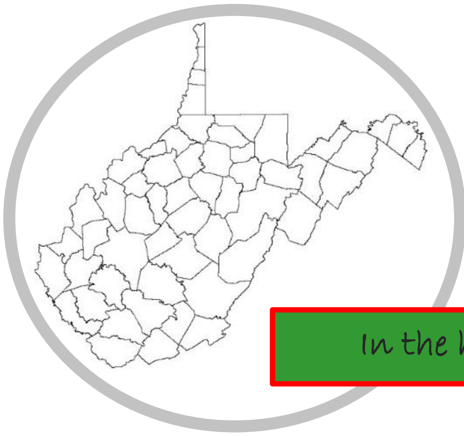


The West Virginia Chapter of The American Chestnut Foundation

NEWSLETTER

In the heart of American chestnut's natural range

June 2020

Table of Contents

Chestnut Chats

Beginning in April, **Lisa Thomson**, President and CEO of TACF and **Sara Fitzsimmons**, TACF's Director of Restoration, hosted hour-long chats on Friday mornings, beginning at 11:30 am. The chats were informal and one way to stay connected during the stay-at-home orders during the Covid-19 pandemic. The 24 April chat featured four TACF volunteers who talked about how they became interested in American chestnut.

Cathy Mayes is the former president of the VA chapter. She is an attorney, who did international consulting. She lives in a small, rural area of VA and she spent much of her work-life indoors. She was a recent retiree when she joined TACF and she was not necessarily interested specifically about chestnut, but she joined a local native plant organization. She knew enough about chestnut to write an article for the native plant organization newsletter and her relationship with TACF began. She began her work with the VA chapter by doing office work, land negotiations, recruiting volunteers and purchasing equipment. Cathy was interested in more than just chestnut but how chestnut could improve the environment. American chestnut fits her lifestyle and where she lived. How involved you become depends on whether or not you enjoy the people you work with, and it's the people of TACF that she enjoys most.

Cathy Mayes, Virginia chapter

Dr. Leila Pinchot works for the U.S Forest Service in Delaware, OH. She works both on elm and American chestnut. She first became interested in American chestnut at the age of 16 when her father showed her a 10" tree near her eastern PA home. Leila's father told her that there is a good community of people working on chestnut. That led her to volunteer with Dr. Sandra Anaganostakis at the Connecticut Agricultural Experiment Station in New Haven, CT. She worked summers with Sandra measuring chestnut trees and assessing chestnut blight. Leila's father suggested that she call the President of TACF (Marshal Case at the time) and ask him about the organization. That conversation led her to TACF's research farms in Meadowview, VA. She then worked for Sara Fitzsimmons prior to obtaining her Ph.D at the University of Tennessee with Dr. Scott Schlarbaum. Leila has three chestnut research projects in Pennsylvania. Those projects are: (1) different silvicultural practices; (2) how site quality impacts American chestnut and other species and site effect on blight severity; and (3) deer impacts on chestnut, looking at browse incidence.

Leila said that chestnut brings her closer to her father and she has appreciated the opportunities TACF has provided her.

Dr. Leila Pinchot, Ohio chapter

Dr. Brian Roth is at the University of Maine and he has been a TACF members since 2012. He loves TACF because he gets to use all the tools he learned as a forester.

Those tools include genetics, plant pathology, and nursery management. He stated that TACF could not do its work without citizen scientists. He loves TACF because he can reach a lot of people who have a love of trees. He connects with students, school groups, conservationists and foresters.

There are quite a few living American chestnuts in Maine. Brian enjoys hunting for large trees and he felt the best way to find large American chestnuts was to first develop a chestnut suitability map. The map included data on soil type, precipitation, elevation, etc. and he put together his own map of Maine with the help of students from the University of Maine. Once the map was developed, the took off in an airplane when chestnut was in bloom to try and spot large, flowering chestnuts. Then, on the ground, they looked for the trees that they had spotted from the air. By luck, they found the tallest, living American chestnut recorded in North America. The tree is 100' tall. It does not have a large diameter as it is competing with other forest trees for light, but it grew quite tall. He estimates the age of the tree to be 90-100 years old.


Dr. Brian Roth, Maine Chapter

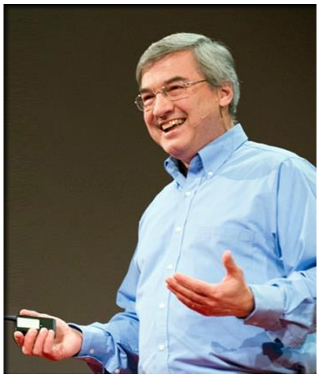
Charley Tarver is a forester and the founder of Forest Investment Associates (FIA), a registered investment advisory firm, headquartered in Atlanta, Georgia. Until his retirement, he served as the firm's President and CEO. FIA specializes in timberland investment management for large institutional investors. He is a graduate of the School of Forestry and Wildlife Sciences at Auburn University, where he has served on the Advisory Council and Development Committee. The University named him outstanding alumnus in 1992.

Charley grew up in lower Alabama and his first exposure to *Castanea* (the chestnut genus) was to Allegheny chinquapin, where he and his siblings collected the nuts when they were young. Fast-forward 35 years and Charley was at a national forestry organization in Washington, DC where he met Don Willeke, one of the founding members of TACF. Charley said that Don nearly talked his ear off about chestnut and TACF. Charley admitted that most of what Don said went in one ear and out the other. That being said, Don planted a seed in Charley's mind that day. Twenty years ago, Charley and his wife built a house in southwest Georgia for quail hunting. Charley's wife offered to have a table made for Charley and she asked him what type of wood he wanted for the table and Charley replied, chestnut. That started his foothold with American chestnut. When Charley built a house in Highlands, NC (as southwest GA was too hot), he found lots of chinquapins in his 30 acres. He met Lisa Thomson at a meeting at the Jones Ecological Research Station. The Jones Center seeks to understand, demonstrate and promote excellence in natural resource management and conservation on the landscape of the southeastern Coastal Plain of the United States. At that meeting with Lisa, Charley learned a lot more about the story of American chestnut. He has planted backcross chestnuts on his property in NC, and he envisions seeing blight-resistant American chestnuts growing throughout the chestnut's native range.


Charley Tarver, NC chapter

Dr. William (Bill) Powell of the State University of New York is one of more than 100 individuals who have worked more than 30 years to develop a genetically-engineered American chestnut tree. This collaborative work has involved scientists, students, technicians and citizen scientists. The status of the genetically-engineered tree is still under review by the USDA-APHIS (Animal Plant Health Inspection Service). Bill points out that chestnut has 30,000 gene pairs. The addition of the oxalate oxidase [OXO] gene from wheat (that breaks down oxalic acid produced by the chestnut blight fungus into hydrogen peroxide and CO_2) is comprised of only a few genes. American chestnut, therefore, retains 100% of its original traits.

The oxalate oxidase gene is a plant protectant and not a pesticide.



Dr. William Powell, NY chapter

Bill Powell addressed a question posed from those who raise honeybees. Many beekeepers treat their hives with oxalic acid to control Varroa mites. Beekeepers are concerned if the genetically-engineered chestnuts will pose a threat to their treatment regime. Powell stated that they have looked specifically at OxO in pollen, and they didn't see any directly harmful effects against bees.

As far as interfering with Varroa mite treatments, the only way they understand this could have an effect is if there is an adequate quantity of active OxO enzyme present in beehives, such that the enzyme would come in contact with the oxalic acid before the acid contacts the mites. This would require bees moving live tissue to their hives, which does happen with pollen. Transgenes are expressed at very low levels in pollen, so it is very unlikely that there would be enough active OxO enzyme to degrade oxalic acid, especially before the acid comes in contact with the mites. This will be interesting to study once they are able to collect enough transgenic pollen to do studies like this. Based on what we understand at this point, it doesn't seem like a substantial risk.

TACF's Specific Science Plan for State Chapters

The following is a lengthy and detailed plan that involves all 16 state chapters of TACF. There is a good deal of information around ink disease (*Phytophthora cinammomi*), an issue that is mostly problematic for our southern chapters. However, ink disease has been found in the Waddell orchard in Preston County, so the work on ink disease also is relevant to WV.

Explanation. The chapter-specific science plan explanation provides greater details and clarifications for the current breeding strategy and protocols.

Objective 1. Maximize blight resistance in chapter breeding programs. The American Chestnut Foundation's traditional breeding program was built upon research which suggested that blight resistance was a relatively simple trait, controlled by only 2 to 3 genes. Through decades of backcrossing and, now, integration of genomic analysis, we have learned that blight-resistance is a complex trait that is controlled by many genes. As a consequence, backcrossing to American chestnut has partially diluted blight resistance. TACF science staff has outlined steps to maximize blight resistance in chapter breeding programs in light of the recent findings.

I. Increase stringency of selection in BC3/BC4 orchards. We would like all chapters to work with their regional coordinators to phenotype their current backcross selections (BC1, BC2, BC3, and BC4 generations) for traits indicative of blight resistance/susceptibility. TACF national is currently genotyping chapter backcross trees to determine how much of their genome was inherited American v. Chinese chestnut. We will pair the phenotype and genotype information to determine which of the current backcross selections have moderate resistance and which have inferior resistance. We recommend conserving the inferior backcross trees for their American chestnut diversity, but not using them as parents in the breeding program.

1. Inoculate with weak and strong strains of *Cryphnectria parasitica* (SG 2,3 and EP-155 strains) and make phenotypic selections per existing protocol.

2. Phenotype selections that have been inoculated at least two years ago to assess their long-term field resistance.

3. Please have all orchards/trees in dentataBase by spring 2020 prior to TACF genotyping the backcross trees.

4. Collect tissue for genotyping from selected trees that we do not currently have in our collection. We have tissue for most chapter backcross selections made in 2017 or before, but we do not yet have

tissue for selections made after 2017.

5. Work with regional coordinators to use phenotype and genotype data to increase stringency of selection in BC3/BC4 orchards and refine BCxF2 planting decisions. Based on phenotype/genotype analysis, we will recommend not advancing some of the BC3/BC4 trees that were selected previously based on canker severity ratings.

a. If these newly unselected BCx trees are contaminating open pollinated crosses, harvest seeds from unselected trees and move progeny to a separate location. Cut the tree down after progeny are planted. The progeny can be mixed in germplasm conservation orchards (generally not preferred) or planted in separate locations.

b. If the unselected trees are not contaminating open pollination now, there is no need to cut these trees down. If unselected trees are the sole survivors of a particular backcross line, preserve these trees for future crossing with transgenic trees.

6. Intercross the BCx trees that have resistance phenotypes and that inherited >5% of their genome from Chinese chestnut; plant progeny from this reduced set of BC3/BC4 selections in seed orchards.

7. Conduct a limited number of controlled crosses between your BC3 and BC4 selections and F1 and BC1 trees to create BC1 and BC2 trees that have greater potential for blight resistance and Phytophthora resistance. Add plot(s) in each seed BCxF2 orchard block with BC1 and BC2 progeny. These earlier generation hybrids may contribute additional resistance alleles from different Chinese chestnut parents and improve the overall resistance of the population. TACF staff will send pollen from a number of these earlier generation hybrids with good resistance at Meadowview Research Farms. You might also use chestnut hybrids with higher levels of blight resistance from other locations as parents of your seed orchard trees. Other locations with earlier generation hybrids (i.e., BC1 and F1) include Lesesne State Forest in VA and the CT Agricultural Experiment Station.

II. Select the 1% most blight resistant trees in seed orchards (BC3F2 and BC4F2 generations).
We intercross the BC3/BC4 selections to improve blight resistance in the following generation (BCxF2). The BCxF2 progeny potentially inherit genes for blight resistance from both BC3/BC4 parents. However, due to the dilution of blight resistance through multiple generations of backcrossing to American chestnut, we expect that the selected trees in BCxF2 seed orchards will have intermediate blight resistance. In the previous generation (BC3/BC4), we recommended increasing the stringency of selection and conducting a limited number of controlled crosses between BC3/BC4 selections and F1 and BC1 trees to improve the average level of resistance in BCxF2 seed orchards. Next, we outline steps to accurately select the 1% blight resistant trees in BCxF2 seed orchards. By culling all but the most blight resistant trees, we will maximize blight resistance of the seed coming from these orchards. Blight resistance may be further improved through additional generations of selection in orchards or naturally in the forest.

1. Include American chestnut, F1, and Chinese chestnut controls in seed orchard to assess the level of resistance of the BCxF2 selections relative to controls. Remove controls (especially Chinese and American chestnuts) after you have completed selection of best BCxF2 trees.

2. Inoculate BCxF2 and controls with the weakly pathogenic SG2,3 strain when most trees in 150 tree plot are greater than 1 inch in ground line diameter. Rogue the most blight susceptible trees.

3. Wait a year or two for remaining trees to grow and inoculate a second time with a more pathogenic strain of *C. parasitica* (Weekly or EP-155). Further cull seed orchard plots to < 20 trees per plot.

4. Conduct small stem assays on a subset of your selection candidates for the purpose of developing genomic prediction models for progeny blight resistance. Collect ~ 50 BCxF3 seeds from 50 – 100 BCxF2 mother trees and send to 10 Meadowview (or another location) for small stem assays to assess resistance of seed coming from orchard.

5. Collect young leaves for genotyping the mother trees whose progeny are in the small stem assay. The genotyping will be used to develop genomic prediction models for the blight resistance of

progeny of other trees in the orchard.

6. Collect young leaves from remaining selection candidates for genomic selection. Cull the orchard down to the 1% most blight resistant trees based on genomic data.

Objective 2. Conserve a range-wide collection of wild American chestnuts in germplasm conservation orchards to prepare for outcrossing and diversifying transgenic blight-tolerant American chestnuts. Breeding transgenic blight-tolerant American chestnuts with susceptible wild-type (WT) trees is potentially an efficient method to rescue the genetic diversity and adaptive capacity of the American chestnut population for large-scale restoration. We would like to chapters to conserve a total of 1,000 WT American chestnuts in germplasm conservation orchards (including current collections) to prepare for outcrossing and diversifying transgenic populations. If federal regulatory approval is granted to release transgenic trees, we would like to chapters and TACF staff to outcross transgenic trees to wild trees over three to five generations to increase regional adaptation and minimize inbreeding in transgenic blight tolerant populations (see Westbrook et al., 2019 for more details on the plan to diversify transgenic populations). Getting started on germplasm conservation now gives us time to find new sources American chestnuts, develop our skills with graft propagation, and test the efficacy of hypovirulence and other methods to keep blight-susceptible American chestnuts healthy for use in breeding.

1. Take inventory of survival in your current germplasm conservation orchards

2. Scout and find WT American chestnuts. a. Record locations in TreeSnap™ or with Tree Locator Form. Collect samples for species ID/verification. b. Flag or mark trees that you want to revisit in winter for scion collection

3. Collect nuts in the fall

4. Collect scion wood in winter

5. Conserve trees in orchards with biocontrol:

a. Mud-packing

b. Mixed strains of hypovirulent C. parasitica

c. Super donor strains*

*Note: while not yet available, the super donor strains of hypovirus hold the most promise. These strains were developed at the University of Maryland and are being tested primarily at West Virginia University. They are still regulated and require a permit to release, but work is under way to assess their regulatory status and we are hopeful they may be used by our program in the future.

Objective 3. Combine resistance to chestnut blight and Phytophthora root rot. American chestnut is highly susceptible to the soil borne pathogen, *Phytophthora cinnamomi*, which causes root rot that kills plants. The range of *P. cinnamomi* is limited by prolonged freezing temperatures. Historically, this pathogen has affected American chestnuts in the southeastern U.S. As winters warm, *P. cinnamomi* is spreading north and is expected to reach 11 New England by 2080. Combining resistance to phytophthora root rot with resistance to chestnut bight is essential for restoring the American chestnut. The American Chestnut Foundation is collaborating with Clemson University and the U.S. Forest Service to screen American chestnut backcross hybrids for resistance to *P. cinnamomi*. To date, most of the *P. cinnamomi* resistance screening has been conducted on American chestnut backcross families from Southwest Virginia. Some American chestnut backcross families inherited moderate to high levels of resistance to *P. cinnamomi* from Chinese chestnut. To increase the climate adaptability of backcross trees selected for *P. cinnamomi* resistance, TACF would like to screen backcross families from TACF's chapter breeding programs ranging from Maine to Georgia. Annually, for the next 5 to 10 years we plan to screen 4,800 backcross seedlings for resistance to *P. cinnamomi* at the U.S. Forest Service Resistance Screening Center in Asheville, NC. We expect that approximately 5% of the seedlings will survive infection. Survivors with resistance will be planted at field sites where *P. cinnamomi* is present in the soil. Once these resistant trees grow large enough to flower, they will be bred with backcross or transgenic trees with resistance to chestnut blight to combine resistance to the two diseases. Chapters can get involved in the *P. cinnamomi* resistance screening effort simply by sending seeds from backcross trees to Meadowview to screen for root rot resistance at the U.S. Forest Service Resistance Screening Center. We ask for assistance from chapters in the South in identifying field sites with *P. cinnamomi*.

1. Collect minimum 50 nuts from untested Graves lines and other resistance sources besides 'Clapper'. Descendants of 'Clapper' have minimal resistance to *P. cinnamomi*. Send seed to Meadowview research farms for storage over winter.

2. Identify potential orchard sites where *P. cinnamomi* is present in the soil. Send soil samples to Dr. Steve Jeffers at Clemson University, who will test the soil for *P. cinnamomi*.

Objective 4. Plant reintroduction trials to determine if current levels of disease resistance and American chestnut characteristics are sufficient for restoration. We will assess restoration trials planted in the last decade and plant new restoration trials with our best material to date to determine if intermediate blight resistance is sufficient for backcross trees to compete and reproduce in Eastern forests. With sufficient blight resistance, it is our hope that natural selection will continue to improve blight resistance and competitive ability of backcross populations in the forest. In preparation for large-scale reintroduction, we would like to plant reintroduction/restoration trials to test how varying silvicultural treatments influence survival and blight resistance. For restoration plantings, TACF staff will allocate specific families and work with collaborators to design experimental plantings. However, TACF is looking for collaborators who will install, manage, and collect data on the plantings. Chapters may help us identify landowners and agency cooperators to manage these restoration plantings.

New WV Plantings in 2020

There were eleven new plantings of backcross American chestnut trees established in WV in 2020, bringing the total number of plantings in the state to 68. One of the new plantings is with the Army Corps of Engineers at Sutton Lake. WV-TACF Board of Director member**, Jimmy Jenkins** suggested the site as his son, Harrison, is employed by the Army Corps. **Harrison Jenkins and Brandy Acord** planted six backcross chestnuts and a germplasm conservation orchard (GCO) comprised of 10 American chestnuts (from chestnut trees in Greenbrier and Hardy Counties).

Harrison Jenkins planting a chestnut at Sutton Dam.

The intricate fencing used to protect seedlings at the Sutton Dam.

West Virginia now has 11 GCOs. While the goal for each GCO is 100 American chestnuts (10 trees each from 10 different sources), most orchards have fewer than 10 American chestnuts. However, like the old adage, "how do you eat an elephant—one bite at a time", we have to start with the seedlings we have. The one exception is Jeff Kochenderfer's planting in Parsons; he has about 90 American trees. The more WV members inform us of American chestnut sightings in the state, the more material we will have in the future.

Two additional new chestnut plantings are pictured below.

A new chestnut planting in Jefferson County.

A new chestnut planting in Harrison County.

WV Chapter Summer Intern

The WV chapter is hiring a college intern this summer**. Logan Hosaflook**, a senior in Forestry at Glenville State College, will primarily scout the state for new sources of American chestnut (as per Objective 2 on page 5). WV chapter members were asked this spring for sites with American chestnut and many members responded. Logan will work mainly for his father, a consulting forester, so he will only be able to work 3-4 days per month for the chapter. Look for a report of Logan's work in a fall newsletter.

Remove Nut Prior to Planting

When planting chestnut seedlings that have been reared in pots, the protocol is to remove the nut prior to planting. The nut is a wealth of nutrition that contains protein, carbohydrates and fats, making chestnuts (and acorns) a favored food for many animals (squirrels, chipmunks and raccoons. While the chestnut may appear rotten, the following picture gives a very different impression when the nut is removed from the root system and cut in half. Surprisingly, while the exterior may appear dark, the inside reveals a creamy white nutritious meal for wildlife. If the nuts are not removed, wildlife can destroy the seedling in search of the nut-meat.

Chestnut seedling ready for planting with nut attached.

Chestnuts from seedlings that were removed prior to planting and cut in half. The nut-meat is attractive to wildlife.

Meadowview Research Farm

Meadowview, Virginia is home to The American Chestnut Foundation's Research Farms. This property and its facilities are used to preserve, study, and breed American chestnut trees for resistance to the blight fungus. Meadowview includes tens of thousands of trees at various stages of the breeding process, planted on more than 150 acres.

An aerial view of the Meadowview Research Farm in southwestern Virginia.

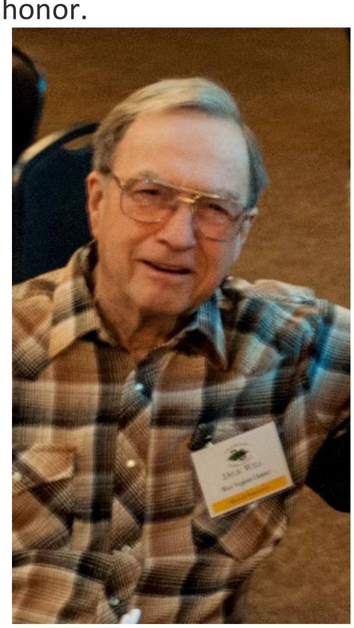
1989: The first research farm was established to formally execute the backcross breeding program. Chestnut trees have been planted, crossed, and grown on this farm for more than three decades, and it continues to function as one of two seed orchards. More than a decade later, the property was filled to capacity with chestnut trees at various stages of backcrossing.

1995: A generous donation enabled the purchase of land nearby, now known as the Glenn C. Price Research Farm and home to the farm staff's offices, laboratory, maintenance shop, nursery facilities, and backcross orchards.

2010: The Glenn C. Price Laboratory was dedicated and is used to conduct on-site research.

2015: An operations building was constructed near the offices and laboratory on the Glenn C. Price Farm. The facility boasts a large 3,300 square foot indoor workspace with overhead heating, a drive-thru garage bay, pesticide and herbicide storage and mixing area, a large walk-in cooler used for seed storage, and is equipped with an eyewash station, emergency response equipment and often houses machinery and containerized chestnut seedlings in winter. In addition, the building is used as a general workspace for large tasks such as seed processing, comfortably accommodating large workgroups of volunteers and students. TACF has hosted a number of company celebrations and dinners, classes, and presentations in the facility.

2017-18: TACF greatly improved the operation's capabilities by constructing a greenhouse and neighboring shade houses to increase the capacity and ability to grow seedlings for research and breeding. In spring 2018, TACF board of directors recognized chairman emeritus **Dick Will** for his years of unwavering support, advocacy, and leadership by dedicating the newly constructed greenhouse in his honor.



Dick Will, former chairman of the board of TACF.

Dick and his wife live part of the year in their home in West Virginia's eastern panhandle.

More than 7,500 chestnut seedlings can be reared in the Meadowview greenhouse.

As the bedrock of TACF's traditional backcross breeding program and a center for chestnut blight research, Meadowview Research Farms works closely with collaborative partners including colleges and universities, state and federal government agencies, volunteer conservation groups, and private industries who assist us in moving closer toward our unwavering goal to return the American chestnut to its native range.

Emotional Ties to American Chestnut

For many older Americans who can remember American chestnut before or doing its demise, there is a very emotional tie to this tree. One such memory comes from **George Unger** in Grand Haven, Michigan. George was born in Hungary in 1904, the same year that chestnut blight was discovered on large American chestnut trees at the Bronx Zoological Park in New York City. In the late 1980s when George was in his 80s, he recalled the importance of American chestnut to him and his family. George reminisced that he and his siblings would venture out into the forests in Michigan and collect fallen chestnuts in burlap sacks. George said that his parents helped to haul the sacks out of the woods and carry them to the train depot where the chestnuts were shipped to Chicago. Street vendors in Chicago roasted chestnuts in 55-gallon drums and sold the hot, roasted nuts on the city streets.

A chestnut roaster.

George recalled emotionally that the money collected from the chestnuts provided his parents enough money to purchase George and his siblings shoes for the winter. Mr. Unger wept at the loss of the American chestnut tree.

A young boy exuberant over new shoes.
Photo courtesy of boardpanda.com