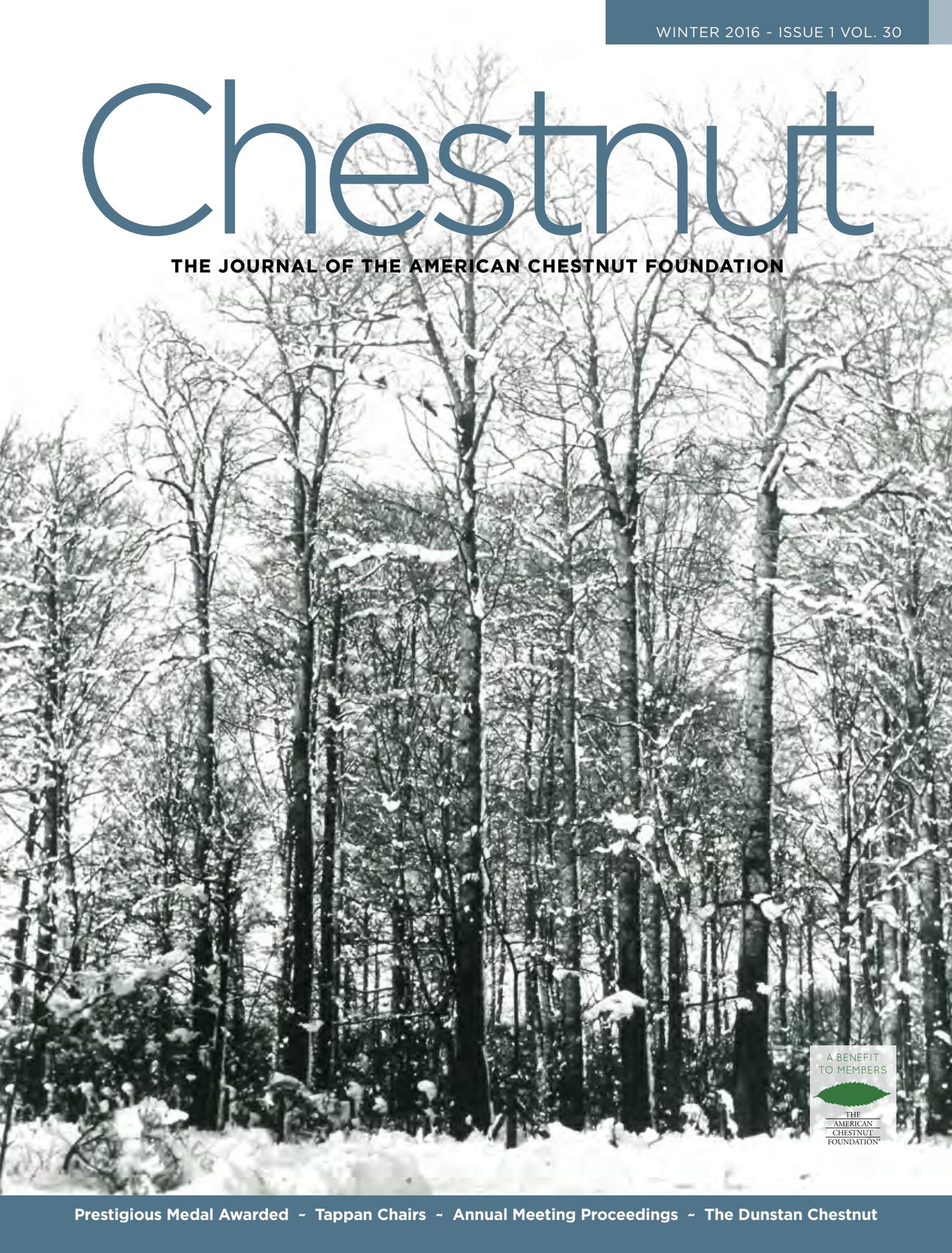


# Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION



A BENEFIT  
TO MEMBERS



THE  
AMERICAN  
CHESTNUT  
FOUNDATION

# Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

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A BENEFIT  
TO MEMBERS



THE  
AMERICAN  
CHESTNUT  
FOUNDATION



Lisa Thomson

President and CEO

## DEAR CHESTNUT ENTHUSIASTS,

This holiday season, I am fully immersed in all things chestnut, and I am seeing signs of them everywhere! Walking the streets of New York City recently, I passed a vendor selling roasted chestnuts and heard the famous line “chestnuts roasting on an open fire” beautifully sung by a passerby at the same moment. As a seasonal treat, Starbucks sold Chestnut Praline Lattes. Local friends here in Asheville, a chef, a brewer, and a chocolatier, reached out to us to include chestnut flavors in their concoctions. In December, Maine supporters celebrated finding the tallest American chestnut tree at 115 feet, and a local farm to table restaurant hosted a chestnut-themed dinner that evening. Chestnuts are certainly part of our culinary culture, so I hope you enjoy the soup recipe on page 32 as a warm and hearty meal this winter.

It has also been an exciting time for the Foundation. Thanks to our friend and advocate Chuck Leavell, keyboardist for the Rolling Stones and award winning tree farmer, we launched our first online video campaign via the web and social media, including Chuck's own Mother Nature Network. Response has been overwhelmingly positive, and we look forward to having other luminaries help us spread the word of the innovative work of the Foundation. If you haven't seen the video yet, make sure to visit our website or our Facebook page to hear Chuck share his chestnut story.

Along with a strategic planning process, we are working hard to bring together all available scientific tools and partners. We are continuing to refine our traditional backcross breeding program while strengthening our partnership with the New York Chapter's effort to support the biotechnology research at SUNY-Environmental Science and Forestry. There is also promising work being done by our colleagues at West Virginia University and the University of Maryland on hypovirulence to weaken the blight fungus. We have received feedback from our members and supporters who would like choices to support these different methodologies, with the goal being the same: bring back the species in our eastern forests. These advances in the scientific community were showcased at a recent joint meeting with The American Chestnut Foundation and the Schatz Center for Molecular Tree Genetics at Penn State. I hope you enjoy reading the proceedings from these renowned scientists on pages 12-20, many of whom have worked for decades to find the key to this critical forest restoration challenge.

Some of these challenges may take years, even decades. But we are patient and determined to see this vision through. This issue of Chestnut is dedicated to those persistent and talented scientists whose curiosity and dedication are the reason The American Chestnut Foundation exists. Please join me in thanking them for their important work!

Wishing you a wonderful start to 2016,

Lisa Thomson, President and CEO  
The American Chestnut Foundation



Follow me on Twitter (@MadameChestnut).

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# Sweet Chestnuts

Sweet chestnuts (*Castanea sativa*)  
in the Knerthenborg forests of Denmark.  
Photo by F.M. Knuth, circa 1940s.  
Image courtesy of the Connecticut  
Agricultural Experiment Station.

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THE  
AMERICAN  
CHESTNUT  
FOUNDATION®

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## 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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## THE AMERICAN CHESTNUT FOUNDATION

# Awarded Prestigious Medal

by Massachusetts Horticultural Society

The Massachusetts Horticultural Society (MHS) awarded The American Chestnut Foundation (TACF) with the Jackson Dawson Memorial Award medal for 2015. Founded in 1829, MHS is the oldest, formally organized horticultural institution in the United States.

On October 15th, President and CEO Lisa Thomson attended the Honorary Medal's Dinner at the MHS headquarters in Wellesley, MA to accept the prestigious Jackson Dawson Memorial Award. The award praised the Foundation for its exceptional skills in the propagation of woody plants and work to restore the American chestnut to the eastern woodlands.

Every year, MHS hosts the Honorary Medal's Dinner, a 116-year tradition, to celebrate and honor leading figures of horticulture for their dedication to excellence within the field and for the public good.

"This tradition gives us the opportunity to recognize the outstanding contributions of horticulturalists, plant innovators, and those who have made significant contributions to the enjoyment and appreciation of plants and the environment," said Katherine MacDonald, MHS president of the honorary medal's committee.

The Jackson Dawson Memorial medal is awarded for skill in the science or practice of hybridization or propagation of hardy, woody plants. It was named for Jackson Thornton Dawson, regarded as one of the world's greatest gardeners of his time. Dawson immigrated to the United States from England in 1849, settling in Andover, MA. After serving with the Massachusetts Nineteenth in the Civil War, Dawson became a U.S. citizen and began working at the Bussey Institute under Francis Parkman. Shortly after, he became involved in



Lisa Thomson, center, TACF President and CEO, with Katherine Macdonal, President of the Massachusetts Horticultural Society and Wayne Mezitt, Chairman of the Board, Massachusetts Horticultural Society. Photo courtesy of the Massachusetts Horticultural Society.

the development and establishment of the Arnold Arboretum and served as Superintendent of the Arboretum for 43 years.

Past recipients of this respected award include: The New England Wildflower Society and Nasami Farm, Robert L. Johnston, Jr., founder of Johnny's Selected Seeds, and Dr. Elwin R. Orton, Jr. of the Orton Patents.

MHS provides information on horticulture and related sciences, disseminating this information through

its library, education programming, exhibitions, and community outreach initiatives. It also has successfully championed many important issues throughout its history, such as the introduction of food plants (Concord Grape, 1853), the garden cemetery movement (Mount Auburn Cemetery, 1831), the school garden movement (1880s), the adornment Boston's back alleys by establishing home gardens (1930s), the victory garden movement (1940s), and the garden history movement (1990).



# Tappan Chairs

and TACF: A Handcrafted Partnership

By Stephanie Parker

One of the American chestnut's many great qualities is its beautiful, high quality, rot resistant wood – wood that is currently quite hard to come by. But when the Vermont/New Hampshire chapter harvested an almost completely unwormed American chestnut in Berlin, VT, the opportunity arose to use this precious wood to make something truly special.

A four slat Tappan rocker, similar to the model of Tappan rocking chair presented to Dr. Fred Hebard at TACF's 2015 Annual Meeting. Photo by Adam Nudd-Homeyer.



The current home of Tappan Chairs in the late-1800s with Tappan Chairs on the porch, back when it was a parsonage. Photo by Adam Nudd-Homeyer.



VT/NH chapter member Randy Knight (left) and Adam Nudd-Homeyer (right) display chestnut wood stored at Knight's barn in Perkinsville, Vermont. Photo by Yuri Bihun.



To that end, Vermont/New Hampshire chapter president Yuri Bihun got in touch with chair-maker Adam Nudd-Homeyer, the owner of Tappan Chairs, in Sandwich, NH, a nearly 200-year old manufacturer of high-quality, handmade, wooden chairs.

"We were in touch with Adam for more than 18 months before we pulled the trigger, waiting for just the right woodworker and opportunity to use this precious wood," Bihun says. Nudd-Homeyer was excited, and nervous, for the opportunity.

"There was a big unknown of how it would perform," Nudd-Homeyer says of the wood. But he relished the opportunity to work with such a rare material.

"While an exotic wood may take some work to import it, at least, is available. Unwormed American chestnut, on the other hand, must first be found! It's almost like finding a prehistoric animal still alive and hiding midst the wild."

Nudd-Homeyer first became involved with TACF in 2014, and quickly volunteered to lend a helping hand to the effort

by contributing a beautiful Tappan chair, made of American chestnut of course, to a fundraising campaign. He ended up crafting two wonderful rocking chairs, one which was presented as a retirement gift for TACF's Chief Scientist

Emeritus, Fred Hebard, at TACF's national conference, and another that will be auctioned off to one lucky TACF donor.

He chose to create two tall-backed, five slat rocker chairs, a popular and classic style for Tappan, which invoked the imagery of the chestnut leaf, especially its hooked-edge shape, as influence.

## For a chance to win

Nudd-Homeyer's American chestnut Tappan chair, join, renew, or upgrade your existing TACF membership to the \$250 level or above between February 1 and March 30, 2016. You will be automatically be entered into a drawing for this beautiful chair, valued between \$2000-\$3000.

After he finished, he gave them the ultimate test: a vigorous rocking. "Using a rocking enthusiasm not to be tried at home, I was pleased to discover that while American chestnut is relatively soft, it is incredibly springy and supple. The result was a pair of pleasing, and functional, chairs. And it was a good thing - I had to leave for the national conference, with them in hand, the next day!"



# THE AMERICAN CHESTNUT FOUNDATION EARNS Four-Star Rating

FROM CHARITY NAVIGATOR



The American Chestnut Foundation (TACF) is proud to announce its recent four-star rating for 2015 from Charity Navigator, America's largest and most influential charity rater. 2015 marks the ninth year that TACF has earned the organization's highest rating.

Since 2002, using objective and data-driven analysis, Charity Navigator has awarded only the most fiscally responsible organizations its four-star rating. In 2011, Charity Navigator added 17 metrics, focused on governance and ethical practices, as well as measures of openness, to its ratings methodology. These "Accountability & Transparency" metrics,

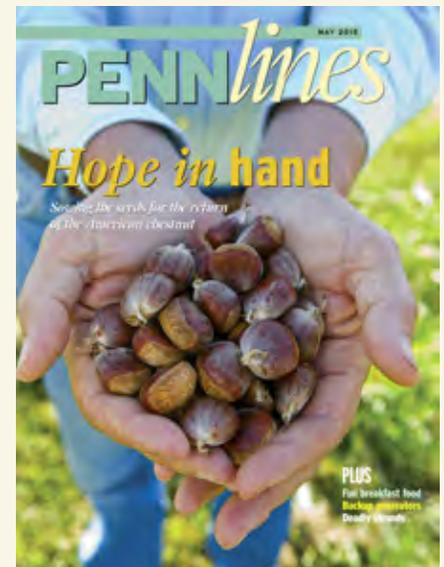
which account for 50 percent of a charity's overall rating, reveal which charities have "best practices" that minimize the chance of unethical activities, and whether they freely share basic information about their organization with their donors and other stakeholders.

Charity Navigator is also a 501(c)(3). This ensures that their ratings remain objective. Furthermore, in their commitment to help America's philanthropists of all levels make informed giving/social investment decisions, Charity Navigator does not charge users to access their data. Registration is a free service. **TACF's rating is available at [charitynavigator.org](http://charitynavigator.org).**

"HOPE IN HAND: SOWING THE SEEDS FOR THE RETURN  
OF THE AMERICAN CHESTNUT"

## Earns Top Honor

Congratulations to Penn Lines magazine, published by the Pennsylvania Rural Electric Association, for its recognition at the 2015 National Electric Cooperative Statewide Editors Association Willies Awards. The publication's May 2015 article, entitled "Hope in Hand: Sowing the Seeds for the Return of the American Chestnut," earned a first-place honor for feature writing. TACF certainly thinks the subject is award-winning, and we appreciate the opportunity to work with Penn Lines writer Kathy Hackleman to bring more attention to this effort.



The American chestnut tree discovered in Lovell, Maine stands at 115 feet in height and nearly 16 inches in diameter. This tree is now considered the tallest living American chestnut tree within the native range. All photos by Jared Westbrook.

## NORTH AMERICA'S TALLEST LIVING AMERICAN CHESTNUT TREE DISCOVERED IN MAINE

Scientists, forestry officials, and members of The American Chestnut Foundation (TACF) officially recorded the tallest living American chestnut tree in North America on Wednesday, December 2nd. Measuring at 115 feet, the tree resides in Oxford County, near the town of Lovell, Maine.

University of Maine forest scientist and TACF member Dr. Brian Roth discovered the tree earlier in the summer as he and one of his graduate students flew over the area in a small plane. At the time, the tree was in bloom, and the two passengers spotted it immediately. The official measurements taken in December proved what was already suspected when the tree was first spotted this summer.

At 115 feet, the 'Lovell' tree is the tallest American chestnut tree in North America, and is second only to the tallest known specimen in the world, a 121-footer located in an arboretum in Belgium. "If this keeps growing, it will surpass that," Roth said.

TACF's President and CEO Lisa Thomson and Quantitative Geneticist Jared Westbrook, along with representatives from the Maine Forest Service and the University of Maine Foundation, were present for this special event. TACF representatives spoke with media about the tree's significance and the research efforts to bring the species back. It is estimated that there are only a few dozen large, surviving trees such as this one left in the Maine woods.

The 'Lovell' tree resides on land bequeathed to the University of Maine Foundation by the Volk family. Douglas Volk (1856 - 1935) was a famous American portrait and landscape painter with works that can be found in most American collections. The Volk family owned the property for over 100 years before donating it to the University Foundation.

**Photo captions  
(top to bottom):**  
TACF President and CEO Lisa Thomson poses next to the discovered American chestnut tree in Maine.

Local press was present during the official measurement of the large, living American chestnut tree. Dr. Brian Roth was one of the TACF representatives interviewed.

Maine Forest Service Forester Shane Duigan measures the diameter of the tallest chestnut tree in North America in Lovell, Maine.



TACF WELCOMES ITS NEW DIRECTOR OF DEVELOPMENT

# Victor Hutchinson

IN SEPTEMBER, 2015



“As the director of development, my role is to raise financial support for the foundation...”

**Chestnut: What initially attracted you to TACF?**

**Vic:** I believe that the plight of the American chestnut tree and the Foundation’s efforts to restore it to the forests and ecosystems of North America is truly an American story. The tree itself contributed much to the success and thriving nature of the settlement of this country. By providing food for wildlife and livestock, rot resistant materials for shelter, as well as the energy and nutrients it returns to the earth, the tree was indispensable. I knew that I wanted to be part of the Foundation’s efforts to restore the chestnut to the country and help make it a true American success story.

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

**Vic:** As the director of development, my role is to raise financial support for the foundation by linking people with a passion for the American chestnut tree to the scientific research and species reintroduction efforts of the Foundation. The past and future successes of the Foundation are not possible without the people that help us.

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

**Vic:** I grew up in the small town of Youngstown, New York, located at the mouth of the Niagara River and Lake Ontario. I attended Alfred State College (SUNY) and Bowling Green State University, where I received my Associates and Bachelor’s degrees in Electrical Engineering Technology. I later returned to school at Clemson University, where I earned my Masters in Business Administration. After graduate school, I worked at the Appalachian Hardwood Center at West Virginia University as an Extension Specialist, helping members of the state’s wood industry with energy conservation, resource utilization, and business development issues. I went on from there to work in development with the School of Medicine at West Virginia University and the Regional College of Veterinary Medicine at Virginia Tech. Most recently, I have been running my own business providing consulting to business and industrial clients on energy usage and electric utility billing best practices.

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

**Vic:** I think my favorite part of working for TACF is being a part of a noble conservation effort. Knowing that people in the future will be walking through forests full of American chestnut trees, and that I will have been a small part of that, brings me great satisfaction and joy. I also very much enjoy interacting with others and helping them achieve goals that they would not be able to achieve alone. I could not ask for a better organization to work for!

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

**Vic:** I hold both United States of America and Italian citizenship. I was raised in a large Italian family. I know, with a name like Hutchinson, how is that? My Grandfather, Big Vic Cheavacci, was a big part of my life and I idolized him and his brothers. So, a few years ago, after many years of paper work, I was able to obtain my dual citizenship with Italy.

**Chestnut: Favorite activity to do in your free time?**

**Vic:** During most of my free time you will find me on my mountain bike. Riding along the trails and getting to see areas that most people never get to. Staying fit and enjoying a ride, there is nothing better than that, in my opinion. I plan to take advantage of the great riding that Asheville and western North Carolina offer.



## John Wenderoth

Born and raised in the suburbs just outside of Baltimore, it was the summers on his grandfather's farm when John Wenderoth found his passion for nature. The farm, located along the coastal plain near the rivers that feed the Chesapeake Bay, was where Wenderoth learned about the birds and trees of the forest. It was these experiences that led him to Penn State University to study forestry.

After graduating in 1963, he went to Duke University to study chemistry, math, and botany. At Duke, Wenderoth was introduced to the chestnut blight fungus causing cankers on a common southern oak. This piqued his interest in researching the destructive aftermath of invasive species. However, his time at Duke was cut short by a call to join the Peace Corps. For more than four years he served as a volunteer in Nepal, where he worked on reforestation, education, and erosion control.

He returned to the U.S. to complete his master's degree at the University of Pennsylvania before starting a job at the Economic Research Service, a USDA agency just outside Philadelphia. "I worked as an economist and planner involved in resolving land-use conflicts," Wenderoth explains. "Many people thought the agency was a front for the CIA."

When he retired in 2009, he looked for a way to engage in something meaningful. He read a book about the loss of the American chestnut, which encouraged him to work with The American Chestnut Foundation. "The Pennsylvania chapter is a welcoming group, so in short time I was invited to join the board."

Two years ago, Wenderoth became the chapter's vice president, which led to him being elected as chapter president this past January. He is about to enter his second, and final, year in this role.

"During the first year, I visited more of Pennsylvania than ever before, and I become more interested in learning about genetics and understanding how to promote public appreciation of the significance of the loss and restoration of the American chestnut," he says. "And I'll continue to work with our chapter's committees when my time as president is over."

"What amazes me the most about John is the level of dedication he brings to the organization," says Sara Fitzsimmons, the North Central Regional Science Coordinator. "You only have to work with him once and it's easy to see how he has poured his heart and soul into this work. He's one of rare individuals that enjoys both the boots-on-the-ground work and the development/fundraising side of what we do."

Almost three years ago, Wenderoth and his wife Annette relocated to the Wilmington, Delaware area. "I may be the first chapter president who does not live in their designated state," he laughs.

His other interests include gardening, particularly in establishing self-sustaining landscapes, and spending time with his six children and ten grandchildren.

"What amazes me the most about John is the level of dedication he brings to the organization. You only have to work with him once and it's easy to see how he has poured his heart and soul into this work."

SARA FITZSIMMONS,  
NORTH CENTRAL  
REGIONAL SCIENCE  
COORDINATOR

# GEORGIA CHAPTER MILESTONES: 10 years of Breeding

## AND THE FIRST SELECTED BACKCROSS LINES

By Martin Cipollini and Royce Dingley

The Georgia chapter, which initiated its first backcrosses in 2005, has finally begun producing its first lines of selected  $BC_3F_2$  (and  $BC_4F_2$ ) seeds for seed orchards.

Berry College GA-TACF intern Royce Dingley harvests  $BC_3F_2$  burs from a selected  $BC_3$  tree at the Berry College backcross orchard near Rome, GA. The line being collected is one of the first five selected backcross lines produced by the Georgia chapter. Photo by Martin Cipollini.

This achievement comes in the face of several challenges, including serious *Phytophthora* root rot and Asian ambrosia beetle problems in many orchards, along with a relative dearth of wild American chestnuts capable of flowering in Georgia, and in the number of “Graves”  $BC_2$  hybrids available for the chapter to work with at TACF’s Meadowview Research Farms.

In May of this year, three backcross orchards that had been blight-tested were culled, including those at Berry College, University of Georgia Horticultural Research Farm, and Reinhardt University. Roughly 1100 nuts from five maternal lines were collected in September and will be transferred to a “Graves” seed orchard under development by the Kentucky chapter, part of the chapter’s “cross-pollination” work. We hope that seeds from these five maternal lines, as well as additional lines from two other backcross orchards that have been blight-tested (Callaway Preserve and University of Georgia Mountain Research and Education Center), will be planted in one or more seed orchards in Georgia next year.

Another milestone reached this year is that the chapter made what is hoped will be the final crosses done with Meadowview backcross trees for the completion of its blight-resistance breeding program. The first seeds will go into a seed orchard the same year that the final backcrosses for seed orchards are being made. This is not ideal, but given the challenges the small chapter has faced, it is progress nonetheless. Finally, this past summer, the chapter continued a program started several years ago to breed for *Phytophthora* root rot resistance via the production of new  $F_1$  and  $BC_1$  crosses. Two  $F_1$  lines and two  $BC_1$  lines were produced this summer. The chapter is encouraged by the progress made so far, recognizes the huge contributions made by members and numerous volunteers over the years, and hopes that chapter members will continue to support the remaining efforts for the breeding program to complete its major long-term objectives.

Curt Laffin



# 2015 ANNUAL MEETING VOLUNTEER SERVICE AWARDS

Each year, the awards committee of TACF reaches out to chapter presidents and asks them to nominate a stand-out volunteer from their state. From there, the committee selects one recipient from each region. The selected Volunteer of the Year recipients are honored for their outstanding volunteer work in restoring the American chestnut tree to the eastern woodlands.

The 2015 Volunteer of the Year Award recipients are as follows:

North Central: Kristen Russell-Stewart

New England: Curt Laffin

Mid-Atlantic: Doug & Stacey Levin

Southern: K.O. Summerville

Doug & Stacey Levin



Kristen Russell-Stewart



K.O. Summerville





# Selecting Disease Resistant American Chestnut

BACKCROSS HYBRIDS WITH GENOMIC TESTING

By Dr. Jared Westbrook, TACF Quantitative Geneticist



In the quest to restore the American chestnut, the challenge for TACF scientists is to select the most blight resistant backcross hybrid chestnut trees from seed orchards at Meadowview Research Farms. The selected trees will then serve as a seed source for the restoration of the American chestnut in its native forest environment. To introduce blight resistance from Chinese chestnut into American chestnut, Chinese chestnut x American chestnut hybrids were backcrossed to American chestnut over three generations to recover an average of 94% of the genome of American chestnut while retaining genes for blight resistance from Chinese chestnut.

There are multiple genes at different locations in the hybrid chestnut's genome that underlie blight resistance. At each of these locations, backcross hybrid trees are expected to have inherited one gene variant from Chinese chestnut that contributes to blight resistance, and another variant of the same gene from American chestnut that does not contribute.

Backcross hybrids, therefore, have intermediate blight resistance between Chinese and American chestnut.

The genomic research that is underway is a proof-of-concept for use of DNA sequencing technology to select blight resistant trees. If we are successful in developing

a low cost genetic test that can accurately predict blight resistance, we may use genetic testing to select blight resistant trees from seed orchards that are currently being established by TACF's state chapters. Genomic tests may also be used to select trees that are resistant to *Phytophthora* root rot disease, which is a threat to chestnuts growing in the Southern coastal plain. Maintaining a high level of genetic diversity will be essential for the adaptability of the species across a large range in the face of climate change.

Selecting trees with the highest genetic resistance to blight is not as straightforward as inoculating trees with the blight fungus and selecting the trees with the smallest cankers. Canker size is influenced by both genetic and environmental factors. Environmental factors such as year-to-year variation in weather and competition from neighboring trees may

have large enough effects on canker growth so as to obscure the underlying genetic effects on blight resistance.

One method to precisely select trees that are most genetically resistant to blight is to plant the progeny of trees in the seed orchard and select the trees whose progeny have the smallest average canker

size. Currently, about 10,000 trees with varying genetic resistance to blight remain in TACF's seed orchards at Meadowview. Our objective is to select the 500 most blight resistant trees. It is not feasible to progeny test all 10,000 trees to complete our selections.

Predicting a tree's blight resistance from its DNA sequence may accelerate and increase accuracy in selecting the tree with the greatest genetic resistant to blight. Genomic sequencing will be useful for assessing how much of the genetic diversity that remains in extant American chestnut trees has been captured by TACF's breeding program.



Dr. Ronald Sederoff of NC State University is one of the featured keynoters at Saturday evening's banquet. All photos by Ruth Gregory Goodridge.

## Proceedings of the 2015 TACF Annual Meeting (Presenters listed in alphabetical order)

**Albert Abbott, Ph.D.** *Biological Research Team Leader, Forest Health Research and Education Center, University of Kentucky; "Leveraging forest tree genomics and genetics resources to mark and identify genes for resistance to important forest tree pathogens and pests"*

The recently established Forest Health Research and Education Center at the University of Kentucky (Lexington, Kentucky, USA) is focused on utilizing genetics and comparative genomics resources of key forest and fruit tree species to identify, mark, and characterize genes for resistance to major tree pathogens such as *Phytophthora cinnamomi* (Pc), the oomycete pathogen causing *Phytophthora* root rot (Prr) of chestnut. In addition to being a serious tree pathogen, Pc is one of the most important plant pathogens worldwide. This pathogen infects ~1000 plant species including agricultural crop plants, landscape plants, and forest trees, causing significant losses both in the wild and under cultivation. Currently, no resistance has been identified in most of the important forest and plantation tree species. We have identified resistance to *P. cinnamomi* in hybrid progenies of American chestnut (susceptible) and Asian chestnut species (resistant) that appears to be conferred by one or a few resistance locus/loci. In this report, we present our QTL mapping data and comparative genomics analyses to mark and characterize the genetics of resistance to this important forest tree pathogen in chestnut, and discuss the potential strategies we are investigating to rapidly mobilize this resistance into susceptible chestnuts.

**Catherine Bodénès, Ph.D.** *Researcher, National Institute for Agricultural Research (INRA), France; "Comparative genomics between oak and chestnut"*

Oaks and chestnuts belong to the Fagaceae family and their divergence is estimated to have been around 70 million years ago. Intensive efforts have led to the development of genomic resources for oaks and chestnuts, including the creation and sequencing of numerous genomic and cDNA libraries, the development of molecular markers based on SSRs or SNPs, and the genome sequencing and assembly. An 8k-SNP gene-based array was designed for two European white oaks species, *Quercus robur* and *Q. petraea*. This array was used to genotype more than 1,050 full-sibs from two intra-specific (*Q. robur* and *Q. petraea*) and two inter-specific mapping pedigrees, and to create a composite gene-based linkage map comprising 5,460 SNPs. In chestnut, a transcriptomic-based genetic map of Chinese chestnut created with 1,400 SSR and SNP markers (Kubisiak et al., 2012) and a genetic map of European chestnut created with about 400 markers (RAPD, ISSR, SSR, EST) (Casasoli et al., 2001, Casasoli et al., 2006) are available. QTL detection was also performed for several adaptive traits (height growth, bud burst, water-use-efficiency, disease resistance) for both genera (Casasoli et al., 2006, Brendel et al., 2008, Derory et al., 2009, Kubisiak et al., 2012). Comparative genetic mapping demonstrates that macro-colinearity is highly conserved between oak and chestnut and that some QTLs co-localized between these two closely related species.

**Nathaniel Cannon** *Doctoral Candidate, Integrative Biosciences, Penn State University; "Comparison of the Genomes of Chestnut Species"*

The Chinese chestnut 'Vanuxem' reference genome provides the platform for characterizing introgression in the TACF back-cross breeding program at high-resolution and accuracy. It is now possible to develop a high throughput, low-cost system for assessing the amount and chromosomal location of Chinese vs. American genome sequence within trees at any stage of backcrossing. The requirements for introgression analysis are known chromosomal positions of sequences, what the similarities and differences are between the genomes of different chestnut species, and bioinformatics tools for rapid assessment of results. To that end, we have ordered the Chinese chestnut genome scaffolds (fragments) into continuous sequences for each of the 12 chestnut chromosomes (called "pseudochromosomes"). We then generated whole genome sequence data for *C. alnifolia*, *C. crenata*, *C. henryii*, *C. ozarkensis*, *C. sativa*, *C. seguinii*, five genotypes of *C. dentata*, five genotypes of *C. mollissima*, and five *C. dentata* x *C. mollissima* backcross individuals. We then mapped the sequences from each of these individuals to the reference pseudochromosomes for Chinese chestnut. We will report what was learned about genome-level variation between species and how this approach can be used to identify species-specific loci in backcross individuals. A trial study was conducted in collaboration with Sara Fitzsimmons to compare the genomes of blight-susceptible vs. -resistant offspring and their parent trees in the BC<sub>3</sub> trial at the PSU arboretum. This trial study is the basis of the hands-on workshops being offered on day two of the meeting.

**John Carlson, Ph.D.** *Professor of Molecular Genetics; Director, Schatz Center for Tree Molecular Genetics, Department of Ecosystem Science and Management, Penn State University; "The Chestnut Genome"*

To provide a foundation for genome-wide marker-assisted selection in the TACF backcross breeding program, and for discovery of genes for resistance to the chestnut blight fungus (*Cryphonectria parasitica*), we produced a genome sequence for Chinese chestnut (*Castanea mollissima*). Version 2.2 of the Chinese chestnut genome covers over 98% (784 Mbases) of the genome of the cultivar 'Vanuxem' genome. A framework of over 14,000 scaffolds (pieces) of the genome were ordered along the chestnut genetic linkage map into "pseudochromosomes" representing whole-chromosome sequences. A total of 38,146 genes were identified in the Vanuxem genome, confirmed with gene expression data. To narrow the search for blight resistance genes, DNA clones covering the three blight resistance QTL were sequenced to great depth. Across all three QTL regions, 782 genes were found. The QTL genes encode proteins with a variety of molecular functions and biological processes, including approximately 200 classified as stress-related genes, among which several were selected as potential blight resistance genes.

**Rita Costa, Ph.D.** *Senior Researcher and Head of Biology Laboratory of Forest Research, Portuguese National Institute for Agricultural and Veterinary Research (INIAV); "How genetic and genomic tools may improve resistance to Phytophthora cinnamomi in chestnut"*

Portugal is a major exporter of nuts of European chestnut, *Castanea sativa* (Mill), placed in the top three of exporter countries in Europe. Nut production is very important to

the economy in northern regions of the country. Root rot, caused by *Phytophthora cinnamomi* (Rands) is the most destructive disease affecting *Castanea sativa*, reducing by half the productivity of Portuguese chestnut orchards. Therefore, breeding for resistance to this pathogen is important for improving resistance of the host, which will positively impact the economy of the agricultural sector in Portugal. A research program was implemented using combined genetic and genomic approaches for a better understanding of the molecular mechanisms underlying resistance of *Castanea* spp. to *P. cinnamomi*. A set of interspecific crosses was performed between three species of *Castanea* with different susceptibilities to *P. cinnamomi*: European chestnut, *Castanea sativa*, susceptible, and two Asian species: *C. crenata* and *C. mollissima*, both resistant. Two full-sib pedigrees were produced, SC (*C. sativa* x *C. crenata*) and SM (*C. sativa* x *C. mollissima*), a mapping family where genotyping with EST-SSR and SNPs markers developed from Chinese chestnut EST sequence has been performed as well as phenotyping regarding resistance to *P. cinnamomi*. A genetic map was produced and two QTLs have been identified so far. A set of 20 differentially expressed genes were also selected in transcriptomes, challenged with *P. cinnamomi*, which was further validated by digital PCR, and a new set of EST-SSR markers was designed in these genes. The final goal is to identify genomic regions correlated with ink disease resistance in *Castanea* genus to develop molecular markers for marker-assisted selection. These new tools will aid in the selection of improved genotypes from the breeding program, an asset for the European market, which presents a high deficit of improved plant material for plantation, namely rootstocks, with a consequent positive impact on orchard productivity.

**Angus Dawe, Ph.D.** Associate Department Head and Associate Professor of Molecular Mycology, New Mexico State University; “Banquet-omics: Looking at genomes to understand how pathogens make a meal out of chestnut”

A coordinated restoration strategy for the American chestnut must involve a deeper understanding of both the host and the pathogens that seek to destroy it. This talk will present a brief overview of the state of genome sequencing efforts of the chestnut pathogens *Cryphonectria parasitica* (the blight fungus) and *Phytophthora cinnamomi*. It will also cover the ways the genomic information can be used to better understand the interaction of the blight fungus with chestnut, and whether insights into the fungal genome can improve efforts to employ hypovirulence as a counteractive measure.

**Jason Holliday, Ph.D.** Assistant Professor of Forest Genetics and Biotechnology, Forest Resources and Environmental Conservation Department, Virginia Tech; “Genomic selection to advance backcross breeding in American chestnut”

Next generation sequencing now makes it possible to uncover most or all of the heritable variation relevant to a trait of interest, and to use these genotypes for de novo prediction of phenotypes. This approach, known as genomic selection, relies on next generation sequencing to identify informative polymorphisms. The most commonly used library preparation method for genomic selection in structured populations is restriction enzyme-based methods, which recover a fraction of the genome near recognition sites for the chosen enzyme in a random and



Dr. Kim Steiner introduces the fifteen speakers participating in Saturday evening's expert Q&A session.

repeatable manner (often referred to as genotyping-by-sequencing (GBS)). The TACF backcross breeding program is applying this technology for genomic selection with the goal of developing high-resolution genomic selection models for blight resistance. In addition to building predictive models for blight resistance, this project will enable precise quantification of the extent of Chinese chestnut background in a given tree (which can then be minimized in the selection stage) as well as the ability to select resistant individuals with diverse genetic backgrounds, thereby increasing health of deployment populations.

**Nurul Islam-Faridi, Ph.D.** Research Molecular Cytogeneticist, Southern Institute of Forest Genetics, Southern Research Station, USDA Forest Service; “Chestnut Molecular Cytogenetics - an update”

The American chestnut (*Castanea dentata*), once known as the King of the “Appalachian Forest,” has been nearly decimated by chestnut blight caused by an invasive fungal pathogen (*Cryphonectria parasitica*). Scientists at TACF and other organizations have been working since the early 1980s to transfer the blight resistance gene(s) (through backcross breeding and genetic engineering) into American chestnut from Chinese chestnut (*C. mollissima*). Chinese chestnut has been genetically and physically mapped, and is currently being sequenced to facilitate these improvement approaches. Plant genomes generally contain high amounts of repetitive DNA that hinders the construction of genetic and physical maps and ordering of scaffolds during genome sequence assembly. Specific DNA sequences (gene and/or molecular markers) can be physically localized onto specific chromosomes using fluorescence in situ hybridization (FISH), thereby facilitating the correct ordering of maps and sequence scaffolds. We are using FISH to construct a cyto-molecular map of Chinese chestnut using 16 to 20 BAC clones from each linkage group (LG) that will provide centromeric positions and short and long arm assignments for each LG. The cyto-molecular map delineating the structural details of the chestnut chromosomes will help to improve our understanding of the chestnut genome, allowing for powerful comparative studies that will better inform gene discovery and mapping, interspecific breeding, and genetic engineering.



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Photos clockwise from upper left:

TACF Intern Aadish Shah pollinates chestnut trees at the Cataloochee Ranch orchard. Photo by Dr. Hill Craddock.

Cataloochee Ranch orchard in 2011.

Students from Charles D. Owen Middle School assist each other during the planting of the conservation orchard. Photo courtesy of Brittany Krasutsky.

Atlanta History Center. Photo courtesy of Marty Cipollini.

Those present during the official measurement of the large, living American chestnut tree discovered in Lovell, Maine on December 3. Photos courtesy of the University of Maine Foundation.



**Antoine Kremer, Ph.D.** *Senior Scientist, National Institute for Agricultural Research, Bordeaux, France and*  
**Ronald Sederoff, Ph.D.** *Distinguished University Professor and the Edwin F. Conger Professor of Forestry and Environmental Resources, North Carolina State University;*  
*“Major events in the molecular genetics of forest trees: past present and future”*

Interspecific differentiation in oaks has been a recurrent concern in evolutionary and population genetics in oaks, due to the high genomic permeability by gene flow. Here we present results obtained by whole genome approaches implemented in four temperate European white oak species (*Quercus petraea*, *Q. robur*, *Q. pubescens*, *Q. pyrenaica*). These species are interfertile, but occupy contrasting ecological niches even under sympatric conditions. We therefore anticipate that species differentiation may result from ecological divergence and reproductive isolation. Whole genome scans were implemented in two different ways: by high density SNP genotyping, and by whole genome resequencing using a pool-seq strategy. We compare the results obtained with the two methods, and provide an overall genomic landscape of species differentiation in white oaks. Cross comparisons of pairwise species divergence works to disentangle genes likely to be involved in adaptive divergence or reproductive isolation. We further discuss evolutionary scenarios that may have contributed to the extant maintenance of interfertile white oak species.

**Scott Merkle, Ph.D.** *Associate Dean of Research and Professor, Warnell School of Forestry and Natural Resources, University of Georgia;* *“Don’t call them clones: How hybrid backcross chestnut varieties can enhance TACF’s restoration mission”*

For the past five years, our lab has been part of the Forest Health Initiative (FHI), a multi-institution research project that has as its mission to demonstrate the application of biotechnological tools to address forest health threats in the U.S. The first FHI target species is American chestnut (AC), and we have been employing embryogenic chestnut cultures to address several FHI objectives focused on restoration of the species. While testing the effectiveness of dozens of candidate genes for blight resistance in transgenic chestnuts has been the most high-profile part of the project, another goal is to facilitate clonal testing of conventionally bred material from TACF’s breeding program using somatic seedlings—trees regenerated via somatic embryogenesis (SE). However, the application of SE to chestnut may have implications for restoration beyond clonal testing. SE has already demonstrated the potential to enhance forest productivity in southern pine plantations where this “varietal forestry” approach has been tested. Prior to the beginning of the project, only pure AC material had been propagated via SE, so the potential to propagate advanced generation hybrid material was unknown. Open-pollinated (OP) seeds from TACF B<sub>3</sub>F<sub>2</sub> seed orchard parents were used to initiate cultures in 2010 and 2011, resulting in embryogenesis induction at rates that were not significantly different from those for AC. The first B<sub>3</sub>F<sub>3</sub> somatic seedlings were planted in clonal field tests in 2013, along with conventional B<sub>3</sub>F<sub>3</sub> seedlings. Selected B<sub>3</sub>F<sub>2</sub> parents were crossed in 2012, and while embryogenesis induction rates were lower than for OP

seeds, embryogenic cultures were produced for most crosses. The first somatic seedlings from the control-pollinated material grew well in the greenhouse and were planted in field tests in 2015. Now that we have confirmed that SE can be applied to propagate B<sub>3</sub>F<sub>3</sub> material, all the pieces are in place to apply the varietal forestry approach to blight-resistant chestnut. Just as varietal forestry has shown the potential enhance the productivity of southern pine plantations, applying the varietal approach to TACF B<sub>3</sub>F<sub>3</sub> and other hybrid backcross chestnuts could both enhance restoration efforts and induce interest by landowners in growing chestnuts by making elite chestnut varieties available to them for commercial timber and nut production.

**C. Dana Nelson, Ph.D.** *Supervisory Research Geneticist and Project Leader, USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics; Co-Director, Forest Health Research and Education Center, University of Kentucky;* *“Mapping Disease Resistance QTLs in Chestnuts”*

Several researchers over nearly two decades have been working to genetically map the quantitative trait loci (QTLs, i.e., genes) in chestnut (*Castanea* spp.) responsible for resistance to chestnut blight (CB) and more recently *Phytophthora* root rot (PRR). Resistance QTLs for both CB and PRR have been mapped in several populations originating from various Chinese chestnut (*C. mollissima*) ancestral sources using a series of technologically advanced genetic marker systems, including DNA sequencing. Results from these studies will be described and summarized, and the current status of using the QTL information in backcross breeding and genetic engineering will be presented and discussed.

**William Powell, Ph.D.** *Professor and Director, Counsel on Biotechnology in Forestry College of Environmental Science and Forestry, State University of New York;* *“Screening putative resistance-enhancing genes in transgenic American chestnut”*

The American chestnut (*Castanea dentata*) and chestnut blight is the classic example of what happens when our forests succumb to exotic pests and pathogens. Because of its environmental, economic, and social importance, many tools have been brought to bear on the chestnut blight problem. We have focused on enhancing blight resistance by producing both transgenic and cisgenic American chestnut trees, adding only two to five genes to the approximately 45,000 genes in chestnut genome. The most promising of the transgenes encodes an oxalate oxidase (OxO) from bread wheat (*Triticum aestivum*). According to leaf and small stem assays that predict the level of blight resistance, this OxO gene has raised resistance levels at least as high as those found in the blight-resistant Chinese chestnut (*C. mollissima*). We have also discovered two promising cisgenes from the closely related Chinese chestnut: a laccase-like gene and a proline-rich protein gene, both of which can partially enhance blight resistance when overexpressed in American chestnut. This means the trees have significantly less necrosis in leaf assays than the wild type American controls, but significantly more necrosis than the Chinese chestnut controls. We will report on the progress made with all genes tested to date.

**Jeanne Romero-Severson, Ph.D.** Professor of Quantitative Genetics and Genomics, Biological Sciences Department and Director, Tree Genetics Core Facility, Forest Conservation and Tree Genetics Program, University of Notre Dame; “Seed orchard DNA fingerprinting: The double-edged sword of the open access gene pool”

The prime directive of a tree breeding program is know your parents, inside and out. This is especially challenging for a breeding program conducted on chestnuts. The genetic background of chestnuts in the United States can include contributions from at least four species, all of which can form fertile interspecific hybrids with each other. In cultivated chestnuts, this “open access” gene pool has resulted in a morass of misidentification and mistaken impressions that hinders development of the perfect cultivar: a locally adapted, stress-resistant, reliable producer with an orchard tree form, bearing easily peeling, good-keeping, and delicious nuts. American chestnut restoration programs have a different “ideal” chestnut in mind, but the prime directive still applies. Using EST-SSR markers developed from Chinese chestnut EST sequence, Romero-Severson and colleagues have discovered many homonymies (same name, different genotypes) and synonymies (different names, same genotype) in the chestnut cultivars grown at the University of Missouri Center for Agroforestry, including some cultivars with both homonymies and synonymies. This cultivar collection represents most of the cultivated germplasm in the United States. Ascertaining the degree of interspecific ancestry and detecting relationships among close relatives in cultivated and wild trees will require more resources, but this effort, when combined with the performance data that already exists, has the potential to deliver much better trees in a shorter period of time, whether these be hybrid cultivars for fresh market chestnuts, or American chestnut trees for restoration projects.

**Margaret Staton, Ph.D.** Assistant Professor, Department of Entomology and Plant Pathology, University of Tennessee, Institute of Agriculture; “Online Genomic Resources for Chestnuts and other Hardwood Trees”

The hardwood genomics website and database (HWG, <http://hardwoodgenomics.org>) was established to house and provide public access to a diverse set of genetic and genomic resources for important woody angiosperms, particularly forest trees. Available chestnut data include the draft whole genome sequence of the Chinese chestnut, and targeted sequencing data obtained from the three major genomic regions (quantitative trait loci, QTLs) associated with *Cryphonectria parasitica* resistance. The site hosts additional sequence data such as transcriptomes generated from various American and Chinese chestnut tissues, genes identified from the genomic sequence, and whole genome resequencing data from a variety of *Castanea* species and intercrossed hybrids. Users may interact with the sequence data by searching with gene function keywords, downloading the assembled sequence files, using BLAST to find homology to their own sequence, or browsing the reference genome with the genome browser software Jbrowse. Tracks for exploration on Jbrowse include the gene sequences, aligned proteins from other model plant species, and variants identified between chestnut species. Via the genome browser, users may structurally and functionally



Dr. Jeanne Romero-Severson answers questions during Saturday evening's expert Q&A.

annotate their own genes of interest with the software webApollo. The annotations may be submitted back to the community in order to help to improve the current and future genome annotation versions. In addition to sequence data, the chestnut genetic markers, genetic map, and *Cryphonectria*-resistant QTL map can be found on the HWG and visualized with the software CMap. The HWG includes a number of other important hardwood tree species that may be compared to chestnut, including northern red oak, black walnut, and green ash. Future expansions of the HWG include integration with the online bioinformatics analysis portal Galaxy and interactivity with other tree databases, including Dendrome, the Genome Database for Rosaceae, and the Citrus Genome Database.

**Jared Westbrook, Ph.D.** Quantitative Geneticist, The American Chestnut Foundation; “Current progress toward breeding for blight resistance in American chestnut and the role of genomics in future breeding and selection”

For over 30 years, TACF has used backcross breeding to introgress blight resistance from Chinese chestnut into third backcross–intercross hybrids ( $B_3F_2$ ) that inherited an average of 15/16 of their genome from American chestnut. The backcross method was implemented based on the hypothesis that blight resistance alleles from Chinese chestnut are incompletely dominant and segregate at two to three loci. Assuming Mendelian segregation, 1.5% to 6.25% of  $B_3F_2$  individuals were expected to be fully homozygous for blight resistance alleles. A first round of selection against blight-susceptible individuals has been carried out by inoculating  $B_3F_2$  trees with a weakly pathogenic strain of *Cryphonectria parasitica* and culling individuals with significant canker expansion. Progeny testing for blight resistance was initiated in 2009 and has been completed for 265  $B_3F_2$  parents from the Clapper and Graves sources of resistance. Between 10% and 40% of the variation in canker size after inoculation of  $B_3F_2$  progeny with a highly pathogenic and a weakly pathogenic strain of *C. parasitica* was attributable to additive genetic effects of resistance alleles. This result indicates that substantial genetic variation in blight resistance remains among

$B_3F_2$  trees after phenotypic selection against susceptible individuals. The most blight resistant  $B_3F_2$  trees that have been progeny tested have canker size breeding values that are intermediate between Chinese chestnut and American chestnut. It is unclear if all resistance alleles from Chinese chestnut have been retained through backcrossing and if more than two to three loci control blight resistance. In seed orchards at TACF's Meadowview Research Farms, more than 8000 Clapper and Graves  $B_3F_2$ s remain from which to make the final selections of the 500 most blight resistant individuals. Genomic selection has the potential to accelerate and to increase accuracy in selection for blight resistance. Genomic sequencing also has the potential to address fundamental unanswered questions: Do Clapper and Graves  $B_3F_2$ s segregate for blight resistance at different genomic loci? What segments of the Chinese chestnut genome have been retained through backcross breeding, and do these segments vary among backcross lines? Resolving these uncertainties will inform future breeding decisions. If blight resistance has been lost through backcrossing, and resistance alleles differ among lines or sources of resistance, blight resistance may be enhanced through controlled crosses and recurrent selection.

**Fiorella Villani, Ph.D.** Senior Researcher, Institute of Agroenvironmental and Forest Biology, National Research Council, Italy; "Chestnut Genomics Research in Italy"

CNR-IBAF has been a partner and coordinator of several EU projects addressing the genetics of forest trees with particular focus on chestnut. Within this context, an overview of genetic and genomic research activities on *Castanea sativa* in relation to major evolutionary factors and anthropogenic impacts is reported. Different study scales and approaches, from landscape genetics to genetic mapping, were applied and integrated in order to predict the future dynamics of the chestnut ecosystem and to develop strategies for its conservation. The genetic diversity and structure of natural and cultivated *C. sativa* was evaluated on a pan-European scale across the whole distribution range. The integration of genetic results with environmental data, through GIS support, highlighted the main *C. sativa* gene pools and introgression zones. Variation and phenotypic plasticity of adaptive traits were studied in selected geographic provenances grown in common garden trials and ex situ germplasm collections. Results related to phenology, growth, drought tolerance, and *Dryocosmus kuriphilus* resistance are summarized and discussed in relation to environmental variation of the original sites. Genome regions and molecular basis of chestnut phenology were identified by genetic and QTL mapping and further elucidated by an ongoing transcriptome study. The overall results are discussed in view of future research and applied perspectives.



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# WHAT CAN YOU TELL ME ABOUT THE Dunstan Chestnut?

By Sara Fern Fitzsimmons and Dr. Bill Lord



...there are some seven to 10 species of chestnut in the genus *Castanea*. “The Plant List” gives 179 names given to *Castanea* species throughout the years .

The most common question I get as an employee with The American Chestnut Foundation is, “Can I have some chestnut trees?” And my response is invariably, “What kind of chestnut tree?”

You may all be snickering, knowing that pretty much anyone would want an American chestnut, but that isn’t always the case. We also have to be careful to make the distinction between a “pure” American chestnut, collected from properly identified wild specimens and/or their progeny, and the trees that we are creating with our breeding program for disease-resistance.

There are multiple goals for landowners when they decide to plant chestnut trees. Some landowners simply want to feed wildlife. Some want to feed themselves or others with a commercial crop of chestnuts. Others want a timber tree to grow in their woodlots. And some would prefer that those goals be fulfilled by a native species, while others are indifferent. Depending on what an individual’s goals are, one or more type of chestnut might be suitable.

Depending on who you talk to, there are some seven to 10 species of chestnut in the genus *Castanea*.<sup>1</sup> “The Plant List” gives 179 names given to *Castanea* species throughout the years.<sup>2</sup> Most of these are synonyms; for example, *Castanea dentata* has been assigned up to eight different names, such as *C. americana*. All of these species can form combinations to make viable progeny, although each amalgamation interbreeds with varying levels of success. This makes for a staggering number potential combinations, especially when one starts making backcrosses, intercrosses, and any number of crosses between, among, and within those species and crosses.

Dr. Sandra Anagnostakis of the Connecticut Agricultural Experiment Station (CAES) keeps a running catalogue of named chestnut cultivars,<sup>3</sup> a list that currently has 1,327 entries.<sup>4</sup> While people have been breeding chestnuts for centuries, Europe claims the most named cultivars (701) with Italy being the top (361). Some variation of “marrone,” the Italian word for “brown” and “chestnut,” appears 135 times in these cultivar names.

In the US, there are have been several prolific chestnut breeders. Some notable names on that list are Luther Burbank, Arthur Graves,<sup>5</sup> and R.T. Dunstan. While any of these names might ring a bell for our readers, if the amount of calls I get is any indication, I suspect that the name Dunstan is of particular interest.

Dr. Robert Dunstan was a member of the Northern Nut Growers Association (NNGA) who resided in Greensboro, NC. Another NNGA member, James Carpenter, sent Dunstan some scion wood from an American chestnut in Salem, OH. Because the Salem tree had not yet contracted the blight while nearby American chestnuts had, Carpenter believed it to have resistance to blight.

Dunstan took the scion from the Carpenter American chestnut and top-grafted it to chestnut rootstock. Around that tree, Dunstan had planted the name Chinese chestnut cultivars Meiling, Nanking, and Kuling.<sup>6</sup> These trees were allowed to open-pollinate and Dunstan reportedly took F<sub>1</sub> progeny from the Carpenter tree, presumably after it was pollinated by one or more of those three *Castanea mollissima* cultivars.

These F<sub>1</sub> seeds were planted and grown to flowering. At this point, the trees were backcrossed to all four trees named above. Thirty of the resultant seedlings were taken by Dunstan to his retirement farm in Florida. These trees showed a mix of American and Chinese traits. Because the F<sub>1</sub> trees were produced via open-pollination, the lineage of the offspring cannot be given as a percentage of its parents. Six of the resulting BC<sub>1</sub> seedlings were further propagated and named.

In the 1980s, Bob Wallace, Dunstan’s grandson, commenced planting a 500 tree chestnut orchard on Dunstan farm in Alachua, Florida. The four main BC<sub>1</sub> cultivars, Revival, Willamette, Carolina, and Carpenter, were to create the orchard. Revival, Willamette, and Carolina were patented and received the first patents ever given to chestnut tree varieties, along with Heritage. Seeds from these trees were germinated and planted, and 220 of these seedlings were grafted to the named cultivars.

<sup>1</sup>Anagnostakis S. 2009. Identification of Chestnut Trees. The Nutshell. 63(3): 11-22. <http://www.northernnutgrowers.org/ChestnutID.pdf>.

<sup>2</sup>The Plant List. 2013. Version 1.1. Published on the Internet; <http://www.theplantlist.org/> (accessed November 23, 2015).

<sup>3</sup>The International Society for Horticulture Science (ISHS) maintains a database of contacts, by genus, of those who curate cultivar registries: <http://www.ishs.org/sci/icra.htm>

<sup>4</sup><http://www.northernnutgrowers.org/CultivarsofChestnutA-Z2014Sandy.pdf>

<sup>5</sup>The “Graves” tree, a named BC<sub>1</sub> tree residing at CAES, which has been used for one of TACF’s major sources of resistance.

<sup>6</sup>Our readers might be interested to know that each of these cultivars have been introduced as sources of resistance in TACF’s breeding program, either and Meadowview and/or through work in the Chapters.

List of accepted,  
named  
*Castanea species*<sup>2</sup>

*Castanea dentata*,  
American chestnut –  
North America

*Castanea pumila*,  
Allegheny chinkapin –  
USA

*Castanea x neglecta*\*  
– Named hybrid of  
*C. dentata* x *C. pumila*  
– USA

*Castanea ozarkensis*,  
Ozark chinkapin – USA

*Castanea sativa*,  
European chestnut –  
Europe

*Castanea mollissima*,  
Chinese chestnut –  
China

*Castanea seguinii*,  
dwarf Seguin chestnut  
– China

*Castanea henryi*,  
Henry chinkapin –  
China

*Castanea crenata*,  
Japanese chestnut –  
Japan



At Chestnut Hill Nursery, Wallace has made selections based upon large nut size, good nut production and growth habits, and most importantly, blight-resistance. He did not select trees to propagate based upon American timber tree traits. His goal was to develop a U.S. chestnut orchard industry, with emphasis on producing in abundance nut of large size and good taste.

The orchard produces seed by open pollination, producing seedlings that are sold to the public. Bob's main market is orchardists, individuals who want a chestnut tree on their property and as a bountiful food source in wildlife plantings. Wallace believes his 500 tree orchard is reaching maturity with the tallest seedling trees reaching a height of 60-feet. The grafted trees are shorter, standing at 25 to 30-feet. He harvests about 5,000-7,500 pounds of nuts per year from this planting.

There has been confusion before regarding whether or not a "Dunstan" chestnut is an American chestnut, going back to the very early years of TACF. A 1988 advertisement for the Dunstan chestnut as "the return of the American chestnut" prompted then TACF president Phil Rutter to write to Chestnut Hill Farms for clarification. The resulting letter from Bob Wallace was published in the Journal of TACF,<sup>7</sup> and repeats much of the same history presented here.

So, is the Dunstan hybrid an American chestnut? No. If performance of the parent trees is good indication, these trees will not be able to compete with the native trees in our eastern US woodlands. That said, Dunstan trees are great for those wanting nut production for wildlife. In addition, they are commercially available, including at many "big box" stores.

Note the Dunstan trees sold are seedlings. If you want to get a tree that will look exactly like a parent tree and/or be exactly a certain cultivar, you need to get a tree that has been vegetatively-propagated. For chestnuts, that usually means a grafted tree. Most trees sold or otherwise distributed in the US, however, are seedling trees. This means that the tree will exhibit characteristics of both parents. Even if the seed was collected from a known and certified cultivar, the resulting tree is not that cultivar.

The American Chestnut Foundation is one of the best places to go for American chestnut seedlings and the best place for a potentially blight-resistant American chestnut. **For more information one where to purchase chestnut trees, including some grafted cultivars, Dr. Anagnostakis curates a list of suppliers accessible at: <http://www.ct.gov/caes/cwp/view.asp?A=2815&Q=376838>.**

<sup>7</sup>The Dunstan Hybrid Chestnuts. 1989. Journal of The American Chestnut Foundation. 3(1): 38-39. [http://acf.org/pdfs/resources/journal/journ\\_vol3-1.pdf](http://acf.org/pdfs/resources/journal/journ_vol3-1.pdf)



# Five-year Graft Compatibility

## IN AN AMERICAN CHESTNUT BREEDING ORCHARD

By Jim McKenna and Brian Beheler

ABSTRACT: Grafting of chestnut is possible, but fraught with uncertainty because graft unions tend to fail over time and the intended scion is lost. This is a condition known as delayed graft incompatibility. We grafted American chestnut (*Castanea dentata*) trees for the Indiana chapter in 2009, and planted numerous grafted trees per clone into an orchard to examine the graft compatibility of 23 clones. After five years in the field, we measured graft compatibility and growth to estimate the magnitude of delayed incompatibility in American chestnut.

## INTRODUCTION

Grafting is defined as “the uniting of two plants to grow together as one.”<sup>1</sup> The practice allows for cloning and multiplying a single tree asexually through vegetative means, to avoid seedling variation. Grafting is effective among a single species or in some cases among closely related species in the same genus such as *Juglans cinerea* (butternut) grafted onto *J. nigra* (black walnut) rootstock. For chestnut, the same species or hybrid must be used as the scion and rootstock, and some genotypes will graft more successfully than others. For example, Italian and California chestnut growers have found seedlings of a

example of this is “Blackline Disease” in Persian walnut (*J. regia*) grafted onto black walnut rootstock. For most of the last century, California walnut growers considered this problem to be delayed graft incompatibility. But in the 1980s, researchers found that it was instead caused by the differential susceptibility of the scion and rootstock to a previously unknown virus.<sup>4</sup> A variety of physiological and biochemical differences between scion and rootstock, such as the change from a juvenile to a mature state, can effect delayed incompatibility too.<sup>5</sup>

We created an experimental grafted American chestnut breeding orchard in 2010 with the aim to plant numerous

Figure 1



One of the new Indiana American trees that scion wood was collected from. This tree, ‘Wagner,’ is near the town of Fort Ritner, Lawrence County, Indiana, and measured 8” DBH after the 2008 season. Photo by Brian Beheler.

Grafting is defined as “the uniting of two plants to grow together as one.”<sup>1</sup>

grafted cultivar make the best rootstocks for successfully grafting additional copies of that cultivar.<sup>2,3</sup>

Along with genotype variation for initial graft success, delayed graft incompatibility in chestnut happens over years. Its occurrence and time-interval can only be determined by trial and error. In some cases, diseases and physiological differences between scion and rootstock have been found to contribute to delayed incompatibility. A widely known

grafted trees per clone on a relatively tight spacing, expecting delayed incompatibility to occur so that successful grafts would ultimately be more widely spaced for good seed production. Our larger goal for this orchard is to support the Indiana chapter breeding program to: 1) conserve and perpetuate American chestnut genotypes native to Indiana, 2) produce a second BC<sub>3</sub> series of lines with new TACF resistant sources to augment our ‘Clapper’

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- Soiless mix used was Metro Mix 560 with Coir.

Figure 2



A whip and tongue graft of the clone 'Brems' in its first season in the orchard at Purdue. Photo by Jim McKenna.

Figure 3



The "Duke American Breeding Orchard" mid-summer in 2010. Photo by Jim McKenna.

lines, and 3) provide new American trees to hybridize with Asian chestnuts to aid fundamental resistance research and breeding. Our first  $BC_3$  'Clapper' lines were pollinated on American chestnuts scattered all across Indiana, a slow and costly process, requiring nearly 13 years to produce 20 breeding lines. If we can manage the delayed graft incompatibility problem, this orchard could allow us to create a new set of 20  $BC_3F_1$  lines in just a few years.

## METHODS

Dormant shoots were collected from Indiana trees in the winter of 2008-09 (**Figure 1**). Unrelated and mixed bare root 1.0 American chestnut seedling rootstocks from several pure American chestnuts were mixed and grown at the Indiana DNR, Division of Forestry Nursery (IN-DoF) in Vallonia, Indiana. Trees were dug in February 2009, potted into 10-liter pots with soilless mix,<sup>6</sup> and grown in a greenhouse managed by the Hardwood Tree Improvement and Regeneration Center (HTIRC) at Purdue University. After rootstocks flushed, whip-and-tongue grafts were made for scions and rootstocks that matched in stem diameter (**Figure 2**); in some cases, when only thin (-1/8") scion sticks were available, larger rootstocks were bark grafted. Up to 10 grafts per clone were made when enough scion wood was available. Rootstock shoots, or suckers, were removed as they formed, and by summer, the trees were transferred to a 50% shade house. The trees were kept well-watered with pH adjusted water (5.8) containing dilute fertilizer. Seedlings from four American chestnuts were potted in similar fashion and included in the orchard to compare with grafted trees. Grafted trees and seedlings

were overwintered in a walk-in cooler kept at 36°F. The orchard was planted 15 × 12-feet, in a completely randomized fashion.

## RESULTS & DISCUSSION

The initial graft take in 2009 was good, ranging from 50% - 80% success. We rogued out weaker grafts and planted the best ones in May of 2010. Most trees survived and grew well their first year (**Figure 3**). Note that while we did get at least one decent graft per clone to start, there was clear variation in the number of grafts that took per clone initially. While many of the clones that initially grafted relatively well did persist for five years, others did not. Survival of the scions of the grafted trees, our measure of graft compatibility, varied widely from 0% - 75% (**Table 1**). The most graft failures (40%) occurred by the end of the second season and after that, began to level off. When we measured and evaluated the trees last fall after five years in the orchard, about 20% more had failed, and we had in total 41% of the grafted trees that we started with still growing (**Figure 4**). The seedlings survived much better than the grafts, and growth (height, DBH = diameter at breast height and GLD = ground line diameter) were comparable between seedlings and grafts. Unfortunately, we had mortality of seedlings and added stress to many grafted trees from ambrosia beetle attacks in the second and third years.

Last season, after five years, grafted trees set more seed than the seedlings (**Table 1**), and in much greater abundance. This is to be expected since grafted mature tissue remains capable of flowering, unlike juvenile tissue

**Table 1: Five-year graft compatibility of grafted American chestnut clones and survival of seedling families planted in the IN-TACF Duke Energy American Chestnut Breeding Orchard at the Forestry & Natural Resources Department at Purdue University, West Lafayette, Indiana. Courtesy of Jim McKenna. (Height, DBH = diameter at breast height and GLD = ground line diameter).**

American Chestnut ( <i>C. dentata</i> ) [Clones]	N 2010	N 2014		Height (ft)	DBH (in) <sup>1</sup>	GLD (in) <sup>2</sup>	% Seed 2014	Survival (%) <sup>3</sup>	Graph Compatibility <sup>4</sup>
Seig #2	4	3		17.0	1.9	4.8	67%	75%	++
Hoyt	7	5		17.5	2.6	5.2	60%	71%	++
Brems	6	4		13.5	1.6	3.7	25%	67%	++
IB1 Burger	3	2		16.4	1.8	4.1	50%	67%	++
Lawson	5	3		16.4	2.2	4.3	33%	60%	+
Sones #1	7	4		13.8	1.6	3.8	80%	57%	+
Birchwood	2	1		16.0	2.0	4.4	0%	50%	+
Byler	6	3		13.5	1.0	3.3	67%	50%	+
IB2 Burger	2	1		12.3	1.5	2.8	100%	50%	+
Roselawn 4	2	1		16.3	2.0	3.7	0%	50%	+
Wagner	4	2		12.8	1.3	2.6	100%	50%	+
Burger R3-T5	5	2		14.4	1.5	3.5	0%	40%	-
Burger R1-T6	3	1		20.2	2.8	4.6	100%	33%	-
Krider Park	6	2		12.7	1.7	3.3	100%	33%	-
McCosky	3	1		15.6	3.1	4.3	100%	33%	-
Sones #2	4	1		12.4	1.5	4.1	100%	25%	-
HCSF 1	4	1		8.1	0.9	2.4	0%	25%	-
McCosky Cross	4	1		10.8	0.8	2.8	0%	25%	-
Johnson	5	1		16.0	2.0	4.4	100%	20%	-
Becky's Best	4	0		-	-	-	-	0%	--
Dr. White	1	0		-	-	-	-	0%	--
Nicolson JWSF	4	0		-	-	-	-	0%	--
Roselawn 1	4	0		-	-	-	-	0%	--
<b>Total</b>	<b>95</b>	<b>39</b>	<b>Avg.</b>	<b>14.5</b>	<b>1.8</b>	<b>3.8</b>	<b>57%</b>	<b>38%</b>	
			<b>SD</b>	<b>2.8</b>	<b>0.6</b>	<b>0.8</b>	<b>42%</b>	<b>24%</b>	
<b>[Seedlings]</b>									
Lauber 1	3	1		-	-	-	-	33%	
Lauber 2	4	3		10.6	1.4	3.2	67%	75%	
B. Blystone	5	3		16.1	2.7	4.6	33%	60%	
C. Burger	5	3		10.8	1.2	3.6	0%	60%	
<b>Total</b>	<b>17</b>	<b>10</b>	<b>Avg.</b>	<b>12.5</b>	<b>1.8</b>	<b>3.8</b>	<b>33%</b>	<b>57%</b>	
			<b>SD</b>	<b>3.1</b>	<b>0.8</b>	<b>0.7</b>	<b>34%</b>	<b>17%<sub>s</sub></b>	

1 = Diameter at breast height, 4.5-feet from the ground;

2 = diameter at ground level below graft unions;

3 = survival of the scion or seedling tree;

s4 = relative 5-year compatibility where(++) represent good grafting clones, (+) are fairly compatible, (-) are poorly compatible, and (--) are incompatible.



Figure 4

A clear example of a well-healed American chestnut graft in its 6th season in fall of 2015. Photo by Brian Beheler.

Figure 5



The “Duke American Breeding Orchard” in early fall of 2015. Photo by Jim McKenna.

of seedlings that is incapable of flowering. For breeding, this is a very useful feature of grafted trees. In some cases, grafted trees will set fruit the year they are grafted! This year, a similar percent of the clones and seedlings set seed again. Surviving grafts are now well spaced out and the orchard is ready to begin utilization to cross new lines (**Figure 5**).

We have successfully grafted a number of  $BC_3F_1$  resistant parents onto random  $BC_3F_2$  open pollinated seedlings in the past and planted those at two different sites. Much like the present American orchard, all grafted trees started out well but within three years, every scion died. Both sites were heavy clay and not very well drained. Our new American chestnut orchard is on a very well drained loamy soil, and a good chestnut soil appears to be critical to limit delayed graft incompatibility. Similar findings have been noted by IN-DoF Vallonia staff for northern red oak (*Quercus rubra*), which also suffers from delayed graft incompatibility.

#### ACKNOWLEDGEMENTS

We thank the Duke Energy Foundation, the USDA Forest Service Northern Research Station, the Fred M. van Eck Foundation at the HTIRC, the Department of Forestry & Natural Resources at Purdue University, and the IN-TACF Chapter for funding this project. New trees were located and identified by a number of foresters with the Indiana DNR Division of Forestry, and by Wakeland Forestry Inc. We thank Burk Thompson, Andy Meir, and Michael Loesch-Fries for their efforts helping to establish and maintain this orchard.

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## My first encounter with a chestnut came in 1937, when I was 10 years old and it was long dead.

My family lived at the foot of the Catoctin Mountains in central Maryland when I was assigned to help my elder brother chop wood for our kitchen stove. I quickly learned that the chestnut stumps were the easiest to split. Just a light tap of the axe and they would break apart. Making slivers for kindling was a snap, and thus began my love of the great American chestnut.

What I didn't understand at the time was that those logs were super dry. They came from trees that had been dead for many years, victims of the fungal blight that started at the turn of the last century and killed hundreds of millions of what was once the predominant tree species in the Appalachians.

But dead chestnut trees do not rot. Years after their demise and denuded of their bark, they continued to stand and become known as "the grey ghosts of the forest."

The trees were ideal for log cabins and many people used them for that purpose. My parents built one in 1940 to serve as an antique shop and residence. So, like Abe Lincoln, I can say I grew up in a log cabin. I don't know if Lincoln's log cabin was made of chestnut, but it could have been. Most likely the rails he is famed for splitting were as well. Although the Appalachians are the native range of the chestnut, early settlers prized the trees so much that they took them along as they moved west.

By Charles R. Downs,  
TACF member

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# Undying Enthusiasm

By Stephanie Parker

Herbert Darling became involved with The American Chestnut Foundation in 1989 when then-president Phil Rutter gave him some bad news.

“A large American Chestnut tree was found on our property by a hunter,” says Darling of the 80-foot, 21-inch diameter tree the hunter discovered on his home in New York’s Zoar Valley.

“I contacted Phil Rutter, the President of the national organization at that time. He came to look at it and reported it had approximately five years left, which made me mad!”

Darling’s hope is that “[the tree] will be returned to the wild and its native places as soon as possible.”

Luckily in the case of Darling, anger translated into action. First, he hired a helicopter, put pollen in a shotgun shell, and from the helicopter, shot the tree with the pollen. That didn’t work. So he and his son erected an 80-foot scaffold to hand-pollinate the individual blossoms and gather the nuts to keep the species

alive. Unfortunately, by 1995, the tree had succumbed to the unstoppable blight that had already wiped out nearly all of the American continent’s once ubiquitous chestnut trees.

Although Darling had not been very familiar with the tree before his encounter, he understood its importance as a keystone species and knew he wanted to contribute to the fight against the blight.

Scaffold at Zoar 1 chestnut tree, an 80-foot, 21-inch diameter tree a hunter discovered on his home in New York’s Zoar Valley.

All photos courtesy of Herb Darling Jr.

“It provides high quality rot resistant timber and food for the wildlife.” In addition, the tree is a cultural touchstone in America, can provide food for humans as well, and has plenty of carbon sequestration potential.

So Darling, a civil engineer and owner of his own company, Herbert F. Darling, Inc., got to work. And although he was not a research scientist, he used his business acumen and his passion for conservation to co-found the New York State chapter of the American Chestnut Foundation with Arlene and Stanley Wirsig, a chapter that now boasts membership of roughly 500. “We ran the New York State chapter with no staff and an all volunteer group,” Darling says.

And from his son’s help building that first scaffold, Darling’s family has been an integral part of his work. “From day one my family has totally been involved in sharing the work to be done,” he says. “They were at the events, they were planting, harvesting, and they staffed the annual meetings.”

“All the children and grandchildren willingly stepped up to the plate when it came to helping with planting,” adds his wife, Jane Darling, “literally ‘digging in.’”

The two met as college students when he was at Dartmouth and she attended Colby Sawyer. They were introduced by Darling’s sister and will celebrate their 60th anniversary September, 2016.

By far the largest, and most challenging, project that Darling and his New York chapter undertook was researching and fundraising for a genetic response to the American Chestnut Blight through the State University of New York’s College of Environmental Science and Forestry (ESF). The first challenge was that the American Chestnut Foundation’s national board rejected the idea. Their response to the loss of the tree through a program of cross-breeding American and Chinese chestnuts in the hopes of creating a more blight resistant plant.

Darling and the Wirsig’s believed, however, that The American Chestnut Foundation needed a multi-pronged approach and to invest in more than one strategy to restore the American chestnut. And although the genetic response was vetoed by the national organization, the state chapter was permitted to move ahead with the research.

Plant science biotechnology was still an emerging science, and had not been tried yet with forestry, so no one knew quite what to expect. “First thing is that we thought it was only going to be 10 [years],” says Darling, “and it turned out to be 25. So that was disappointing to all of us, but it just made us work harder. Because we were gonna get this thing, no matter what.”

And get that thing they did. In 2014, the scientists at ESF solved the problem of blight resistance by splicing a wheat gene with the chestnut. They chose this wheat gene because it is eaten daily by humans all around the world, so they knew it was safe, but it is not one of the genes associated with gluten. In addition, it is a natural defense gene that helps protect the wheat from disease, which they hoped would be true for the chestnut as well.

This hypothesis proved correct and this first line of trees to demonstrate this enhanced blight resistance



Herb Darling Jr. (left), his wife Jane (middle), and daughter Kathy (right) plant chestnut seedlings on the William White Farm, a seed orchard for the New York chapter.



2000 Annual Meeting with former TACF President Marshall Case.

was dubbed the Darling4, in honor of Darling’s hard work and contributions to the successful project.

Now, Darling’s hope is that “[the tree] will be returned to the wild and its native places as soon as possible.”

The new blight resistant tree is not available to the public yet. It must first go through testing at federal labs, which could take up to five years.

Beyond his pivotal work in the ESF transgenic project, Darling also created several educational cartoon characters to raise awareness and enthusiasm about the American chestnut among the younger generation. The main characters, chestnut-headed Charlie Chestnut and Ginny Gene, personifying the wheat gene that helps chestnut trees fight off the fungus, face off against Buster Blight, who is eager to destroy Charlie’s grandmother, a full-grown and vulnerable tree.

Darling is also currently on the Board of Directors of The American Chestnut Foundation, where he served as Chairman for six years and was awarded Chairman Emeritus status. He remained president of TACF-NY from its inception in 1989 until 2015 when he became its President Emeritus.

“Herb’s passion for the American Chestnut has instilled in all of us the importance of the environment and his determination to make this earth a better place,” Jane Darling says proudly. “And he has done just that!”

# Potato, Mushroom, and Chestnut Soup

Jul's Kitchen, [julsKitchen.com](http://julsKitchen.com)

Photo courtesy of  
Jul's Kitchen.



Prep time: 7 mins  
Cook time: 50 mins  
Total time: 57 mins

## Ingredients

2 tablespoons extra virgin olive oil  
1 medium onion, finely chopped  
500 grams (1.10 lb) potatoes (I used Russet potatoes),  
cut into small, even cubes  
150 grams (5.3 oz) cremini mushrooms, wiped clean  
and chopped  
360 ml (1½ cup) warm water  
1 teaspoon salt  
50 grams (1.8 ounces) pre-cooked chestnuts,  
crumbled

## To Serve

freshly ground black pepper  
fresh parsley, finely chopped  
1 tablespoon crumbled chestnuts

## Instructions

- ❶ In a medium pan, sauté the onion with 2 tablespoons of extra virgin olive oil until soft and translucent.
- ❷ Add the potatoes and mushrooms, stir and cook on low heat for about 10 minutes.
- ❸ Dissolve the salt in the warm water and add it to the pan.
- ❹ Cook on low heat for about 20 minutes.
- ❺ Add the chestnuts and cook for another 15 to 20 minutes, stirring occasionally, until the potatoes are soft and the soup is thick and creamy.
- ❻ Serve the potato, mushroom, and chestnut soup topped with crumbled chestnuts, chopped parsley, and freshly ground pepper.

# Enjoy!

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AUGUST 1 – NOVEMBER 30, 2015



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