied the genetic enhancement of resin production in loblolly pine stems for use in liquid biofuels, which he successfully presented in November.

It's this rigorous training from his dissertation research that he believes will drive his work. "TACF is a good fit for me. In terms of the skill set I can bring and in terms of the mission, I really like the idea that TACF is restoring a tree so that it can naturally evolve on its own," Jared said.

His three main objectives in his new position are to:

1. Select trees with the highest levels of genetic resistance to chestnut blight, by analyzing the blight resistance of their progeny.

2. Map the regions of the genome that are associated with blight resistance. Find out whether blight resistance is controlled by different genes among different sources of resistance in the backcross breeding program (e.g., Clapper and Graves).

3. Develop marker assisted selection for resistance to chestnut blight and *Phytophthora* root rot diseases. If we precisely map the regions of the genome that are associated with resistance to these diseases, then we can sequence the DNA in those regions of the genome to test for presence or absence of disease resistance genes. Individuals that have greatest number of disease resistance genes should be the most resistant to the disease.

Incorporating marker assisted selection should improve the efficiency of the breeding program. Compared to inoculating trees with the disease-causing fungi, DNA sequencing can be done at an earlier age. If DNA sequences are found to accurately predict disease resistance, then only individuals that are predicted to have the highest blight resistance need to be planted in seed orchards, tested in the field, and included in the breeding program.

"Inserting candidate genes into American chestnut trees that are susceptible to blight, and testing for an increase in blight resistance is the most powerful evidence that a particular gene contributes to blight resistance," he explained.

Jared is looking forward to making Asheville his home and says that Asheville was one of the draws for this position. "I think there is a lot of work to be done in data analysis, which is one of my strengths and what I want to focus on. I can do that anywhere, but Asheville is where I want to be," Jared said. Since Meadowview is a short drive from Asheville, Jared will be making frequent trips to the Farm on an as needed basis.

Energetic and determined, hard science is Jared's passion, but hardly his only one. He avidly practices ashtanga yoga and loves to salsa and swing dance, hike, and bike.

The Breeding Program IN MAINE

by Eric Evans, Maine Breeding Coordinator

The current focus of the TACF Maine chapter's breeding program is to harvest seeds (B_3F_2) from the most blight-resistant trees in the third-backcross (B_3) orchards, and then plant them in the seed orchards. This will constitute the 5th generation of Maine's six-generation breeding program.

Searsport: Maine seed orchard in Searsport on the property of Small Woodland Owners Association of Maine.

Winthrop: Four foot tall Restoration Chestnuts 1.0 in two feet of April snow in a seed orchard in Winthrop, ME. (SWOAM property)

Photos by Eric Evans

After evaluation and selection for blight resistance and American type, the seed orchards will then produce seeds (B_3F_3 – the 6th generation) for chestnut testing and restoration plantings in Maine's forests, starting in 2020.

In spring 2014, we planted 8,850 B_3F_2 chestnut seeds from the Merryspring, Camden and Highmoor, University of Maine Experiment Station, Monmouth orchards into the seed orchards in Phippsburg, Searsport, and Stetson. This brought the total seed orchard plantings to 22,350. Last October, we harvested over 9,600 seeds from the Highmoor and Merryspring orchards. This spring, we will draw from these to establish 33 new plots of 150 seeds in each of our Stetson, Phippsburg, and Searsport seed orchards. This will bring the total seed orchard plantings to over 27,000 – the midpoint of the goal of 54,000 hybrid chestnut trees in seed orchards by 2020.

Last June, we injected live, lab-grown blight fungus into the bark of more than 200 trees in the orchards in Veazie, Bradley, and Unity to begin the process of evaluating their blight-resistance. This year, the Maine chapter will continue evaluation of blight resistance and American type, and expect to harvest more B_3F_2 seeds from the Monmouth, Camden, and Hope orchards. We also have plans to inoculate the trees in the Morrill and Lovell orchards.

The Maine chapter will need many volunteers to help plant the 9,600 seeds at Phippsburg, Searsport, and Stetson this coming April and May. To sign up, please email MaineTACF@gmail.com. They will also need help with this June's inoculations in Morrill and Lovell.

Donating a day of your time pay off ten-fold – rewarding you with new friendships, skills, knowledge, and bragging rights that you helped restore this valuable species.

American Chestnut Restoration
Join Our Team!

The American Chestnut Foundation is pleased to introduce "Chestnut Restoration," a custom design by Martin Webster for the Quilt Trails series of Western North Carolina. This unique design captures the distinctive shape and dentate margin of American chestnut leaves using a simple geometric pattern. The alternating colors of the leaves represent the changing seasons. Symbolically, the upward pointing green leaf (on left) represents the American chestnut as the dominant tree of eastern North American at the beginning of the twentieth century. The downward pointing yellow leaf represents the tree's tragic decline due to chestnut blight. The upward point green leaf (on right) represents the hope of restoration – bringing blight-resistant chestnut trees back to the native range.

"Chestnut Restoration" is TACF's new membership decal for 2015. Get yours today!
Joining or renewing is easy – simply call 828-281-0047 or visit us on line at acf.org.
Membership also makes a great gift for friends, neighbors, teachers, and loved ones.
In fact, everyone you know will want one of these cool, new decals!

(828) 281-0047 | acf.org

A STATUS REPORT :

The search for genes for resistance to chestnut blight

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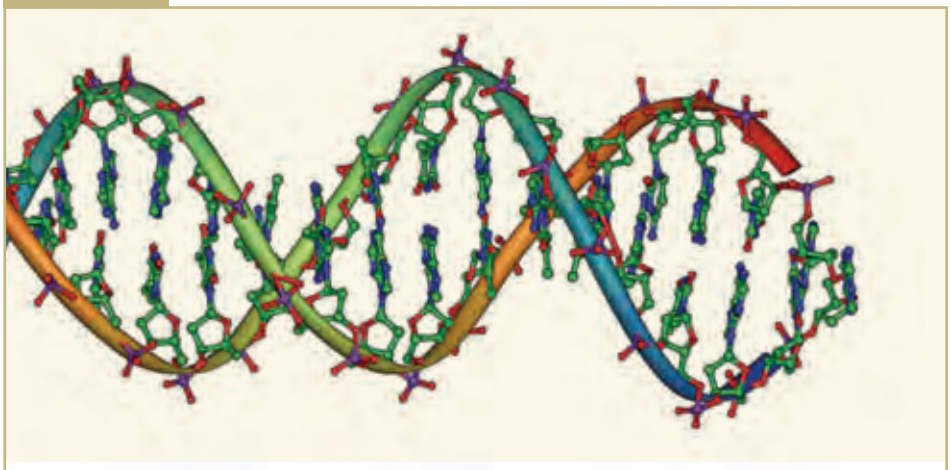
INTRODUCTION: During interactions with the public, we have found that some people are surprised to learn that the genes responsible for resistance to chestnut blight in Chinese chestnut have not yet been identified. Identification and verification of the genes would assist our efforts to breed American-type trees with blight resistance in a number of ways, as well as permit direct cloning of the genes into American chestnut. An important use would be identifying trees in our B_3F_2 seed orchards that had only genes for blight resistance and none for susceptibility. Rapid identification of such trees would accelerate and simplify seed orchard development. A more complete list of the potential uses for this knowledge is in Nelson, et al. (2014). We have been searching for these genes for several decades. What follows is a report of what we have done and what we have found.

Deoxyribonucleic Acid

When we say that we are searching for a gene, we mean that we are searching for a segment of DNA that has a particular function. It has been known since the mid-twentieth century that genetic information in living cells is encoded in molecules of deoxyribonucleic acid, abbreviated DNA. DNA is composed of two complementary polymer strands twisted together in the famous double helix (**Fig. 1**). Each polymer strand is made from four kinds of monomers, and is analogous to a string of beads of four types. There typically are tens of millions of monomers in a DNA polymer.

The four monomers of DNA contain one of four bases, Adenine, Thymine, Guanine, or Cytosine, abbreviated A, T, G and C. Genetic information is encoded in the sequence of these four bases along the polymer strand. Chemically, the bases are alkaline rather than acidic, which is why they are called bases. The two polymer strands of DNA are held together by pairings of complementary bases (A pairs with T and G with C). The two pairs, A-T and G-C, are called base pairs, and the length of pieces of DNA is measured in base pairs, often abbreviated bp. The total length of chestnut DNA is about 800 million base pairs (Mbp).

Figure 1



Model of DNA double helix
http://commons.wikimedia.org/wiki/File:DNA_double_helix_horizontal.png

Chestnut DNA is divided into 12 chromosomes. Each chromosome contains a continuous strand of DNA. Each chestnut cell has two sets of chromosomes, one set having come from the tree's pollen (male) parent, and the other from its seed (female) parent. Thus each tree has two copies of each gene. If the copies differ they are called alleles. These alleles are different (heterozygous) in DNA sequence and can be in function.

Classical Chestnut Genetics

How do we identify a gene? Classically, a gene has been defined based on the inheritance of a trait. In our case, the trait of interest is resistance to chestnut blight. Chinese chestnut (CC) trees are resistant to chestnut blight. American chestnut (AC) trees are susceptible. The first generation (F_1) offspring of a cross between CC and AC are consistently intermediate in resistance. F_2 offspring of crosses between interspecific F_1 trees can run the gamut between highly resistant and

Figure 2

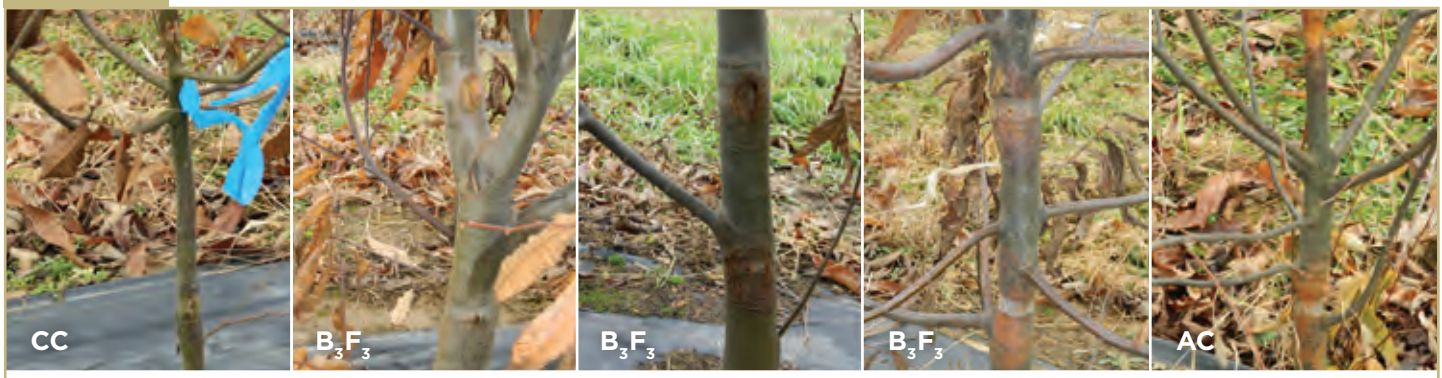


Fig. 2. Blight cankers on Chinese (CC), American (AC) and interspecific hybrid (B_3F_3) chestnut trees displaying variation in blight resistance. The B_3F_3 seedlings are from the same mother (W6-31-92).

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DNA MARKERS

Various features in DNA sequences have been used as markers for genetic mapping. We currently are using two types of DNA markers:

- Simple Sequence Repeats (SSRs)
- Single Nucleotide Polymorphisms (SNPs)

A simple sequence repeat, also called a short tandem repeat, is just what it sounds like: a stretch of DNA where a short motif of one to six base pairs is repeated multiple times. SSRs are highly variable, which makes them useful DNA markers. Thus, a set of only 13 SSRs is sufficient to uniquely identify most humans. In the example shown below, the American chestnut (AC) has four copies of a five-base repeat (tggta), shown in color, whereas the Chinese chestnut (CC) has only three copies. For legibility, only one strand of the DNA duplex is shown. Vertical lines mark identities between the two sequences. Dashes mark the bases missing from the CC sequence.

An example of an SSR:

American Chestnut (AC)

aaatttacctgtgtatggtttggtatggtatggtatggtatgcatgtgtggttaccttggct
aaatttacctgtgtatggtt-----tggtatggtatggtatgcatgtgtggttaccttggct

Chinese Chestnut (CC)

By contrast, a single-nucleotide polymorphism is a single-base difference between DNA sequences. SNPs occur at very high frequency in DNA, but each position has a limited number of variants: at the most four (since there are four different bases in DNA), but more commonly two. In the example shown below, the AC has a different base (A) than the CC (G). Here again, only one strand of the DNA duplex is shown, and vertical lines mark identities between the two sequences.

An example of a SNP, shown in color and uppercase:

American Chestnut (AC)

ctttaagaaAcctcgccgcccatatgagaaggagcggttggatgctgagttgaggct
ctttaagaaGcctcgccgcccatatgagaaggagcggttggatgctgagttgaggct

Chinese Chestnut (CC)

The DNA markers shown here are in expressed genes. The DNA sequence of expressed genes is *transcribed* in the nucleus into a related molecule called RNA. Messenger RNA molecules leave the nucleus to be *translated* into the proteins that make up cellular structures and the enzymes that catalyze the chemical changes needed for the cell to function. We can identify expressed genes by extracting and sequencing the RNA from cells. Sequences of genes expressed in Chinese and American chestnut were acquired as part of a project called "Genomic Tool Development for the Fagaceae," funded by the National Science Foundation between 2006 and 2010.

How do we detect these markers?

When cells grow and divide, the DNA polymer must be copied so that both daughter cells receive a copy. This copying is catalyzed by enzymes called DNA polymerases. Using the polymerase chain reaction one can use short (about 20 bases long), synthetic DNA "primers" to direct a heat-stable DNA polymerase enzyme to replicate millions of copies of particular small regions of chestnut DNA. These small DNA fragments can be separated by gel electrophoresis and detected by staining the DNA.

completely susceptible (**Fig. 2**). By following the inheritance of the trait in progeny of controlled crosses, Graves (1950) concluded that blight resistance is controlled by more than one gene, which complicates identification of the genes. Clapper (1952) concluded that two genes control resistance to blight. Hebard (2006) concluded from canker size data that resistance is controlled by two or three genes.

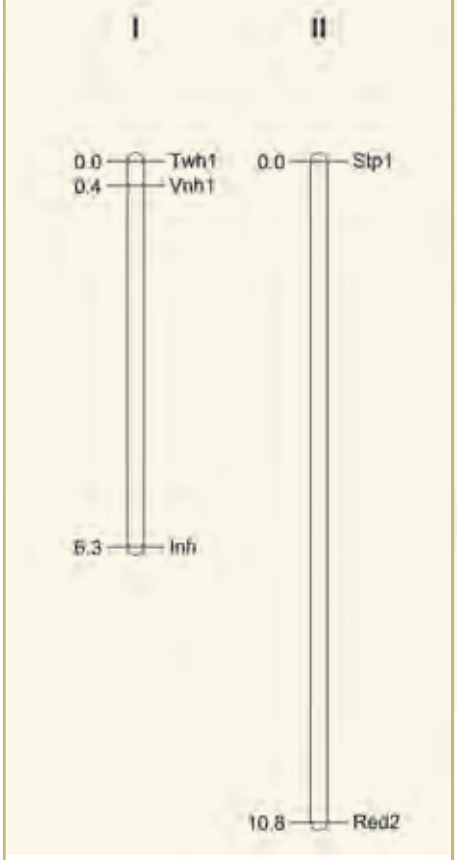
Modern Chestnut Genetics
Genetic Maps

The first step we took to identify genes for blight resistance was to place them on a genetic map.

Genes that are close together in DNA tend to be inherited together. For instance, if brown eye color were frequently associated with brown hair color, one could say that some of the genes controlling those traits are inherited together. The frequency with which two genes are inherited together in related individuals is proportional to their proximity in DNA. Dr. Hebard constructed the first genetic map of chestnut (**Fig. 3a**) based on the co-inheritance of visible attributes such as leaf and twig hairs, stipule size and persistence, and twig color (Hebard 1994a, b). There usually are insufficient numbers of visible traits to give wide coverage and high resolution in genetic maps. For instance, the first genetic map of chestnut did not cover regions associated with blight resistance. Molecular genetic markers overcome this limitation. In particular, variations in DNA yield a virtually unlimited supply of markers (**sidebar**).

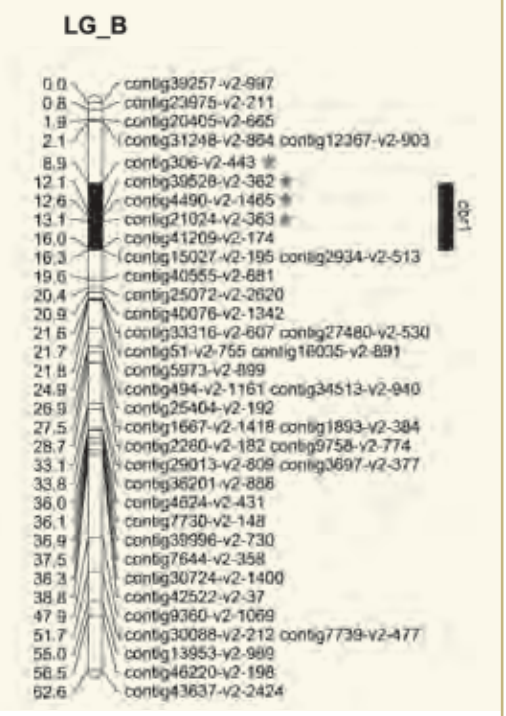
Subsequent genetic maps of chestnut have many DNA markers and contain at least three regions associated with resistance to chestnut blight. Kubisiak et al. (1997) identified three regions in their map associated with blight resistance. These results were confirmed using more markers on the same family (Kubisiak et al., 2013). The location of one of these regions is illustrated in **Figure 3b**. Hebard and Sisco (1999) identified a fourth region from Chinese chestnut governing resistance and also found evidence for two genes for resistance coming from

Figure 3a



First genetic map of chestnut.

Figure 3b



Genetic map of the chromosome containing the region *Cbr*1 associated with resistance to chestnut blight. The vertical bars represent a chromosome or part of a chromosome. The black bar marks the location of *Cbr*1. Horizontal lines indicate the locations of genetic markers, with names on the right and distance on the left. Stars indicate markers assigned to the physical map region shown in Fig 5.

American chestnut in one family of backcross trees. More analysis in additional families and in transgenic trees needs to be done before these genetic associations are confirmed.

It is unclear how the number of resistance genes inferred from segregation data by Graves, Clapper and Hebard mentioned above relates to the number of regions associated with blight resistance on genetic maps. Some of the associations may be spurious, due to the low numbers of progeny and low numbers of markers involved in these maps. Alternatively, multiple factors may be involved with resistance but only a subset needed to confer high resistance to progeny. The additional factors may, however, be crucial to sustainable resistance over the long term.

Genetic mapping is continuing, both to refine mapping of blight resistance in the tree investigated by Kubisiak et al. (1997), and to investigate resistance from other trees. Refinement entails looking at more progeny with more DNA markers.

Physical Maps compared to Genetic Maps

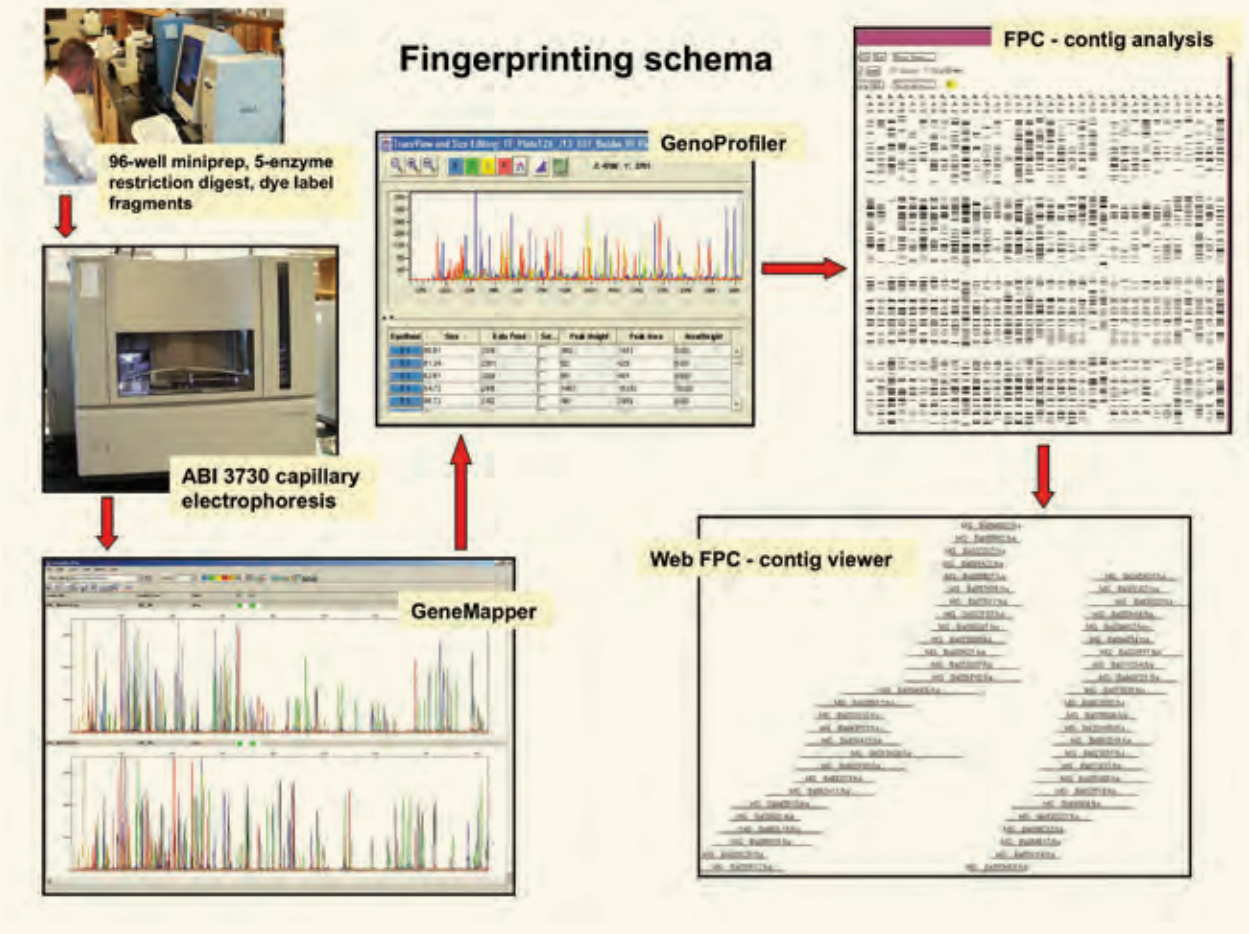
A genetic map is based on the measurements of recombination rates between adjacent genes, not on direct measurements of DNA itself. DNA also can be mapped by direct measurement. Such maps are called physical maps. A common means of identifying the genes controlling a trait is to locate the trait on a genetic map and then find its corresponding location on a physical map.

The ultimate physical map is a complete DNA sequence, but that sequence is still difficult and expensive to determine in a new organism for the first time; chestnut's remains fairly incomplete. Physical maps of lower resolution can be made more readily by a number of methods. A common method in current use begins with molecular cloning of the DNA. Molecular cloning entails extracting DNA from an organism (chestnut, in our case), cleaving the DNA into shorter sequences, splicing the shorter sequences into a vector, and introducing the spliced vectors into a bacterium. By culturing the bacteria, one can store and reproduce the spliced DNA fragments. The spliced DNA in its host bacterium is called a clone, and a collection of such clones a library.

A large number of clones was needed for the chestnut physical map, based on the following considerations. DNA fragments in the clones were a bit over 100,000 bp long. Since the chestnut genome contains about 800,000,000 bp, 8,000 of those fragments would contain all of the DNA in the genome – if they could be cut and spliced systematically. But that is not possible. The fragments are cloned randomly, and in order to determine their positions in the genome, they need to be examined as a group to determine how the individual clones overlap. Numerous clones are needed to get sufficient overlap. To address these limitations, the chestnut libraries were made with 165,000 cloned DNA fragments.

To construct a physical map from these clones, their DNA was extracted and fingerprinted individually (**Fig. 4**). The fingerprinting method used a set of five enzymes called endonucleases, each of which cuts DNA at a particular short, four- or six-base-pair sequence. In this fingerprinting method, one endonuclease is a four-base cutter that makes blunt DNA ends and is included to make the fragments small enough for capillary electrophoresis, and the other four are six-base cutters that make cuts with ragged ends. Fluorescent DNA monomers are then attached to the ragged ends with DNA polymerase, a different monomer and fluorescent

Figure 4



Fingerprinting clones for physical map assembly.

color for each of the four six-base cutters. The color allows identification of each monomer when the fragments are separated by size using capillary electrophoresis. The combination of fragment sizes and fluorescent markers is specific to each clone and also allows detection of overlaps between clones. In this way, a physical map of chestnut was constructed using fingerprints of over 126,000 clones (Fang et al. 2013).

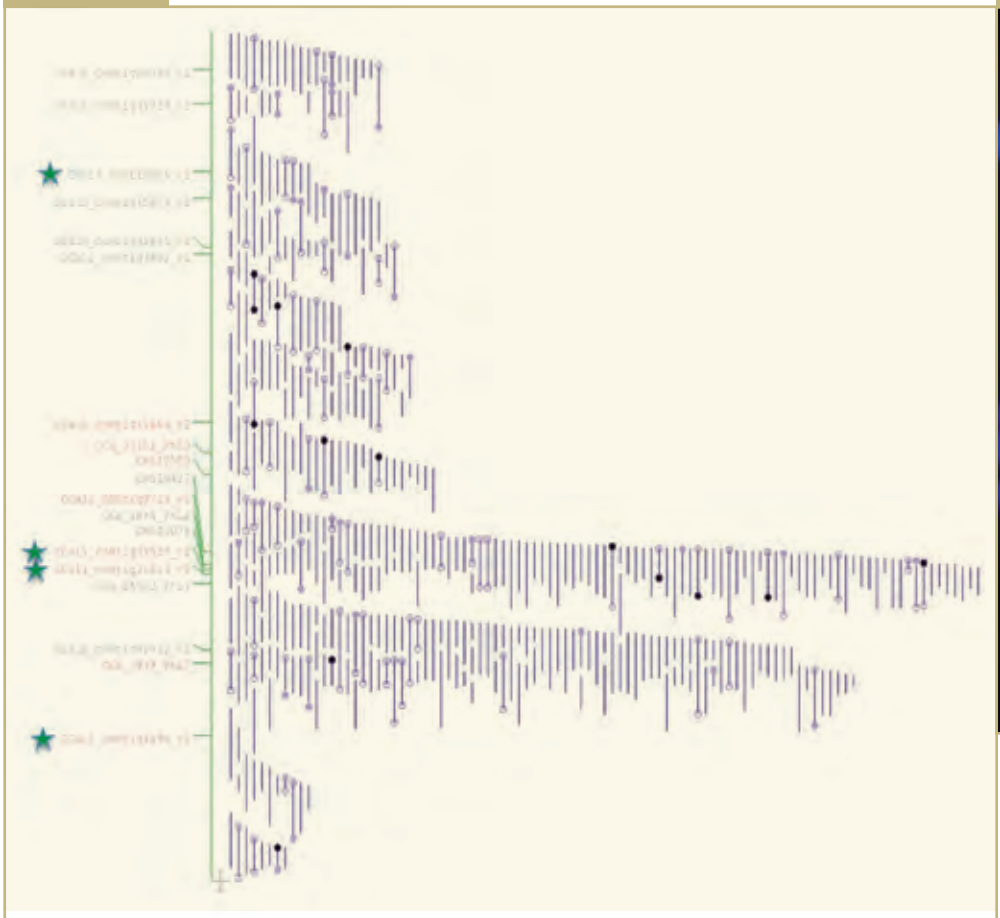
To integrate genetic and physical maps, clones are probed with portions of DNA markers from genetic maps. The clones containing markers reveal the position of the genetically-mapped markers on the physical maps. This is possible because complementary strands of DNA bind to each other with high specificity. A short DNA strand containing about 40 bases will bind only to its complementary sequence in long DNA strands containing hundreds of thousands or millions of bases. It will not bind anywhere else. Probing starts with arraying and growing clones on filters, followed by lysing the bacteria and rinsing, leaving the DNA stuck to the filters, each clone in unique locations. Typically, each filter contains DNA from 18,432 clones. DNA marker fragments are radioactively labeled and applied to clone DNA blotted onto a filter. Clones that bind to radioactive DNA markers will show up as black spots on x-ray film exposed to the filter. **Figure 5** illustrates clones containing DNA markers at the region on the genetic map known as *Cbr*1, the most prominent of the three regions found by Kubisiak et al. (2013) to be associated with chestnut blight resistance.

Cytogenetic Maps

Thus far, we have described two types of physical maps, one based on the direct sequence of DNA bases and one based on overlapping DNA clones. The image of a set of chromosomes under a microscope is a third type of physical map, and the oldest and least detailed, known as a cytogenetic map. The lack of detail can be an advantage in identifying large-scale rearrangements of the genome.

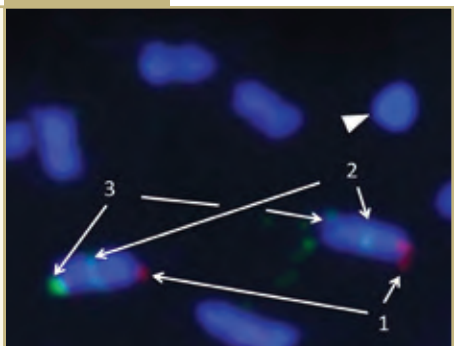
Just as we probed DNA clones blotted on a filter, the chromosomes

Figure 5



Physical map of the region of the chestnut genome containing *Cbr*1. The markers integrating this region of the physical map with the genetic map are indicated by stars. The short overlapping vertical lines on the right side of the figure represent the 417 fingerprinted clones making up this portion of the physical map. A subset of 24 clones spanning the region was selected for sequencing.

Figure 6



Spread of Chinese chestnut chromosomes (stained blue) probed with three physical map clones genetically mapped to the top, middle and bottom of LG_B, respectively:
1, CMCMBD110L01, labeled red;
2, III G6 CD175, and
3, CMCMBB166G01, labeled green.
Note strong red and green signals on two homologous chromosomes. The white arrowhead indicates a broken chromosomal fragment.

themselves can be probed with fluorescently-labeled DNA and the entire set of chromosomes and probes observed under a suitably-equipped microscope. Thus the fluorescently labeled DNA can be located in the genome. Much larger probes, about 100,000 bp long, are required to generate a detectable signal than those used for DNA clones.

Large-scale rearrangements of chromosomes commonly occur during the evolution of new species. When species are hybridized, such as during the first step of backcrossing, the chromosomal rearrangements can interfere with meiosis, the process by which the number of chromosomes is precisely halved to produce eggs and sperm. This, in turn, interferes with the genetic recombination that is used to construct genetic maps, potentially obscuring the location of resistance genes. We had tentative evidence for such an occurrence involving the chromosome carrying the most prominent region associated with blight resistance, *Cbr*1.

To investigate it, TACF provided funding to Dr. Faridi, (Islam-Faridi et al. 2009), work that he has continued after the termination of that grant (**Fig. 6**). To date, the evidence does not indicate that genes for resistance to chestnut blight are entangled in a chromosomal rearrangement, much to our relief.

DNA Sequence Assembly

We cannot presently take a chromosome and sequence its DNA from one end to the other. Rather, with current "next-generation sequencing" technology, the DNA is fragmented and sequenced in small segments, or reads, usually less than 500 bp long. As with the construction of a physical map from fingerprinted clones, the sequenced DNA segments are random, and overlapping reads are needed to assemble a sequence. It's like trying to assemble a puzzle with a million pieces, and on top of that, not all the pieces are present in the box. In effect, you have been handed a big box with dozens of incomplete copies of the puzzle all mixed together! And repetitive sequence is like having lots of pieces of blue sky. Consequently, it really helps to be able to narrow down the region of interest using the integrated genetic and physical maps. Sequence information from the clones can guide assembly of sequence for the entire strand. Using these techniques, the DNA sequence in three regions associated with blight resistance has been assembled and analyzed to identify all the sequences that could encode proteins. This yielded

Table 1

Region	Reference Base[1]	Reference Base[2]	Susceptible Base[3]	Log10(p)[4]	Predicted protein change[5]
Cbr1	G	G	A	-4.11	Trp to STOP
Cbr1	A	A	G	-3.89	Ser to Gly
Cbr1	G	A	G	-1.42	Gly to Arg
Cbr1	C	C	T	-3.53	Arg to Gln
Cbr1	C	A	C	-3.40	Glu to Asp
Cbr1	G	T	G	-4.45	His to Asn
Cbr1	G	G	A	-3.53	His to Tyr
Cbr2	G	A	G	-3.56	Asp to Asn
Cbr2	G	G	C	-3.46	Pro to Ala
Cbr3	C	C	T	-3.81	Met to Ile
Cbr3	G	G	A	-3.10	Cys to Tyr

Single nucleotides varying between pooled resistant and susceptible F_2 trees and the predicted effect on the protein gene product. In the majority of cases, the sequences from the resistant pool match the Chinese chestnut reference sequence; the exceptions are highlighted.

a list of 782 candidate genes, which is too many to test individually.

To refine this list in 2013, we made two bulks, or pools, of DNA, one from 11 blight-resistant F_2 trees, and the other from 14 susceptible F_2 trees. The bulks were sequenced, and we used the assembled blight-resistance sequences to identify sites where the bulk sequences differed by a single base. **Table 1** summarizes information about 11 sites that we consider most likely to be involved with blight resistance, because they are in predicted genes, and are predicted to alter the protein that is encoded by the gene.

What next?

- We are proceeding to evaluate more trees for these sequence differences to see if the association with blight resistance holds up.
- If the association holds, the gene will be used to transform American chestnut to test its effect on the tree's susceptibility to chestnut blight. William Powell and Charles Maynard (SUNY ESF) and Scott Merkle and Joseph Nairn (University of Georgia), with support from TACF and others, have spent many years of patient work to develop the tissue culture and transformation protocols for chestnut that make this possible.
- We also intend to search the entire chestnut genome with sequences from the resistant and susceptible bulks for additional regions associated with blight resistance.

[1] The base present in the reference Chinese chestnut sequence at this position.

[2] The majority base present at this position in the resistant pool.

[3] The majority base present at this position in the susceptible pool.

[4] Logarithm to base 10 of the probability by chi-square with Yates correction that differences between the resistant and susceptible bulks are not associated with marker genotype. A value of -3 equals a probability of 1/1000.

[5] The predicted change in an amino acid in the protein product of the gene due to the variant DNA sequence. The first variant in the table results in premature termination of the protein sequence.

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Learn more about this type of work and the people doing it at TACF's Annual Meeting in October.

See page 33 for more information.

BARBARA KNAPP

Barbara Knapp's history with trees began with her father, a state forester in charge of research in New Hampshire. By 1956, her family moved to a 10-acre oak forest in Germantown, Maryland, and she still resides on this property today.

After gypsy moths killed a large portion of her oaks in 1988, a forester pointed out that she was very lucky to have an American chestnut tree on her property. In fact, more were discovered, and these surviving American chestnuts have been used in the Maryland breeding program, as well as several Allegheny chinquapin.

"After the timber sale and with increased sunlight, more sprouts were found, and they grew fast. About this time I received a fundraising letter from TACF," said Barbara. "The brochure described the story of the American chestnut, which I had not heard before, and it also mentioned the organization's research farm in Meadowview."

Intrigued, Barbara decided to visit Meadowview with her daughter. Upon arrival, Fred Hebard welcomed them warmly and took them on an extensive tour of the orchard. Barbara was immediately hooked.

"Afterwards, I was involved for several years in various attempts to form a Chapter in Maryland. Despite a few false starts, we actually did get our Chapter going, with Essie Burnworth as a driving force," said Barbara.

Since then, Barbara has been avidly involved, whenever and wherever she can. Her favorite job is starting new orchards, planting nuts, and seeing how fast they grow. As a founding member of the Maryland Chapter, Barbara has not only served as secretary, but she has maintained her membership for 22 consecutive years.

"I also particularly enjoy going to see newly-discovered chestnuts. It is certainly exciting to find a new tree that is actually fruiting in an accessible location and big enough to pollinate," said Barbara.

In more recent years, she attends various workdays and photographs the events. Occasionally, she is able to wrangle her grandchildren to help with plantings and brush hauling. Her daughter, Emilie Crown, has also served as treasurer of the Maryland chapter for several years.

"Barbara exemplifies how one motivated volunteer can push our mission forward. She has a steadfast commitment to participate in chapter activities, a never-ending mission to recruit more members. Her energy and definitely her smile are extraordinary!" said Mid-Atlantic Regional Science Coordinator Matt Brinckman. "At any given moment she is ready to invite people to her home to show them her chestnut trees, photo albums of chapter events, and chestnut artifacts and memorabilia. Then she pins them down to sign up for an event. She is a cornerstone of the Maryland chapter."

Barbara is also a Master Gardener with several of her renowned pieces of garden work featured in various publications.

A visiting prominent chestnut researcher once wrote: "I think that Barbara's property is a gold mine for the Maryland chapter...it is a truly natural ecosystem, and the chestnut trees are native."

"Barbara exemplifies how one motivated volunteer can push our mission forward. She has a steadfast commitment..."

MID-ATLANTIC REGIONAL SCIENCE COORDINATOR MATT BRINCKMAN

POST HARVEST SEASON

Canker Assessment

By Jeff Donahue, Director of Operations, and Eric Jenkins, Technical Coordinator

Following harvest season, field activities at the Meadowview research facility focus on canker assessment and rogueing of the B_3F_2 seed orchards, and progeny tests. Canker assessments consist of two types: initial evaluation of recently inoculated two- and three-year-old trees and observation of naturally occurring cankers on older trees.

Generally young orchard plots are artificially inoculated in June and the blight cankers is assessed and measured in November and December. Evaluation of naturally occurring infections on older trees may continue in January, as weather permits.

The initial assessment of young trees consists of rating the severity of the wound reaction on a scale of 1 to 3, and measuring the length of cankers resulting from the inoculation. Since blight-resistance of B_3F_2 trees can range from poor to a rough equivalent of a Chinese chestnut, often times most of the trees in a plot can be removed.

The severity of cankers from artificial inoculations and naturally occurring infections is used to make selections among trees older than five years of age. Other considerations, such as tree age and presence of other healthy trees within the plot may influence the removal decision.

In the orchards, we have been trying several techniques to remove undesirable trees. These techniques vary based on the trees' ground line diameter (GLD). For young trees of 1-2 inch GLD, we have used a modified T-post puller which can be operated by one person.

For larger trees, we have several alternative techniques available. We can use a tractor to pull trees out of the ground with either a chain or a backhoe. In addition, a mini-excavator can be used to pull them with a chain or to dig them out. The mini-excavator is the most efficient method for removing a large number of trees because it can be moved and set up faster than a backhoe.

> Neighboring superior trees suffer from translocation of herbicides on rogued trees, thus, mechanical means of culling are primarily used.

The actual removal of undesirable trees at Meadowview is a mechanical process that involves a variety of equipment. In 2013, we removed approximately 2,200 trees from various orchards on the property, and due to the large-scale of the work involved, we are always looking for ways to improve efficiency. In 2012, a trial was conducted to assess the feasibility of using chemical means to remove trees without harming adjacent neighboring trees. 40 pure Chinese chestnuts were cut back to approximately six inches in height and the stumps were treated with either a 25% or 50% solution of glyphosate. The trees were five years old and averaged 15-20 feet tall and 3-5 inches GLD. In approximately 90% of the cases, signs of chemical translocation were noted in adjacent trees, with severity increasing with the treatment. Symptoms of translocation consist of rapid blackening of upper crown tip foliage. This indicates that root grafting occurs between Chinese chestnuts planted two feet apart. Due to the possibility of similar problems in our B_3F_2 orchards, we have focused on mechanical methods for removal.

Figure 1

Figure 2

Figure 3

Figure 1. An American chestnut individual in a progeny showing wound reactions from the two strains of blight used with artificial inoculations.

Figure 2. (Left) An example of an undesirable B_3F_2 individual to be removed. (Right) A superior, healthy selection, expected to remain in the orchard.

Figure 3. Modified T-post puller used to remove 1-2 inch (GLD) trees. Chain is wrapped around base of tree while lever is pushed downward.

Figure 4. Tractor-mounted backhoe used to remove undesirable trees. Tree can be pulled out with a chain (upper) or dug out with the bucket (lower).

Figure 5. Mini-excavator used to remove trees. The combination of heavy weight, low center of gravity and track mobility makes it an efficient means of rogueing in seed orchards.

Figure 4

Figure 5

HOW TO MAKE THE MOST OF YOUR Ceremonial Plantings

By Kendra Gurney, New England Regional Science Coordinator

Every year, TACF's State Chapters receive an allotment of Restoration Chestnuts 1.0. This is thanks, in large part, to TACF's sponsor membership program, which makes Restoration Chestnuts 1.0 available to the sponsor member, providing a small number to the member's local chapter.

In addition, some Legacy Tree sponsors also choose to donate their allotted seed to be used locally, which can really make an impact. The Restoration Chestnuts 1.0 that end up in the capable hands of TACF's State Chapters can be used for a variety of purposes – demonstration, outreach, or ceremonial plantings, thank you gifts for dedicated volunteers or donors, even raffle items for Chapter events – but figuring out how to get the most benefit from these coveted chestnuts can take some effort.

Local Nashua Boy Scouts dig a planting site in southern New Hampshire. Photo by The Nashua Telegraph

Many of TACF's State Chapters have used the majority of their Restoration Chestnuts 1.0 for demonstration, educational and outreach plantings. Often sites that have the best visibility, outreach potential or access to the younger generation, may not be appropriate for a full-scale orchard. But a small number of chestnuts and some visible signage can expose a much wider audience to our work with this important tree species.

Pulling off a good demonstration planting does not happen without some thought and planning. The PA/NJ Chapter has developed a distribution committee that evaluates requests for such plantings based on a set of criteria (visibility, viability, public relations, partnership and economic/membership status). The MA/RI Chapter has developed a demonstration planting committee that handles everything from assessing the request to overseeing installation, which is no small task. In addition, some chapters have set guidelines for demonstration plantings that include considerations like the minimum number of trees, requirements for signage, or the type of material to be used (seedlings vs. seeds). Many chapters like to plant their Restoration Chestnuts 1.0 in pots and provide seedlings to hosts, which can be a great approach, assuming you have a good grower or two in your Chapter willing to produce some nice looking trees.

If your Chapter has not seriously considered using Restoration Chestnuts 1.0 for outreach purposes, here are some suggestions.

- First, seriously evaluate all requests, or potential hosts to approach, for the benefit they will bring to your Chapter. The goal of this kind of planting is to increase visibility for the restoration of the American chestnut and bring attention to your local work. TACF has some great

Learn more about TACF's Sponsor membership program: acf.donorshops.com/products/sponsor.php

signage available that can add to the educational value of a small planting (acf.org/orchardsigns.php). Also remember that hosting Restoration Chestnuts 1.0 is still a member-benefit and recipients of these chestnuts need to be TACF members.

- Second, make sure the site is appropriate for chestnut. A couple of sad chestnuts struggling on a wet site or in high pH soil does not create quite the same impact as thriving chestnuts in their full glory. Look for sites that will allow the chestnuts to shine.
- Third, make sure you plant enough trees. Often not every tree will make it and planting a minimum of five will give you a better chance at still having a couple of trees in 5-10 years than planting just two.
- And finally, make sure to follow-up with and continue to engage the planting hosts. If their trees struggle or die, we can often find replacements, but no one will know replacements are needed if we lose touch.

Great Sites for Ceremonial Plantings include:

town, city or state parks

schools, colleges and universities

environmental education centers

natural history museums

summer camps

nature preserves

hiking and recreational trail systems

botanical gardens

arboreta

There are certainly many ways to approach a demonstration planting, and assessing the goals of the host can be a great way to hone in on the number and variety of trees to plant. In some cases, a small planting of five Restoration Chestnuts 1.0 and a simple sign are perfect. In other cases, a row each of American, Chinese, F1 hybrids and Restoration Chestnuts 1.0 and a 3-panel detailed sign might be a better fit. For additional resources, Walt Lange with the Ohio Chapter has developed a great "How-To" that covers many aspects of a good demonstration planting, which can be found here: ecosystems.psu.edu/research/chestnut/breeding/types/demo. Getting these kinds of plantings installed is a great way to put donated Restoration Chestnuts 1.0 to good use for your Chapter, and for the overall restoration of the American chestnut.

If you have any questions about a possible demonstration planting site, please contact your local regional science coordinator. Visit: http://www.acf.org/Staff.php for contact information.

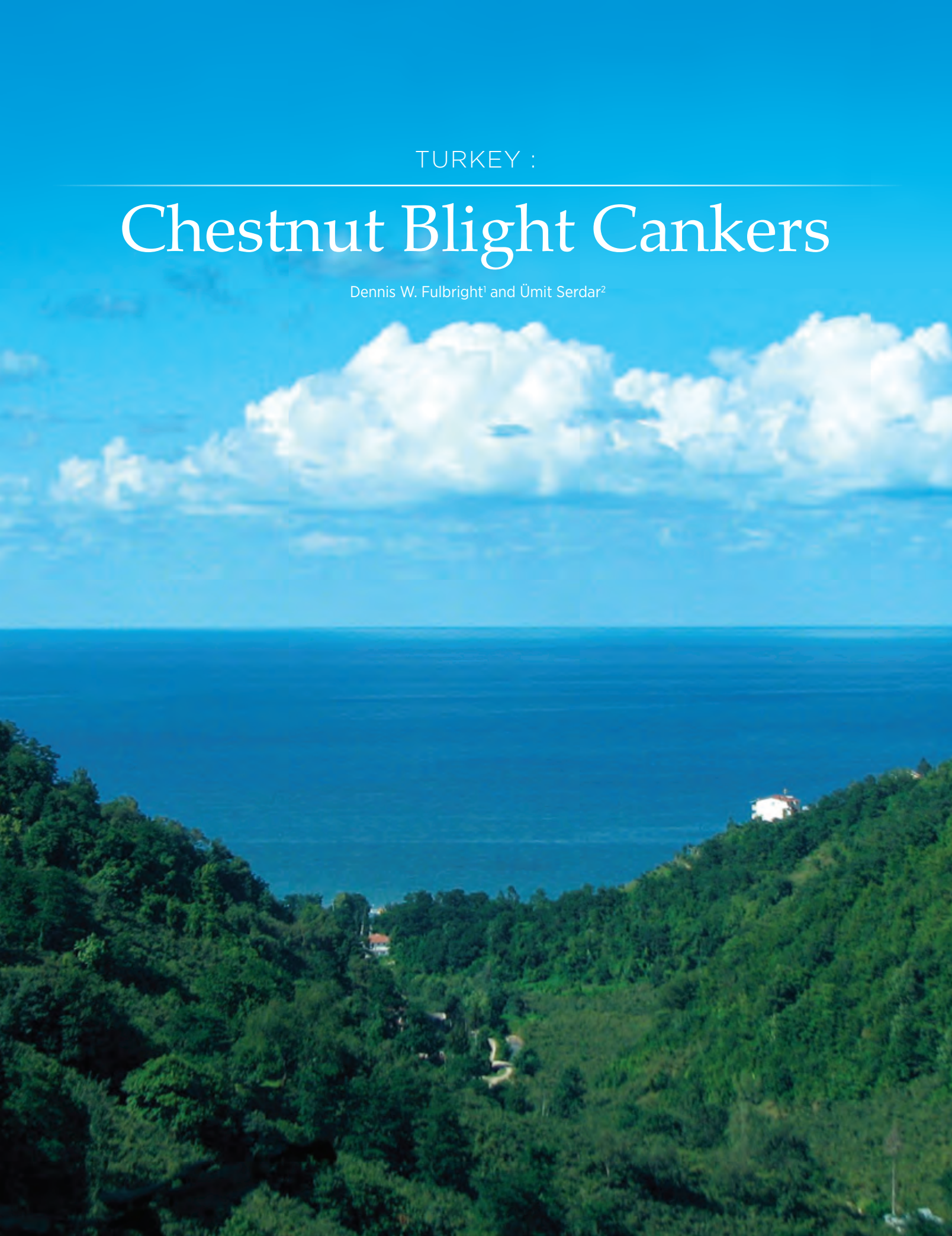
Alvirne High School (Hudson) Biology class, both students and teachers, help plant Restoration Chestnut 1.0 seedlings. Photo by Emily Provencher

Alvirne High School Forestry class students gather around the demonstration planting sites to help Restoration Chestnuts 1.0 take root. Photo by Emily Provencher

TURKEY :

Chestnut Blight Cankers

Dennis W. Fulbright[1] and Ümit Serdar[2]



Have you ever thought about the North American chestnut forest and how it will appear by the end of the 21st century?

How many trees, how close together and how tall will the trees be?

How will chestnut blight (*Cryphonectria parasitica*) infections manifest themselves on the trees?

Will all the trees survive blight, or just some?

How many nuts will the trees produce and will the seed produce a new generation of blight-resistant trees?

To get an idea as to how this future forest might appear, you may want to look to the chestnut forests and orchards of Turkey as they exist today. The Turkish chestnut forest is not perfect as it deals with chestnut blight, *Phytophthora* root rot and various old and new insect infestations. The initial iteration of the restored American chestnut forest in eastern North America won't be perfect either. The parallels between the current Turkish forest and the future North American forest may be surprising and draw strong and insightful comparisons. In 2013 we toured some of the major chestnut tree locations in Turkey and reported on the general conditions and use of the trees (Fulbright and Serdar 2013).

By looking closely at the Turkish chestnut forests and orchards, we may be able to see North America's not-too-distant future in terms of chestnut tree reaction to the chestnut blight fungus. For example, the Turkish chestnut forest is known to be composed of various strains of European chestnut made up of both natural and naturalized populations of the species (Mattioni et al, 2013). Chestnut blight has been present in Turkey for about 50 years, moving across the country from the eastern range of Europe to the western edge of Asia. While reducing the health of the chestnut forest and causing forest management issues, chestnut blight has not completely reduced the trees in the Turkish forests and orchards to remnant sprout populations as it did in North America. In fact, if the Turkish chestnut forest trees are not what they once were, at times, it is hard to tell. Today, chestnut forests and orchards in Turkey remain a vital part of communities where chestnut trees are still commercially utilized for their nuts, wood, and honey; even the catkins are sold for tea. In eastern Turkey, native forests are managed for timber and honey production, and depending on location, wild forest-grown nuts are collected and eaten. Orchards, more common in western Turkey, include both established orchards and forest trees grafted to specific cultivars of European chestnut. The cultivars chosen are based on end use such as fresh-market produce or processing into delicious candies called kestane sekari.

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North America is also full of chestnut diversity representing various species of Castanea including but not limited to American, Chinese, Japanese, and European chestnut as well as the various chinquapins and hybrids. The intermingling of all these species has created a "melting pot" of chestnut species in North America. Once released, TACF pollen will mix with the pollen of other species and the forest that grows 60 or 70 years from now will be a spectacular mix of genes, whether that is desired or not. The same thing will happen with blight, over time. Chestnut killing, virulent strains of *C. parasitica* will interact in various ways with less aggressive, hypovirulent strains constrained and crippled by both native (Fulbright 2007) and introduced hypoviruses (Double 2013) as well as by mutant mitochondria (Baidyaroy 2000) and inhibitory fungi and bacteria (Groome 2001). As the genetics of the trees and pathogen change, and as the climate changes, the disease we know as chestnut blight will surely change.

We are confident changes will occur because a "disease" by definition is the result of the combination of interactions of the three components we just mentioned—the host, the pathogen and the environment (climate included). This is why diseases have often been described as triangles in which the interactions of all components occur simultaneously. A change in any one of these components can influence the outcome of the disease, changes which are often utilized in disease management. Let's take a look.

If the host changes:

When blight-resistant Chinese chestnut replaces blight-susceptible American chestnut in the triangle, we obviously see a different result regarding the outcome of the disease we call chestnut blight. But it is not so clear as to what might happen to chestnut blight when American chestnut is replaced with European chestnut, Japanese chestnut, European x Japanese hybrids, American x Japanese hybrids, or American x chinquapin x European hybrids, or TACF blight-resistant trees, etc. What would we see in terms of disease outcome? Will the changes in disease be subtle or obvious? When utilizing certain European x Japanese hybrid cultivars in orchard situations, blight can be diminished but this is very dependent on the cultivar grown. For example, when using European x Japanese cultivars 'Colossal', 'Nevada' or 'Precoce Migoule' in Michigan orchards, blight can become epidemic within the orchard, but when using 'Bouche de Betizac' or 'Marigoule' chestnut blight appears to be reduced.

If the pathogen changes:

While people are concerned with *C. parasitica* becoming more aggressive or virulent, the fact is that the pathogen is becoming weaker. When looking at the pathogen component of the pyramid we need to consider the constraints the pathogen faces and its aggressiveness. While looking at pathogen component changes we also need to remind ourselves that the pathogen is constrained by hypoviruses, mitochondrial DNA mutations, and inhibitory fungi within the milieu of the canker all leading to a diminished role for the pathogen. For example, when the pathogen is infected with a hypovirus or carrying mutant mitochondria that reduces the growth of the pathogen, making it unable to kill the trees, or hemmed in by inhibitory microorganisms, we commonly see American and European chestnut trees surviving longer than if these constraints were not present. Infections do not necessarily stop appearing, but the result is not always quick death of the tree, which is a change in the expected disease outcome. Now add these pathogen component changes along with host component changes mentioned above to the triangle and we can begin to predict that there will be disease outcome changes.

Environmental changes:

This is the real speculative aspect to disease change as climates and growing zones change through time. A clear-cut vision of what could happen might be found if one looked to the west coast of North America. While a substantial quarantine prevents *Castanea* species and therefore the blight fungus, from entering the Pacific Northwest, growers will tell you that chestnut blight has been present, but it has never developed into epidemics on the chestnut trees planted there. Some suggest it is the warmer temperatures and dryer summer air. It might be that plus the mix of American, European, and other chestnut species and hybrid populations found in California, Oregon, Washington, and British Columbia. What we do know is that environmental aspects of our disease triangle will be changing and climate change needs to be added to the triangle along with changes in host and pathogen.

In the following photos, we show various types of cankers, describe the germplasm on which the infection is occurring, describe the location and perhaps show you the appearance of the near-term future of the North American chestnut forest.

A 20-year-old European chestnut (Castanea sativa) infected by Cryphonectria parasitica. Landowner stated he thinks the swellings were symptoms of hypovirulence since tree is growing well. Others did not think it was hypovirulence because the bark swelling was so large. Grower treats some cankers with ash believing that ash somehow cures the cankers.

How will these changes to the disease triangle alter chestnut blight?

We might get a hint by observing the chestnut trees in Turkey. Even though chestnut blight has been part of the chestnut forest of Turkey for more than 5 decades, chestnut trees appear to react differently to blight from location to location; and in many cases chestnut is, at times, actually winning the struggle. What cannot be assessed is how the Turkish chestnut trees accomplish this feat. Is it the genetics of the tree, the genetics of the fungus, the presence of inhibitory microorganisms, the climatic zones, or a combination of all of these?

This is what we do know about Turkey:

European chestnut (*C. sativa*) genetic diversity exists (Mattioni et al, 2013), hypoviruses and hypovirulent strains of *C. parasitica* have been isolated from most chestnut growing regions of Turkey (Akilli et al, 2013), fungal species inhibitory to *C. parasitica* are present in non-lethal cankers (Akilli et al, 2011), and the tree is growing in many different climatic zones. The outcome of this diversity is a struggling, but surviving, chestnut forest including orchards worthy of a chestnut industry.

As one walks through the chestnut forests there, one must always keep in the back of their minds that the chestnut blight pathogen population with its various levels of aggressiveness is interacting with a tree species with perhaps subtle levels of resistance. The various locations of the chestnut from the Mediterranean climate of

southwest Turkey (Aydin), to the northwest (Bursa), and to the north/northeast represented by Samsun, Fatsa, Ordu, Terme all the way to the Macahel Valley, also plays an important role in disease outcome. The complexity is amazing and so are the trees with their reaction to chestnut blight.

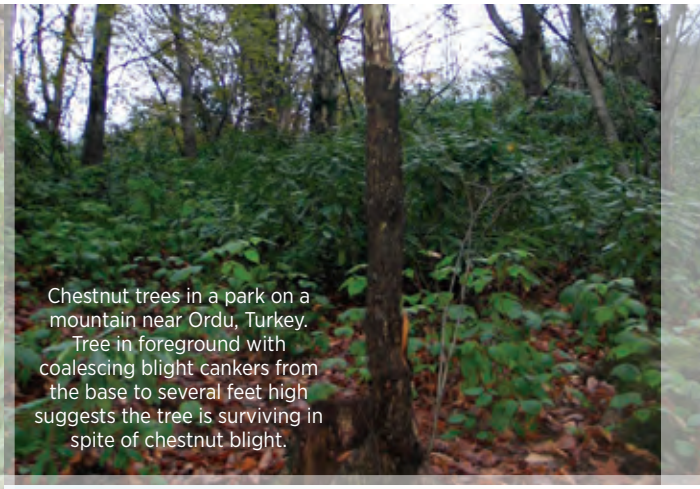
In order to determine how much chestnut is still on the mountainsides around the Black Sea cities of Samsun, Fatsa, Ordu and Terme, all you have to do is look down while walking the mountain paths in November. Look down, because beneath you are thick layers of golden yellow and brown leaves cushioning each step as you walk through the forest–something lacking in North America's eastern forest today. If you look up, you will see the bare stems of fall trees bearing multiple scars where trees wage battle against the chestnut blight fungus. Too numerous to be ignored, cankers are present but in this forest the cankers appear to be benign. At times there is nothing but chestnut stems, leaves, burs and nuts as far as you can see. It is reminiscent of chestnut forests in the Cimini Mountains near Viterbo where chestnut trees, today, easily resist the chestnut blight disease due to hypovirulence. If either site was in North America, Americans and Canadians would be thrilled, whereas Turkey is rightfully concerned about chestnut blight and the fate of their chestnut forests. North Americans would probably be delighted if their chestnut forests could ever look this good again. Yet, for Turkey, these forests represent a step backwards from pre-blight times.

Close up of canker on tree in Figure 1. Even though the pathogen has girdled the stem, enough wound tissue is present to keep the tree alive. Survival is due to hypovirulence, other natural bio-pesticides, tree resistance, environmental scenario or ash treatment.

Summary:

In Turkey, due to the presence of genetic diversity of the tree, inhibiting agents of the blight fungus, and the various locations of the trees, it is nearly impossible to determine the fate of the canker and the tree simply by looking at cankers on stems. We would strongly recommend that members of The American Chestnut Foundation take the trip of a lifetime and go to Turkey to see the chestnut forests during the International Chestnut Symposium in 2016. There are wonderful Roman ruins from the past to see, but make sure you keep an eye to the future of chestnuts on the slopes around you.

Professor Umit Serdar and Turkish forester photograph non-lethal cankers on European chestnut trees while standing on a bed of freshly-fallen chestnut leaves. Non-lethal cankers can be seen on many other trees in the photo.



Chestnut trees in a park on a mountain near Ordu, Turkey. Tree in foreground with coalescing blight cankers from the base to several feet high suggests the tree is surviving in spite of chestnut blight.

In spite of chestnut blight, *Phytophthora* root rot, chestnut Asian gall wasp and other plagues, growers are still planting chestnuts in orchards for nut production. Some trees are pure European and others are European X Japanese hybrids. All are grafted to favorite cultivars for the region.

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In the autumn we waited for the excitement of the first frost and opening of the bur. Trips up the mountain brought home baskets of chestnuts with that distinctive, sweet firmness which lingers in memory.

Marcus M. Gulley, MD

Letter to The American Chestnut Foundation, circa 2006
P. O. Box 4044
Bennington, VT 05201

Dear The American Chestnut Foundation,

In the late 1920s and the early 1930s I lived in a village named Crozet, located at the foot of the mountains north of Charlottesville, Va. The mountains were beautifully green with great chestnut trees that marked our seasons.

In the autumn we waited for the excitement of the first frost and opening of the bur. Trips up the mountain brought home baskets of chestnuts with that distinctive, sweet firmness which lingers in memory. The fun and the flavor mingled. This was especially true with the smaller chinquapins. I could collect a pocket full on my way to school and have a day's worth of good eating, as well as a supply of missiles that bedeviled the girls.

Of course those days preceded anyone's idea of air conditioning other than a breeze through an open window. I would sleep with my head close to the window and enjoyed the panorama of the mountain. It was a great shock to see a line of brown moving day by day across the mountain. The concept of the blight was difficult for a boy to grasp and it took a considerable period of time for me to understand the inexorable march of destruction I was witnessing. I could not believe our chestnut trees would not come back.

My feelings were mixed in a way that a boy has difficulty expressing. They were a mixture of disbelief and vague grief that persist to this day. Mixed with this, of course, was that gift of the human spirit, hope.

I celebrate the persistent energy of the American Chestnut Foundation. I anticipate the day when my nostalgia blends into warm pleasure at your success—and the blacksmith can enjoy the shade.

Very truly yours,

Marcus M. Gulley, MD
Crozet native

'Perfect' Chestnut Scones

(ROASTED CHESTNUTS, DRIED FIGS, AND VANILLA BEAN)

By: Lady and Pups food blogger Mandy Lee: ladyandpups.com/2013/11/08/perfect-winter-scones-eng/

Ingredients:

3.7 oz. (105 grams or 5 - 6 large) dried figs + 1 tbsp. of whole milk

Chestnuts puree:

- 6.3 oz. (180 grams or 1 ¼ heaping cup) of roasted and peeled chestnuts (the weight DOES NOT include shells, and PLEASE trust the weight not the cup
- Seeds from 1 vanilla bean
- 7 - 8 tbsp. of whole milk

1 ¾ cups (228 grams) of all-purpose flour

¼ cup (50 grams) of sugar

1 tbsp. of baking powder

¼ tsp of salt

9 ½ tbsp. (135 grams) of very cold unsalted butter, cubed

¼ cup + 2 tbsp. of whole milk

1 large egg, separated

Turbinado sugar for sprinkling

Use a scissor to cut the dried figs into tiny bite-size pieces, then combine with 1 tbsp. of whole milk and microwave on high for 1 minute to plump up (pause the microwave and give it a stir at 30 seconds). Chill in the fridge for 15 min or until cooled down.

Split open the vanilla bean and scrape out all the black seeds. Process the seed with the roasted and peeled chestnuts in a food-processor, gradually adding in 7 - 8 tbsp. of whole milk until it's pureed as COLD peanut butter-consistency (should be stiff and holds its peak). Set aside.

In a stand-mixer with paddle-attachment, stir flour, sugar, baking powder and salt together just to combine. Add the cubed unsalted butter and mix on low, until the butter is mostly incorporated into the flour mixture, like the texture of coarse meal with larger butter pieces looking like flat disks about 1/2" wide (slightly smaller than a penny). If you are using a pastry blender, the largest butter bit should be the size of small peas with the rest of the mixture looking like coarse meal. Now add the dry figs from the fridge and mix on low until they are separated from each other (they can stick) and evenly spread out. Add all of the chestnuts puree, 1/4 cup of whole milk and 1 large egg yolk (save the egg white for egg wash!). Mix on low for a few seconds to bring the dough together. You may need to add 2 tbsp. more of whole milk in order to do so. DO NOT over-mix. Stop just when the dough seems to have come together, then dust the working surface lightly with flour and transfer dough on top, press all the "loose ends" together with your hand to bring it together.

Pat the dough into a flat disk and plastic-wrap it. Chill in the fridge for AT LEAST 2 hours! This is important NOT ONLY to re-chill the butter inside the dough for puffing, but also to give time for the flour to absorb the moisture in order for the scones to be moist, and not dry and "floury-tasting".

30 minutes before baking, preheat the oven on 400ºF/200ºC. Whisk the egg white with 1 tsp of water until frothy.

Lightly dust the working surface with flour. Unwrap the dough and roll it out into a 1"/2.5 cm thick rectangle. Line a baking sheet with parchment paper and bake AS MANY AS YOU ARE GOING TO EAT. Scone is at its highest value when it's fresh. Plastic-wrap the rest and keep in the freezer.

Brush the top of the scones with egg white-wash and sprinkle with turbinado sugar. Bake in the oven for 17 - 20 minutes until golden browned on top (add 2 - 3 minutes for frozen scones).

Just 10 - 15 minutes on the cooling rack will allow the scones to set and the flavors to "round up".

TACF 25 CONSECUTIVE-YEAR MEMBERS

As a non-profit, TACF thrives on the support of its members. The individuals listed below represent the type of loyalty and dedication that enables TACF to make great strides in restoring the American chestnut. As an organization, we extend tremendous gratitude and honor to our 25 Consecutive-Year members:

James Marshall
James and Susan Ditmore
Roberta Bocock
Frank DeCocco
Willis Land & Goat Co.
Richard Habermann
Henry Heckler
Steven Wright
Marshall Carothers
David and Joyce Foster
John and Helga Radick
Charles Rice
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Dr. Joe and Sandra James
Walter Kloc
Edward and Laurie-Anne Foley
Kenneth and Patricia Summerville
Alan Plisch
Charles Henry Smith
Herbert and Jane Darling
Al Ellingboe
David and Anne Bingham
Michael Doochin
Charles Mackall

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

IN MEMORY & IN HONOR OF OUR TACF MEMBERS

September – December 2014

In Memory of:

Jeanne Augustinsky
By Michael Kenton

Anne Chestnut Bartlett
By Deborah Robina

Virgil R. Beary
By Oliver and Eileen Evans

Shirley Dishaman Bell
By Sarah O'Neill

Pamela C. Binnicker
By Dorothy Reichenbach

Richard T. Blair
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By Rebecca Anderson

James Rogers Sollers
By Deborah and John Fialka

Ivan and Ellery Stancliff
By Ronald Stancliff

George Thompson
By Tim and Michelle Brookshire

Lisa Thomson
By Lewis and Kate Lobdel and Dr. William Lord

Kenji Van Uytricht
By Diana Abrashkin

Save the Date

TACF ANNUAL FALL MEETING

in collaboration with

THE SCHATZ TREE GENETICS COLLOQUIUM

WHAT:
Integrating Genomics Tools in American Chestnut Restoration

WHEN:
Friday, October 23 – Saturday, October 24

WHERE:
Penn Stater Hotel and Conference Center, University Park, PA

Scientists from around the world will present research related to various elements of chestnut genomics including genome sequencing, genetic mapping, marker-assisted selection techniques, and more. This spectacular event will include many hands-on learning opportunities such as DNA extraction in the lab as well as a chestnut genome-sequencing workshop with the scientists who actually did the sequencing. Participants are encouraged to ask genetics-based questions during an open panel discussion with experts. Keynote addresses are scheduled for Friday and Saturday nights. There will also be a field trip to the Penn State Arboretum's BC_3F_2 seed orchard to observe highly-resistant American chestnuts from the PA Chapter's breeding program.

For additional information, please visit: acf.org/AM2015.php

THE AMERICAN
CHESTNUT FOUNDATION
NATIONAL OFFICE
50 N. Merrimon Avenue, Suite 115
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Securing the future of the American chestnut

Please help us to secure the future restoration of the American chestnut tree by remembering The American Chestnut Foundation in your estate planning. In this way, you can continue your commitment to the restoration of this iconic species for the benefit of future generations.

For more information about giving opportunities, please contact us:

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