SPRING 2020 ~ ISSUE 2 VOL. 34

Chestnut

THE JOURNAL OF THE AMERICAN CHESTNUT FOUNDATION

A BENEFIT TO MEMBERS
THE AMERICAN CHESTNUT FOUNDATION

A Special Chestnut ~ Meadowview ~ Germplasm Conservation ~ Male-sterile F_1 Trees ~ Oak Silviculture

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THE AMERICAN CHESTNUT FOUNDATION

Lisa Thomson

President and CEO

DEAR CHESTNUT FRIENDS,

Those of you involved with TACF for years, or are just new to the organization, please join me in recognizing our volunteer leadership: the board of directors, chapter presidents, and committee chairs. Simply stated, this organization would not enjoy its strong reputation or prolific output of work without these effective and talented volunteer leaders.

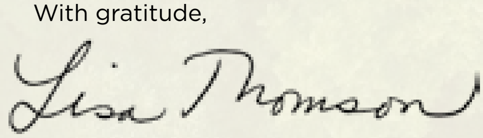
Some examples. Twice a year in person, and once by conference call, our board of directors meet to discuss a variety of issues critical to the organization: science and technology advances, promotion and outreach needs, and non-profit best practices to help ensure TACF earns that coveted 4-Star Charity rating by Charity Navigator (now achieved 6 years in a row). These volunteers are thought leaders from many sectors: science, academia, business, law, and finance to help guide us with their knowledge and expertise. On their own nickel, they travel to these meetings prepared and ready to work. Every month, the seven-member Executive Committee has a conference call to discuss important issues and help in decision-making to help navigate the organization through seas of change. As board chair Brian McCarthy states, "TACF is strong and growing, thanks to a deeply engaged and enthusiastic cadre of talented volunteer leaders."

Chapter presidents and their officers ensure the work of the organization is disseminated to the state and local level. They are the "boots on the ground" who help implement the overall strategic plan and chapter breeding programs. Additionally, task forces are formed for specific needs, such as organizing the public comment period for SUNY-ESF's transgenic tree deregulation. Interested in becoming more involved? Let us know! The optimism of bringing back an iconic species creates a culture of excitement and caring among passionate people.

TACF ended the decade with a splash of positive media attention which may have been one reason for a phenomenally successful end of year appeal and new member activity. At the close of our campaign, we had more than $230,000 in gifts and 427 new members. This is a terrific testament to the appeal of our hopeful story.

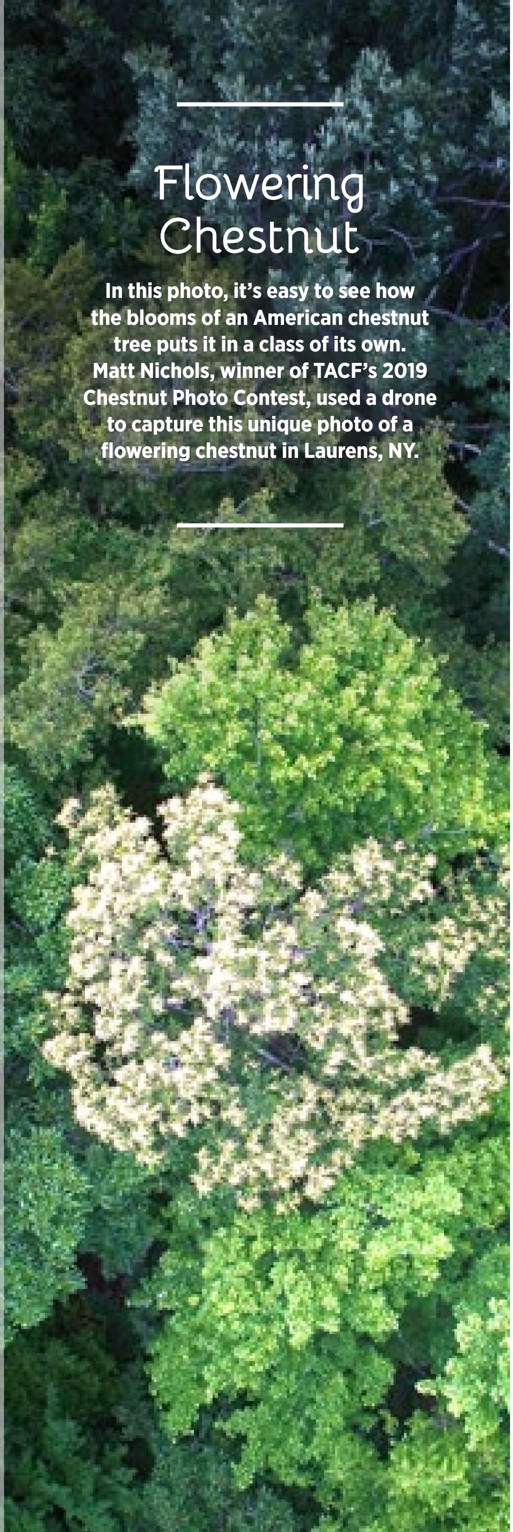
In closing, thank you, dynamic volunteers, donors, and members for helping lead this bold effort. As of press time, we are in the midst of global uncertainty which threatens entire human communities. It is our collective great hope that all of you in the TACF family stay vigilant and safe.

With gratitude,



Lisa Thomson, President and CEO
The American Chestnut Foundation

Follow me on Twitter (@MadameChestnut).



Flowering Chestnut

In this photo, it's easy to see how the blooms of an American chestnut tree puts it in a class of its own. Matt Nichols, winner of TACF's 2019 Chestnut Photo Contest, used a drone to capture this unique photo of a flowering chestnut in Laurens, NY.

At The American Chestnut Foundation, we strive for accuracy in all of our publications. We regret any errors or omissions and appreciate those who bring them to our attention. For the 2020 winter issue of *Chestnut*, we have corrected the following information:

- On page 32 Dr. Charles Burnham was incorrectly placed under the "In Honor" section rather than "In Memory." Dr. Burnham died in 1995.

THE AMERICAN CHESTNUT FOUNDATION™

WHAT WE DO

The mission of The American Chestnut Foundation is to return the iconic American chestnut to its native range.

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TACF Meadowview Research Farms, Meadowview, VA.

Breeding

FOR BLIGHT RESISTANCE

By Sara Fitzsimmons, TACF Director of Restoration

Traditional breeding was one of the first methods scientists used to combat the damage inflicted by chestnut blight in the early 1900s. Thousands of hybrid trees were created and deployed across the eastern United States through the efforts of the USDA and other affiliated research programs. Unfortunately, none of those programs were able find the right combination of disease resistance to chestnut blight along with ecological competence of the American chestnut to allow for species rescue and restoration.

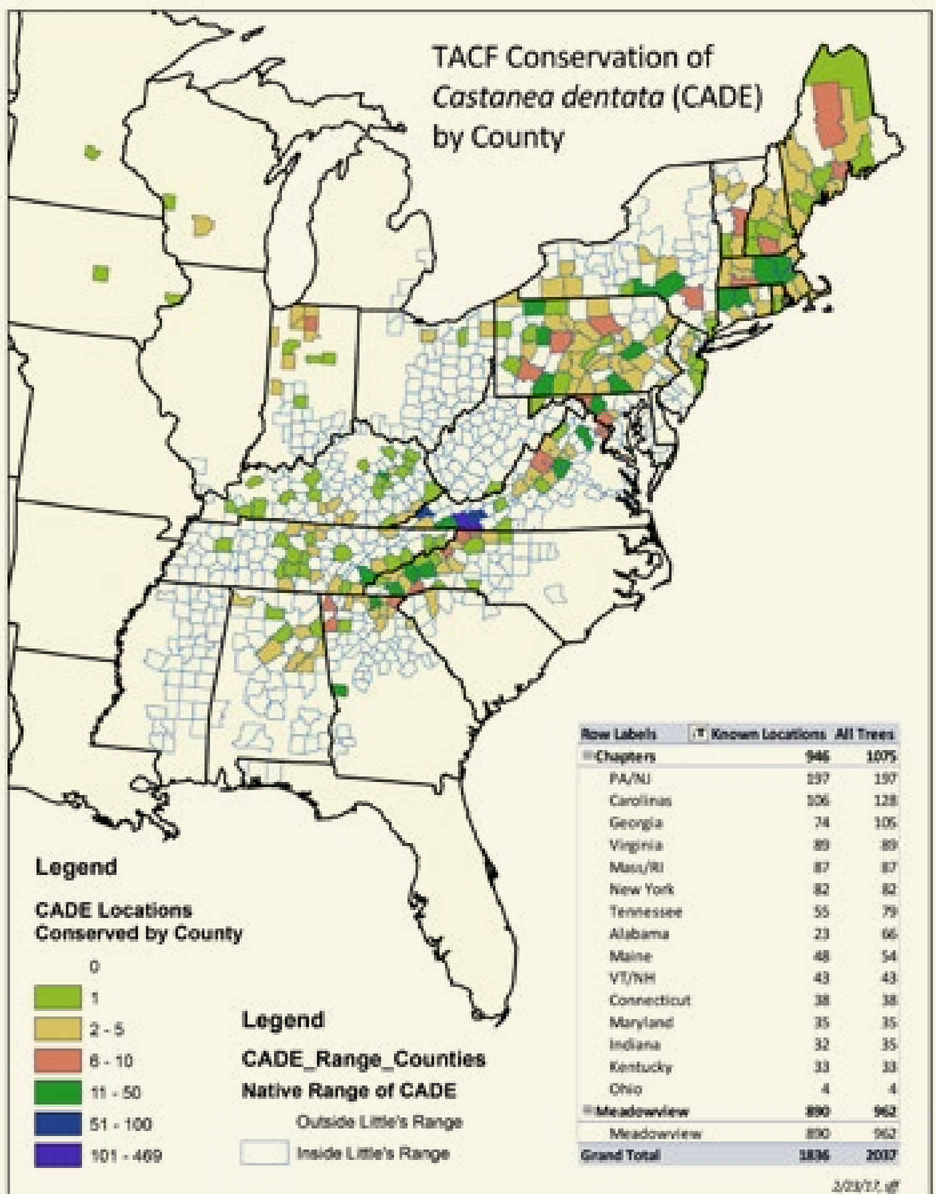
Shortly after the organization was founded in 1983, The American Chestnut Foundation (TACF) initiated continuation of those early efforts, primarily by crossing wild-type American chestnuts with the first backcross hybrids (BC_1) created by the Connecticut Agricultural Experiment Station (CAES). The goal of that program was to generate hybrids that combine the blight resistance of Chinese chestnut with all other traits of American chestnut. The specific method of traditional breeding chosen at that time was called backcrossing.

Through a prescribed set of six generations, American x Chinese chestnut hybrids are backcrossed to dilute as many Chinese chestnut genes as possible while retaining those that confer blight resistance. After nearly 30 years and four generations of breeding for two primary sources of resistance, Clapper and Graves, we have nearly completed selection of the most blight-resistant chestnut hybrids in two seed orchards from our breeding program at our flagship research farm in Meadowview, Virginia.

Through the leadership oversight of TACF's Director of Science, Jared Westbrook, the Foundation has recently incorporated genomic techniques to accelerate selection of the most blight-resistant trees (1. Westbrook et al 2019). That work shows selected hybrids have inherited between 60% and 90% of their genome from American chestnut and exhibit blight resistance on a spectrum that is intermediate between American chestnut and Chinese chestnut. We have planted selected hybrids in over 40 restoration trials in the eastern U.S. to determine if these hybrids have sufficient blight resistance and competitive ability for restoration in eastern forests. We continue to improve blight resistance in our breeding program by inter-breeding the most resistant trees within each generation and selecting the most blight-resistant progeny.

TACF has 16 volunteer-run state chapters ranging from Maine to Alabama. Chapter volunteers have bred chestnut hybrids with local American chestnut trees to incorporate genetic diversity that is critical for the restoration population to adapt current and future conditions. Based on the information garnered

The American Chestnut Foundation Conservation of *Castanea dentata* (CADE) by County



from selection at Meadowview Research Farms, TACF staff and chapters are improving stringency of selection in backcross populations, conserving novel sources of wild-type American provenance, incorporating resistance to *Phytophthora cinnamomi*, and examining performance of hybrids in the forest. Here are the major objectives for TACF's breeding strategy:

OBJECTIVE 1. Maximize blight resistance in breeding programs

TACF is genotyping chapter backcross trees to determine how much of their genome 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.

OBJECTIVE 2. Conserve a range-wide collection of wild American chestnuts

Breeding transgenic blight-tolerant American chestnuts with susceptible wild-type (WT) trees is an efficient method to rescue the genetic diversity and adaptive capacity of the American chestnut population for large-scale restoration. We would like 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, chapter volunteers and TACF staff will outcross transgenic trees to wild trees over three to five generations to increase regional adaptation and minimize inbreeding in transgenic blight-tolerant 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.

TACF works in close partnership with the team of researchers at Bill Powell and Chuck Maynard's (retired) labs at State University of New York, College of Environmental Science and Forestry (SUNY-ESF), the primary investigators to creation of the transgenic American chestnut (part 3 of this series will focus on the details of that project). Maximizing diversity within our restoration populations will give them the capacity to adapt to climate change and other future pressures.

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 Phytophthora root rot (PRR) that kills plants. The range of PRR is limited by prolonged freezing temperatures. Historically, this pathogen has affected American chestnuts in the southeastern U.S., but as winters warm, PRR is spreading north and is expected to reach New England by 2080. TACF is collaborating with Clemson University and the U.S. Forest Service to screen a genetically diverse population of American chestnut hybrid seedlings from TACF's chapter breeding programs for resistance to PRR (2. Westbrook et al 2019). Our hybrids demonstrate a range of resistance to PRR and we plant the survivors at field sites where PRR is present in the soil. Once these trees with resistance to PRR grow large enough to flower, they will be bred with blight-resistant hybrids or transgenic trees to combine resistance to the two diseases.

OBJECTIVE 4. Plant reintroduction trials to determine if current levels of disease resistance and American chestnut characteristics are sufficient for restoration

Restoration trials planted in the last decade will continue to be evaluated and new restoration trials with our best material to date will be initiated. Because blight resistance is more complicated than originally thought, we need to determine whether intermediate blight resistance is sufficient for backcross trees to compete and reproduce in Eastern forests. 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. Eventually, and pending regulatory review, these trials will also include individuals created through transgenic methods.

The Future

Other avenues of research continue including that into hypovirulence and other biocontrol methods, as well as gene editing technologies such as CRISPR. For now, these routes of research take place largely outside of the chapter and volunteer realm, but they will be integrated into chapter-specific breeding plans as soon as they are available. The fourth and final part in our 3BUR series will focus on biocontrol.

To read more, we recommend the following recent publications:

1. Westbrook, J. W., Zhang, Q., Mandal, M. K., Jenkins, E. V., Barth, L. E., Jenkins, J. W., and Holliday, J. A. (2019). Optimizing genomic selection for blight resistance in American chestnut backcross populations: A tradeoff with American chestnut ancestry implies resistance is polygenic. Evolutionary Applications. https://doi.org/10.1111/eva.12886

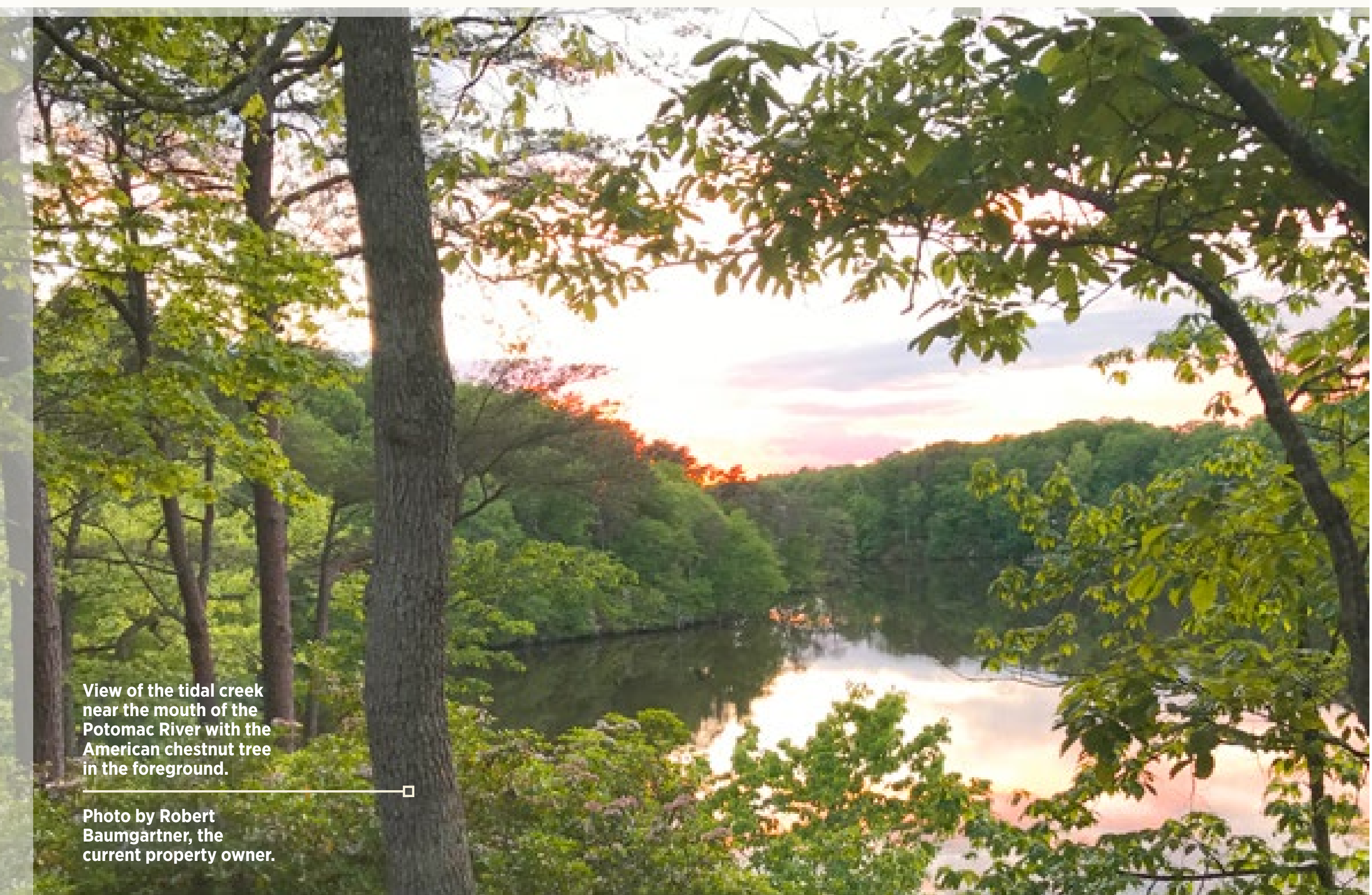
2. Westbrook, J., James, J., Sisco, P., Frampton, J., Lucas, S., and Jeffers, S. (2019). Resistance to *Phytophthora cinnamomi* in American Chestnut (*Castanea dentata*) Backcross Populations that Descended from Two Chinese Chestnut (*Castanea mollissima*) Sources of Resistance. Plant Disease. 103. 10.1094/PDIS-11-18-1976-RE.

A Special Chestnut

By Catherine Mayes, VA Chapter

Virginia's Northern Neck is a wide peninsula of land extending south and east between two great rivers, the Potomac and the Rappahannock. It is in the geological province called the Coastal Plain, but has pockets of terrain that are akin to the Piedmont province – rocky, steep, and loamy. As a result, there are some massive bluffs, including pristine Fones Cliffs, home to a large population of bald eagles.

On a steep slope in Northumberland County, the eastern part of the Northern Neck, covered with mountain laurel and just a few feet above a tidal creek near the mouth of the Potomac River, lives a handsome American chestnut tree with a special history. This is an unexpected place to find chestnuts growing in Virginia.



View of the tidal creek near the mouth of the Potomac River with the American chestnut tree in the foreground.

Photo by Robert Baumgartner, the current property owner.

While there are many wild-type chestnuts surviving in the mountains, and quite a few still in the Piedmont, there are almost none in the Coastal Plain. Though many chestnut sprouts still emerge from old stumps in the mountains of Virginia, few grow past ten feet tall before succumbing to chestnut blight or to ravenous white-tailed deer. So, to find a tall tree in this location is very rare, and one cannot avoid asking, "Is this the one tree in Virginia the blight missed or the one tree that had natural resistance to the blight?"

My inquiries lead to an unexpected explanation. I traced the tree's story back to the 1980s. At that time, the slope was covered with mountain laurel. The landowners cleared the slope and chestnut stumps 3-feet in diameter were discovered there. Robert Lumsden, a plant pathologist and researcher at the U.S. Department of Agriculture (USDA), purchased the property. To his surprise, a sprout emerged from a dormant stump. He watched the sprout grow and, inevitably, it became infected by chestnut blight from a nearby severely infected sprout. From a small canker that had developed at the base of the sprout, Lumsden isolated the fungus in his laboratory at the USDA's Beltsville Maryland Agricultural Research Center. He sent the sample to William L. MacDonald at West Virginia University, now director emeritus with The American Chestnut Foundation (TACF). MacDonald was one of the foremost scientists working on chestnut blight, and in his laboratory, the virulent strain Lumsden isolated was paired with a hypovirulent (virus-infected) strain from Europe. At that time, the hypovirus was thought to control chestnut blight more effectively in European chestnut compared to its American cousin.

MacDonald returned the now hypovirus-infected blight to Lumsden who, with the help of an agile grandson, applied it to the canker on the healthier developing chestnut sprout. As time passed, the untreated sprouts died, but the one treated with the hypovirus continued to thrive.

Robert D. Lumsden with the small healthy tree. Circa 1998.

The tree in summer. Circa 2019.

Dead chestnut with infected sprouts which was the source of infective spores. Circa 1996.

It is now 30 years later. The surviving chestnut has grown to 45 feet tall, 10 inches in diameter, and straight like its ancestors, with no visible die-back in the crown. It has produced flowers and burs but, owing to its isolation, no fertile seed. Lumsden sold the property and the current landowners are keeping an eye on it. A VA-TACF Chapter volunteer collected leaves from the tree for genetic analysis in 2018, as it is probably the lowest elevation tree in the state. The chapter hopes to pollinate the tree in 2020, or collect some of its pollen to pollinate another American tree, to preserve the genetics that may now be unique in the Commonwealth.

The success of Lumsden's experiment of applying hypovirus to a diseased tree offers hope that some – probably not all – wild-type American trees can be saved using a similar technique. Scientists from the University of Maryland and West Virginia University learned a great deal about using hypovirus as a biocontrol in the decades since the Northern Neck chestnut was treated. For example, they discovered that various strains of chestnut blight in the U.S. are not identical to the strains infecting European chestnuts.

There are 64 known types of chestnut blight fungus; these are referred to as 'vegetative compatibility types.' Some hypovirulent strains can fuse with multiple compatibility types and transfer the virus. However, no hypovirulent strain can fuse with all strains of chestnut blight, making biological control in the forest difficult, as many chestnut sites in eastern North America have as many as 25-30 vegetative compatibility types. Donald Nuss (retired from the University of Maryland) used systematic molecular gene disruption and classical genetics to engineer hypovirulent strains with superior virus transmission capability. Nuss knocked out most of the vegetative compatibility genes and referred to these strains as 'superdonors.' In the laboratory, Nuss was able to transmit hypovirus to all 64 vegetative compatibility types. To test the effectiveness of these strains in a forest setting with American chestnut trees, Nuss and colleagues at West Virginia University inoculated the superdonor strains into natural cankers on chestnut trees in western Maryland. They found that 94% of all cankers treated with the superdonor strains were controlled by the hypovirus (bit.ly/hypovirus).

Isolates of the chestnut blight fungus growing on an agar medium. Left, normal orange-pigmented fungus of killing strain of the fungus. Right, abnormal white-pigmented strain that is hypovirulent (virus-containing).

Research into the superdonor strains continues and is led today by Matthew Kasson, Interim Director of the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) and his research assistant, Amy Metheny, at West Virginia University. One roadblock remains: the superdonor strains do not spread well and their potential as biological control agents is still unknown. Scientists continue with work on a universal hypovirus that can spread from canker to canker, tree to tree, forest to forest. As we see in the Northern Neck tree, applying the hypovirus to individual cankers can significantly prolong the life of the one tree, but with millions of American chestnuts infected with chestnut blight, manual application is not a viable solution. We need a biocontrol that will move in the environment and establish itself on other cankers, for example, by infected fungus spores. Ideally, we need a hypovirus that can survive aerial application at the forest level. Our work continues!

2020 American Chestnut Photo Contest

Photo by 2019 contest participant Jacob Pease.

Have you taken a photograph of an American chestnut or American chestnut hybrid that should be displayed on the cover of *Chestnut* magazine? Enter it in TACF's 2020 American Chestnut Photo Contest! Send your best photo(s) to TACF by September 1, 2020 to enter. The winner will receive a complimentary one-year TACF membership and his/her photo will be featured on the cover of a future issue of *Chestnut*.

HOW TO ENTER & CONTEST TERMS:

All entries must be submitted digitally via e-mail or a link to a cloud drive by September 1, 2020;

All entries must relate in some way to the American chestnut;

Entries must include name of photographer and contact information;

Entries must include a full caption including names of subject(s), location, and title;

Entries must be at least 2500 x 3430 pixels and submitted in a jpeg or tiff file format;

Participants are limited to five entries per person;

Entries must be previously unpublished and cannot be entered into another contest.

EMAIL ADDRESS FOR SUBMISSIONS: jules.smith@acf.org

Visit bit.ly/tacf-2020-photo-contest for rules and details.

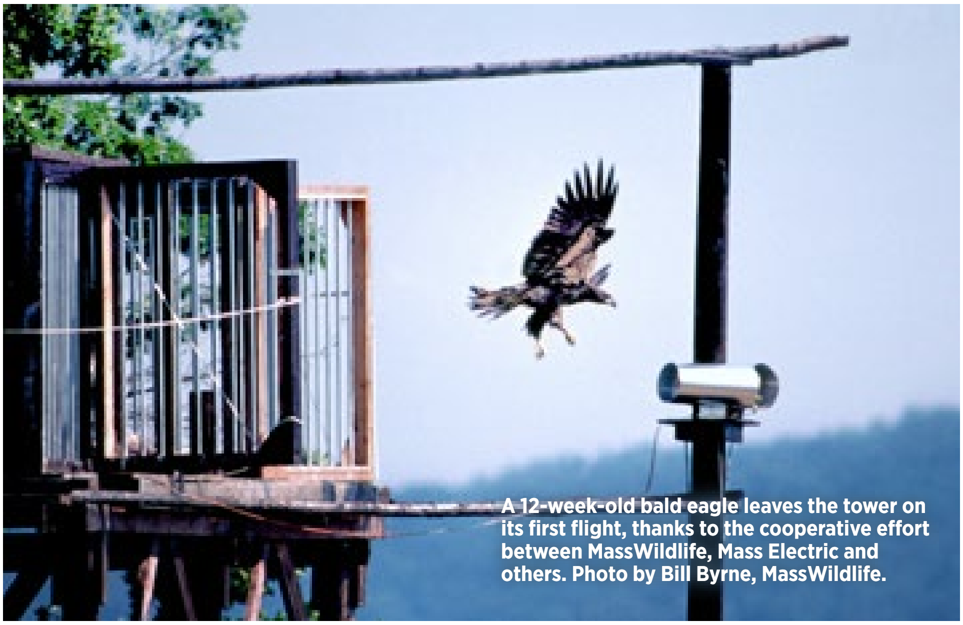
Branching Out:

GROWING PARTNERSHIPS AT THE CHAPTER LEVEL

By Bill Davis, MA/RI Chapter

Rufin Van Bossuyt is a quiet, thoughtful, and passionate champion of the American chestnut tree, especially in southern New England. He is regarded as a cornerstone of the MA/RI Chapter of The American Chestnut Foundation (TACF), having been a founding member, and continuously serving and providing guidance as a member of the Board of Directors. TACF's New England Regional Science Coordinator Kendra Collins states, "The Massachusetts and Rhode Island Chapter has developed an extensive network of partners to support their programs, thanks in large part to Rufin's hard work and ability to build relationships and foster connections to the American chestnut."

Former MassWildlife director Wayne MacCallum (left) and TACF board emeritus Rufin Van Bossuyt, holding American chestnut staffs, stand on the site of the new seed orchard in the 906-acre MacCallum Wildlife Management Area in Westborough, MA. Photo by Kathy Desjardin.



A 12-week-old bald eagle leaves the tower on its first flight, thanks to the cooperative effort between MassWildlife, Mass Electric and others. Photo by Bill Byrne, MassWildlife.

Visitors to the 6,000-seedling orchard in Westborough are greeted with TACF interpretive signage. Like most orchards, this one is protected by gates and an 8-foot deer fence.

Collins continues, “Rufin is an incredible volunteer – both in his capacity to expand the chapter’s network and in his willingness to get his hands dirty caring for the many orchards, follow-up on wild tree reports, and harvest nuts for future planting. And he does it all in his quiet, unassuming manner. TACF is lucky to have him!”

Partnerships are nothing new for Rufin, who was approached by the Massachusetts Division of Fisheries and Wildlife (MassWildlife) when he was working as the System Arborist for Massachusetts Electric, now National Grid, a major utility company in Massachusetts. In 1981, MassWildlife initiated an Osprey Recovery Project as these large, fish-eating birds of prey were in need of suitable nesting structures, which were built using salvaged utility poles and hardware to help their small, 43-pair population rebound from the effects of DDT (a pesticide once used for insect control) and habitat loss.

MassWildlife reached out to Rufin again in 1982 when the Bald Eagle Restoration Project began. Using poles and hardware, an experienced crew from Mass Electric helped construct a 30-foot tower overlooking the 25,000-acre Quabbin Reservoir and artificial eagle nests were built inside protective 8 x 8-foot cages on the tower platforms. Over the next seven years, 41 four- to six-week-old bald eagles were translocated from active, multiple chick nests in Michigan, Manitoba and Nova Scotia, and raised on the tower until taking flight. Both the osprey and bald eagle projects have been tremendous successes with more than 1,000 nesting pairs of ospreys and 70 pairs of eagles calling Massachusetts home today.

Partnering with TACF was another logical step given Rufin and Mass Electric’s commitment to conservation. Mass Electric bucket trucks were used to pollinate and bag flowers on surviving American chestnut trees and collect burs in the wild. Surplus utility poles were used to anchor the corners of deer fences to protect newly planted research and seed orchards. Even after Rufin’s retirement from the utility company the chestnut partnerships continued to grow. The Worcester County Horticultural Society hosted the first research orchard in Massachusetts, at their Tower Hill Botanic Garden in Boylston, while the state Department of Conservation and Recreation joined in with orchards at Moore State Park in Paxton, Wachusett Reservoir in West Boylston, and at Quabbin Reservoir in New Salem. MassWildlife agreed to host a 2-acre seed orchard on the Wayne F. MacCallum Wildlife Management Area adjacent to their field headquarters in Westborough, clearing the area with a bulldozer and brush hog. The orchard is scheduled to be fully planted in 2020, reaching its 6,000-seedling capacity. MassWildlife Director Mark Tisa sees the partnership as a win/win for both wildlife and the people of Massachusetts. “The American chestnut was such an important tree for wildlife and people in the pre-blight era that restoring it on a landscape scale would pay tremendous dividends. MassWildlife is a perfect partner with TACF, considering the agency oversees more than 220,000 acres of protected open space statewide.”

Land Trusts in Worcester and Orleans have become partners, as have the Norfolk County and Bristol County Agricultural High Schools in Walpole and Dighton, and the Tantasqua Regional Jr. and Sr. High Schools in Sturbridge. Conservation-minded groups like the Massachusetts Audubon Society, Harvard Forest, the Norcross Wildlife Sanctuary and others have graciously hosted MA/RI Chapter meetings. Rufin concludes, “What we’ve accomplished to date has truly been a team effort. Everyone within the chapter has contributed in one way or another and our growing team of partners keep bringing additional resources and abilities to the table. I’ve had the pleasure of working with a lot of talented people over the years and we’ve hopefully got the ship sailing in the right direction and that future generations will stay the course.”

ABOUT THE AUTHOR: Bill Davis worked for MassWildlife for 37 years as a technician, biologist and regional supervisor. He met and worked with Rufin Van Bossuyt on Osprey and Bald Eagle restoration beginning in 1981 and helped make the connection between the MA/RI Chapter and MassWildlife which resulted in the Westborough Seed Orchard.

MEADOWVIEW

Collecting, Processing, and Pollinating

By Brandon Yanez-Breeding, Meadowview Research Technician

The three farms involved in the breeding program at Meadowview Research Farms have a mix of trees, some of which will be used as mother trees and others from which we collect pollen. Trees to be used for pollen collection are identified and flagged early in the season. Frequent observation of the growth and development ensures that the pollen-producing male flowers, called catkins, are collected at the peak of bloom. There are two main types of catkins: staminate catkins and bisexual catkins. Staminate catkins have only pollen-producing male floret bundles on the catkin; the bisexual catkins have male florets and female flowers. The female flowers are small and bright green with pineapple-shaped buds that, when pollinated, can grow and develop into a viable chestnut bur. Usually the staminate catkins reach maturity before the female flowers on different mother trees are ready to be pollinated. When catkins are ready to be harvested, a tractor and tow-behind lift are taken to the tree where the catkins are cut from the stems and placed into large brown paper bags. After collection, the paper bags are marked with the parent tree's code, date of collection, and number of bags. Throughout the day the bags are taken back to the enclosed pollen drying room where they are stored, with a dehumidifier situated in the middle. Ideally, the goal is to remove as much moisture from the room and the catkins as possible. This helps ensure the viability and stability of the pollen. Once the catkins have dried, they are processed to obtain the purified pollen. Processing the catkins requires taking the long,

Staminate male catkin ready for harvest.

Bisexual catkin during growth.

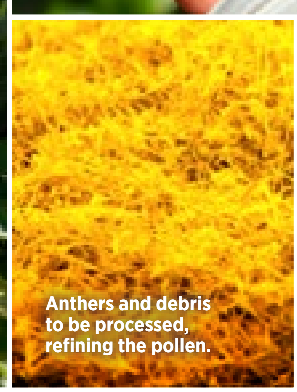
fuzzy flowers and scraping them against a screen to knock off the small anthers. Anthers are sand-sized pods that contain the fine pollen particles. After sieving and removing debris, the pollen is stored with desiccant (a drying agent) to help remove any remaining moisture from the plastic pollen storage vials. From here the pollen is either used in the field for pollinations, mailed to another TACF orchard, or frozen to be used in the future.

Equally important in the breeding program are the trees to be control-pollinated. These trees are identified and marked to be checked later, once leaves and flowers are present. On each tree, the number of female flowers and catkins can vary greatly depending on the tree's health, previous year's production, and environmental conditions. Potential mother trees have to be scouted throughout the early growing season to look for the presence of numerous female flowers. Once a tree is selected for pollination, the next step is to monitor the growth and development to ensure trees are bagged prior to the female flowers entering their receptive stage. Preparing future mother trees for pollination begins by identifying stems that have a significant number of female flowers. Once that happens, the majority of the leaves are cut from stem, leaving a few partial leaves attached near the end of the stem. It is important to ensure that all pollen producing parts of the catkins are removed. Lastly, a brown paper bag is placed over the trimmed and prepped stem and the bag is secured with a twist-tie. This is done to protect female flowers from being pollinated by random sources. At this point in the process, frequent inspection of un-bagged female flowers on the mother tree are used as indicators as to the degree of receptivity of the bagged flowers. Similar to the protocol when collecting catkins, a tractor and tow-behind lift are taken to the mother tree once flowers enter their receptive stage. One at a time the bags are removed and the female flowers are pollinated by hand. Afterwards, the paper bags are placed back onto the stems and the twist-ties are secured. The bags are then marked, indicating which ones have been pollinated.

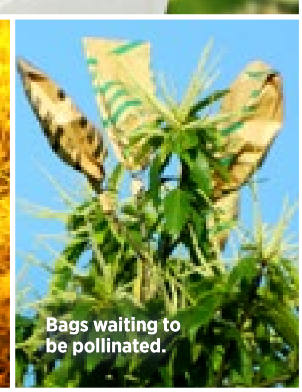
At harvest, the control-pollinated mother trees are heavy with burs. Much like utilizing un-bagged flowers to judge receptivity, observing the development and maturity of un-bagged burs allows farm staff to harvest the control pollinated burs at peak ripeness. Harvested burs are hung in the barn until processed by Meadowview staff and volunteers. From this point, the seeds will be sent to numerous greenhouses, institutions, donors, and members alike to be used in research studies, all with the goal of restoring the American chestnut tree to its native range.

Pollination bags marked as being pollinated.

Female flowers being hand pollinated.



Anthers and debris to be processed, refining the pollen.



Bags waiting to be pollinated.

Jim Gage

CHESTNUT VOLUNTEER MAKES GOOD IDEAS TAKE OFF

By Scott Carlberg, Contributing Author

If you have flown in the past couple decades you can tip your hat to a volunteer in the CT Chapter of The American Chestnut Foundation (TACF). He's Jim Gage, and he cut his teeth professionally on jet engines. Back as far as Boeing 707s, the first evolution of jet engines.

He's a retiree from engineering company and manufacturer Pratt and Whitney. "I started in the engine planning process. I'd be given designs, blueprints, and we had to come up with a way to make the part. Machine it. Design the tools. Make the holes."

Jim took engineers' ideas and made "operations sheets," the recipe to make parts exactly the same every time.

Those engines have powered commercial and military aircraft and carried millions of passengers. As if that is not enough, part way through his career he earned an MBA from Harvard and moved into corporate strategic planning. Looking ahead for the long term, making a path to reach important goals.

After retirement Jim was looking for opportunities that matched his love of outdoors and skills in planning. His brother-in-law, founder of a land trust, mentioned American chestnuts and blight.

That was the spark. Jim saw connections between land trusts and TACF and he set to work with Connecticut Chapter members to create an operational framework for collaboration.

Jim has been the chapter's treasurer and the Northern Connecticut Land Trust's (NCLT) treasurer for more than a decade. By the way, Jim isn't sure when he was elected to the board of the land trust. He says he sat in on meetings starting in 1995. After a while they figured he was on the board and nominated him for reelection.

Jet engines are about thrust. So is Jim's work. He creates connections and energy. Jim forged a strategic link to the NCLT, opening land options for chestnut orchards. He helped create the breeding orchard in Ellington, Connecticut and the seed orchard in Stafford, Connecticut and has acted as support to the orchard managers in both locations.

Jim comes from a line of people who get things done. Real survivors. West Reading, Pennsylvania, set the stage in his youth. Jim's father was a 1926 Yale graduate in investment banking. Just in time for the Great Depression. That tested the family. Then World War II. Another test. "Everyone was involved. Dad sold government bonds. Vets would stay at our house. Mom volunteered at a hospital. I was in elementary school and collected tin cans for scrap."

An all-out effort is the way Jim works on everything. But in his own humble way. "Jim is one of those guys you would never know what he has done. Jim is quiet, smart, hard-working ...the ultimate team member," says former CT-TACF Chapter President Bill Adamsen.

"Aw, it's just a good fit," says Jim about his work with TACF.

Leisure time is just another way Jim sharpens mind and body. "Growing up I wasn't athletic. But I really liked being out in nature. Hiking, skiing." That's one reason Jim lives in New England versus Pennsylvania now.

So, get this: A glacial cirque called Tuckerman Ravine was Jim's favorite place to ski as a young man. It's located on the southeast face of Mount Washington, the highest mountain in the Northeast U.S.

Skiers have to hike up the ravine. No lifts. Lots of rocky chutes drop to the bottom of the bowl. "Extreme skiing, I guess," Jim says. "It's a 1,200-foot big bowl that holds its snow until May because the wind blows drifts off the mountain and into the bowl. The higher up the sides of the bowl, the steeper it is."

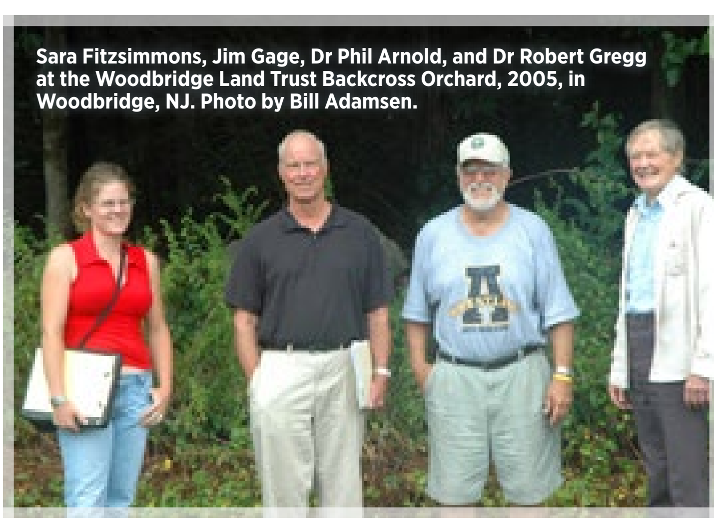
Real steep. About 45-degrees at the rim. One run called The Icefall is 55-degrees. Skiers must jump cliffs as tall as 25 feet. "I took a couple kids there when I was in my 40's," says Jim. "Hiked up to the top and looked down. Actually scared myself." (Jim is now 88.)

Can you tell Jim really likes being close to nature?

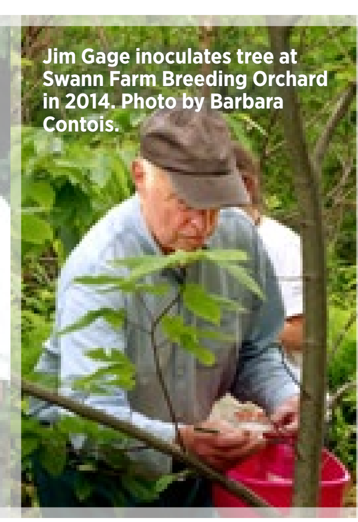
He keeps nature close to his community, too. The American Chestnut Foundation is just a part of that. Jim is an original member of the Ellington (CT) Conservation Commission. He is on the steering committee of the MassConn Sustainable Forest Partnership, public and private forest conservation entities that increase land protection and sustainable forest management in 38 towns in south central Massachusetts and northeastern Connecticut.

A big issue for Jim now is farmland, especially dairy farms. "They're in trouble," he says. "Towns are growing. Farmers are aging out. Some farmers sell to developers. We have to find a balance."

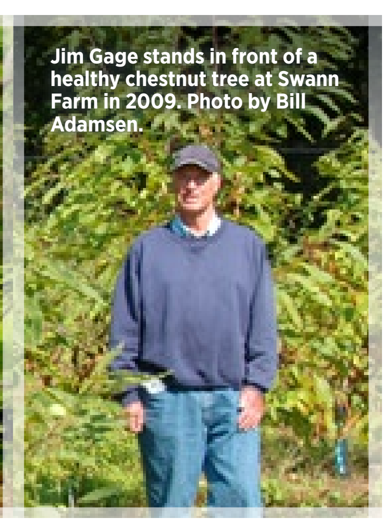
Jim Gage is the one for that job. It is in his nature, and nature is in him. He understands how to make good ideas take off.



Sara Fitzsimmons, Jim Gage, Dr Phil Arnold, and Dr Robert Gregg at the Woodbridge Land Trust Backcross Orchard, 2005, in Woodbridge, NJ. Photo by Bill Adamsen.

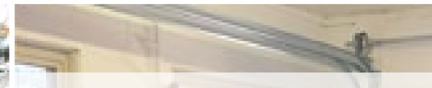


Jim Gage inoculates tree at Swann Farm Breeding Orchard in 2014. Photo by Barbara Contois.



Jim Gage stands in front of a healthy chestnut tree at Swann Farm in 2009. Photo by Bill Adamsen.





NATIONAL VOLUNTEER WEEK AND EARTH DAY



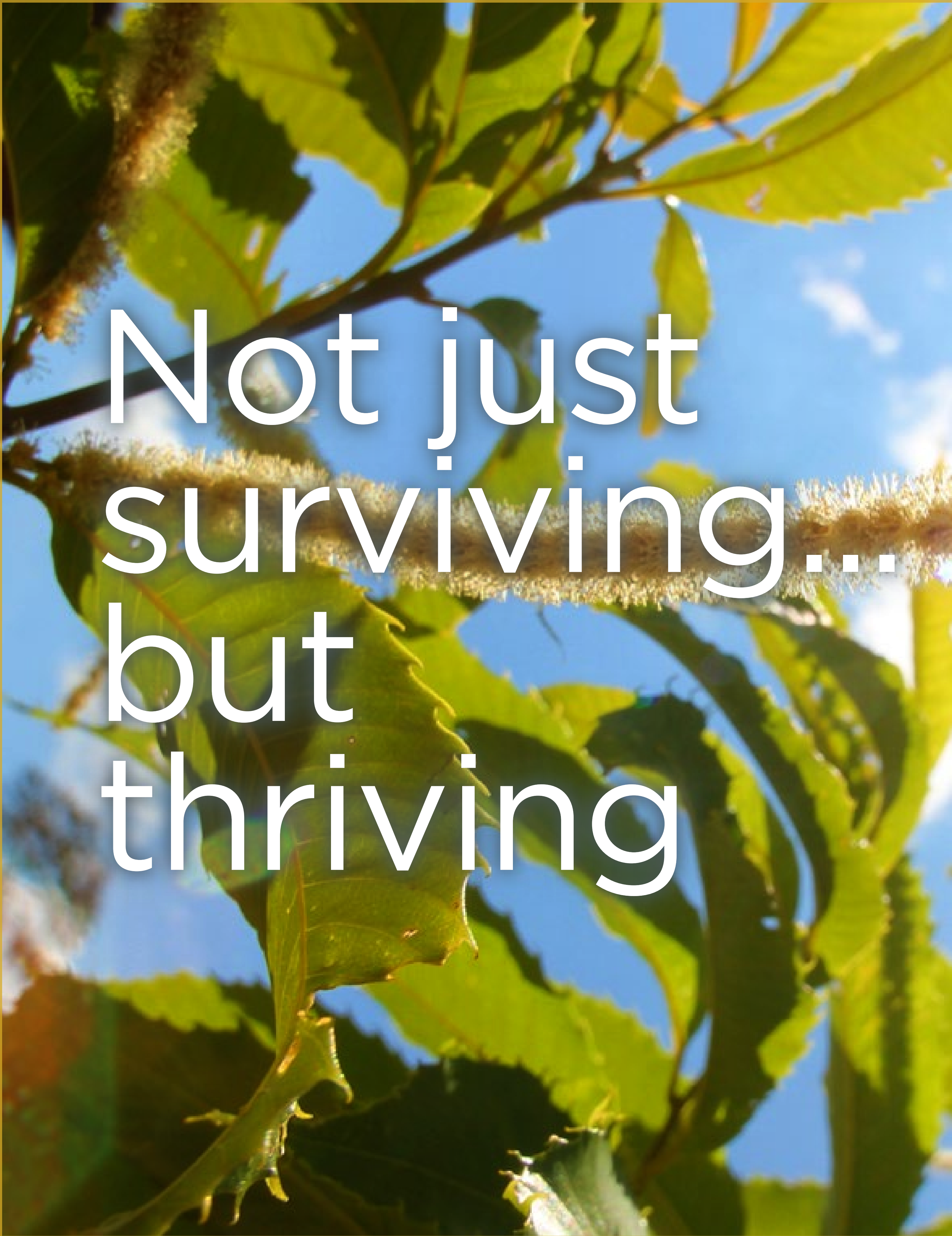
The More We Give, The Happier We Feel

Each year, the month of April celebrates and encourages volunteer participation in two big ways: National Volunteer Week and Earth Day. These special events highlight the hard work and commitment of those who give their time and talents to better the communities in which they live.

The American Chestnut Foundation's many volunteers are an essential part of its mission to restore the American chestnut tree. The progress and accomplishments of TACF would not be possible without the dedication of these selfless humanitarians. We are truly grateful for their exceptional work!






Not just
surviving...
but
thriving

THE AMERICAN CHESTNUT FOUNDATION®

Our organization is almost four decades old. The mission of rescuing the American chestnut from extinction has been a century's long project. We cannot let the COVID-19 crisis constrict our hopeful mission to inaction, as we are determined as ever to keep going, even in the face of uncertainty. Critical work is still getting done from home and in the field (with proper social distancing).

TACF, its staff, and volunteers, are used to uncertainty! Our founders in 1983 took a big leap of faith on a hypothesis. Continued best science practices and discoveries have us closer than ever to solving this complex problem. We will not only survive, but thrive, with your continued support.

PLANNING A Germplasm Conservation Orchard

By Kendra Collins, New England Regional Science Coordinator

The Tyler Arboretum GCO in Media, Pennsylvania uses a blocked planting design, providing good access for pollination. Photo by Sara Fitzsimmons.

Identification and conservation of wild American chestnuts is a priority of The American Chestnut Foundation (TACF). While we have tracked reports of wild trees and assessed leaf samples for most of our organization's history, in recent years we have put more of an emphasis on conservation of wild-type American chestnuts in germplasm conservation orchards (GCOs). American chestnuts conserved in GCOs can be used to better understand and preserve the genetic variation of the species, observe phenological differences or similarities across environmental gradients, and to help diversify transgenic American chestnuts into a population suitable for landscape-scale restoration. Read more on page 16 of our Spring 2017 issue of Chestnut: bit.ly/spring-chestnut-2017.

As with any chestnut orchard, picking an appropriate site is a key to success. A good GCO site should have well-drained and slightly acidic soil, good soil depth, full sun, a dedicated orchard manager, and good access for on-going management and care. Topography is also a consideration, as hand-pollination will likely be required to participate in transgenic diversification efforts. The orchard site should be suitable for safely setting up a ladder or lift, or for a bucket truck to drive on-site.

American chestnuts may be planted in GCOs as nuts harvested from wild trees, grafted seedlings, or in some cases, small seedlings transplanted

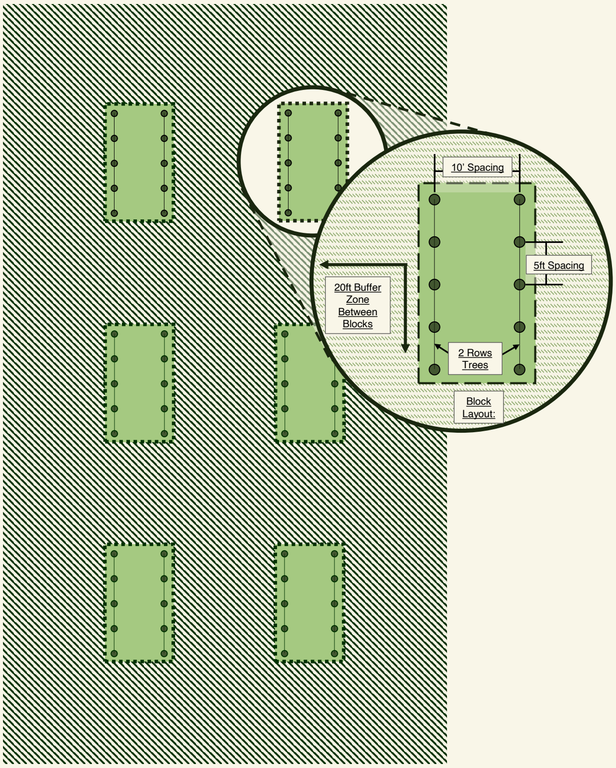
The Tyler Arboretum GCO from above, showing the blocks of different genotypes. Photo by Google satellite view.

from the forest. The standard design for a GCO is to contain 10 unique genotypic sources, with 10 individuals/source, however this design is quite flexible and can be scaled up or down in the number of sources conserved. Not all individuals planted are expected to persist, but on average 50% of a given source will survive long-term. There is no need to plant more than 10 trees from a given source in a single GCO, however sources may be replicated across two or three GCO sites as a back-up.

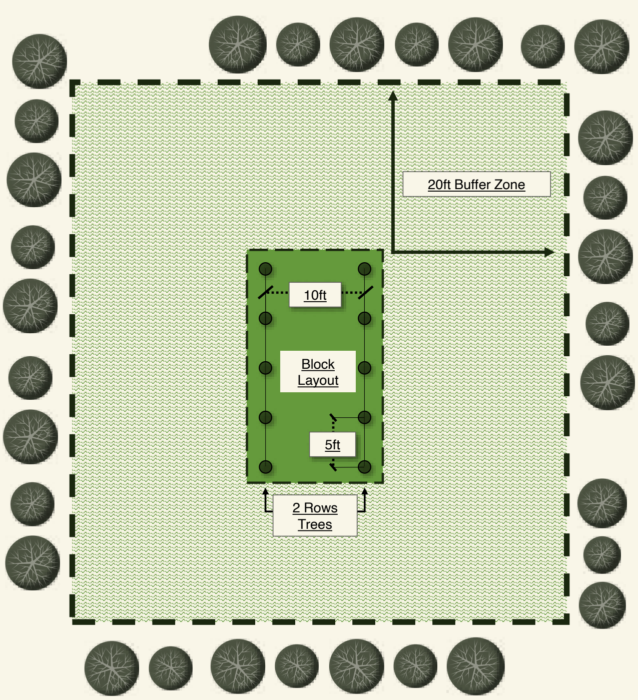
Proper documentation and tracking of what is planted in a GCO is crucial for the trees to be used to support TACF's scientific goals. We need to ensure we have good records as to the source of all trees planted and that those records are well-maintained. The wild tree the nuts or scions were collected from should be identified and documented by TACF's science staff, as should any transplanted wild seedlings. In addition, the trees planted in the GCO should be mapped and recorded in TACF's *dentata*Base. TACF's Regional Science Coordinators can help with these efforts and are happy to provide *dentata*Base training or work with you to ensure wild tree, cross/harvest, and orchard data are properly entered in our program-wide database.

With the goal of accurate tracking of planted sources in mind, there are some layout recommendations specific to GCOs. It is recommended that nuts, grafts, or transplanted seedlings from the same genotypic source be planted together. It is much easier to keep track of sources when they are grouped. The simplest and clearest model is to block sources in groups of 10 individuals. Each block is planted across two parallel rows, with rows 10 feet apart, 5-10 feet between trees, and 20 feet between blocks. This 20-foot buffer around each block should allow for good pollination access by ladder or from larger equipment, though orchard managers will need to resist the urge to plant in that space when the trees are still small. Adding rows in the middle of the planting will inhibit pollination access in the future and makes for a real record-keeping headache. The other option,

PA/NJ-TACF volunteers pollinate American trees in the Tyler Arboretum GCO. Photo by Sara Fitzsimmons.



A blocked layout, illustrated here, is a great way to keep genotypes together for future use. The top diagram shows six blocks together, while the bottom image shows a single block. Blocks can be arranged to accommodate the orchard space you have to work with. Created by Brandon Yanez-Breeding, TACF Meadowview Research Technician.



though less fool-proof, is to stick with a more standard layout 10′ x 20′ layout (10 feet between trees, 20 feet between rows), and plant a new source every 10 spaces.

The only replacements planted when using these recommended layouts should be within the same genotype. It can be very tempting to re-use empty spaces for replacements but if you have an intentional planting design to keep record keeping clear, muddying the waters with replacements from different sources can really complicate things. Everyone intends to keep good records, but with many years of orchard data tracking experience across TACF's scientific staff, we recognize that often these records get lost on desks, forgotten in folders or on clipboards, and several years later when the trees are flowering and ready to pollinate we no longer know what's what and the trees are not of much use in our science programs. Considering the amount of work that goes into finding wild trees, harvesting nuts, making grafts, or transplanting seedlings, it can be very disappointing to have poor or incomplete records and not be able to use the trees for projects where knowing the source is critical.

Germplasm conservation orchards provide a great way to support TACF's science mission and engage with TACF members, collaborators, and supporters. With good site selection and an intentional layout that takes future uses into account, a GCO can play an important role in the restoration of the American chestnut.

TACF New England intern Deni Ranguelova pollinates an American chestnut in a GCO at the University of Vermont's Horticultural Research Center. Photo by Kendra Collins.

PART 2
OF A 3-PART SERIES

Safety Tests

ON TRANSGENIC AMERICAN CHESTNUT

By Andy Newhouse, SUNY's College of Environmental Science and Forestry

EVALUATING SAFETY TO WILDLIFE

As described in the previous issue of *Chestnut*, the transgenic American chestnuts known as 'Darling 58' are being evaluated by regulators, have undergone many types of safety tests, and even more tests are underway. These trees contain a gene called oxalate oxidase, or OxO, which breaks down toxic oxalic acid produced by the blight fungus. OxO genes are ubiquitous in nature, as they are found in many types of plants, mosses, fungi, and bacteria. The previous article in this series described safety to people, in terms of chestnuts as a food product; this second installment will cover safety to wildlife.

As readers of this journal probably know, American chestnuts were incredibly important to a wide variety of wildlife: animals ranging from bees to bears benefited from these trees. It's well known that the productive nut crops provided a stable source of energy and nutrition for many birds and mammals, but other parts of the tree including prolific catkins, leaves, and large stems also provided sustenance and shelter for diverse groups of animals. Therefore, it is essential to consider interactions with wildlife for potential restoration efforts using blight-tolerant American chestnuts. Following are summaries of some of the experiments we have conducted involving wildlife interactions with transgenic American chestnuts.

Monarch butterfly near a flowering American chestnut tree.

Starting from the ground up, the first interaction we will look at involves vernal pools, which are temporary wetlands common in forests of the northeastern U.S. These pools get lined with deciduous leaves and form critical breeding habitat for unique forest inhabitants including insects, snails, and amphibians. We tested one interaction that takes place in vernal pools: wood frog tadpoles consume leaves and associated detritus in the pools, and we know they are sensitive to impacts like pollution or changes in leaf species. Our test (see photo on next page) involved 195 tadpoles, each in its own quart jar containing one type of leaves: transgenic American chestnut, non-transgenic American, hybrid, and Chinese chestnut, and other unrelated tree species controls. The most distinct difference we observed was with one of the controls: fewer tadpoles survived on American beech leaves compared to all other leaf types. There were no significant differences in survival or growth rates between transgenic and non-transgenic American chestnut leaves. In fact, in some conditions the tadpole's development rate was slightly faster with American chestnut leaves (whether or not they were transgenic) compared to all other leaf types.

The next interaction involves compounds called tannins: these chemicals are involved in plant pigmentation, and were used in the leather tanning industry until synthetic alternatives were developed. Tannins are relevant to wildlife because very high concentrations can impart a bitter flavor (this is why acorns don't taste good), but recent research has shown that moderate tannin concentrations can actually be beneficial in some animal diets. We know chestnuts in general have lower tannin content than relatives like acorns, but we wanted to look specifically at tannin levels in transgenic chestnuts compared to non-transgenic relatives. This test was also recommended by the

Tadpole experiment setup at SUNY-ESF. Each quart jar contains 0.8 grams of dried leaves and one wood frog tadpole. Inset: healthy tadpole in a jar with crushed chestnut leaves.

Example microcolony containing five native bumble bees (*Bombus impatiens*) and pollen with OxO. Two connected chambers allow separate feeding and nesting areas.

FDA for their regulatory analysis of the transgenic chestnut, since chestnuts can be used as livestock feed. We had tannins analyzed at an independent testing facility, and results showed substantial variation in tannin concentrations among different types of non-transgenic chestnuts – it's clear that growing conditions or ancestry make a difference. However, transgenic and related non-transgenic American chestnuts showed almost identical tannin concentrations.

Moving up the tree to the canopy level, there have been a few different types of experiments done to look at leaf herbivory by insects. Initial studies have shown that Chinese chestnut leaves may be less attractive to caterpillars than American chestnut leaves, but that transgenic American chestnut leaves aren't substantially different than non-transgenic relatives. However, to turn this around a bit, there are also several invasive forest pests that consume deciduous tree leaves, such as gypsy moth caterpillars. Forest managers use various types of treatments for gypsy moths, including natural biocontrol treatments. This results in a three-level interaction: biocontrols affect gypsy moths, which affect chestnut leaves. We looked in detail at these "tri-trophic" interactions: does chestnut leaf type change effectiveness of biocontrol treatments on invasive insect pests? As with the previously described tests, we observed differences among non-transgenic controls. In this case, Chinese chestnuts showed some differences in caterpillar mortality after biocontrol treatments compared to American chestnut lines. But again, the Darling 58 transgenic chestnut was not significantly different than its non-transgenic relative.

Finally, anyone who has been near a flowering chestnut tree in early summer can easily appreciate that catkins are numerous and very fragrant. Many insects take advantage of these chestnut flowers, including native pollinators like bumble bees, which are currently facing several environmental threats. Chestnut restoration could thus potentially benefit many types of insects that rely on pollen as a source of nutrients or hive-building material, and we know that insects contribute to successful pollination of chestnut trees. Since this is such an important interaction, we looked at potential effects of the OxO enzyme in pollen on native bumble bees. The bees were reared in a series of "microcolonies" made of take-out food containers (example photo at left), each containing five bees. This setup allowed bees to experience some natural social interactions, while allowing us to have enough replicated colonies for a good experiment. Each microcolony was supplied with chestnut pollen containing OxO, or a non-OxO control. We observed survival, body size, pollen use, and reproduction throughout the seven-week experiment, and saw no differences in any of these measures when bees were exposed to a field-realistic concentration of OxO in pollen.

All of these wildlife interaction tests show the same thing we have seen in other types of experiments: there may be variances between different chestnut species, hybrids, or even individuals of the same species, but any changes associated with the OxO transgene are insignificant by comparison. The next installment in this series will describe interactions with other plants and fungi, concluding our summaries of environmental interaction experiments. More detail on these tests and many others are described in our petition to the USDA for nonregulated status of the Darling 58 American chestnut, which should be publicly available soon.

Male-sterile F_1 Trees

AS LONG-TERM CONTROLS IN SEED ORCHARDS

By Paul Sisco, Carolinas Chapter, and M. Taylor Perkins, University of California, Davis

Six-year-old male-sterile F_1 tree in the Carolinas Chapter Clapper seed orchard. Female flowers were bagged to make controlled crosses. Photo by Paul Sisco.

Because environment has a large effect on blight resistance, controls are a critical component of TACF's experimental plantings. Pure Asian, F_1, and pure American chestnut trees are often planted as control trees in seed orchards. At roguing time the Asian and American chestnut trees must be removed so that their pollen does not contaminate seed harvested from the selected B_3F_2 and B_4F_2 trees. In contrast, male-sterile F_1 control trees could remain for the life of the seed orchard, because they could not produce contaminating pollen (**Figure 1**). Seed gathered from these F_1's are also potentially useful in several ways:

To increase the level of blight resistance by producing "Better B_1" trees. We call the offspring of the cross (F_1 x selected B_3F_2) "Better B_1" trees, because they should, on average, have more blight resistance than a B_1 produced by crossing an F_1 with a pure American chestnut.

To add genetic diversity. A diverse group of F_1 trees can be used as controls, utilizing various Asian chestnut cultivars not already included in TACF's breeding program. "Better B_1" trees may thus inherit novel blight resistance alleles (= forms of genes) not found in 'Clapper', 'Graves' or 'Nanking'. The American parents of the F_1 trees can also be chosen to add genetic diversity from the American side.

To add resistance to *Phytophthora cinnamomi*. Crossing B_3F_2 trees back to an F_1 is a way of adding resistance to Phytophthora root rot (PRR), since F_1's are likely to have at least one copy of genes for PRR resistance.

The male-sterile phenotype in chestnut

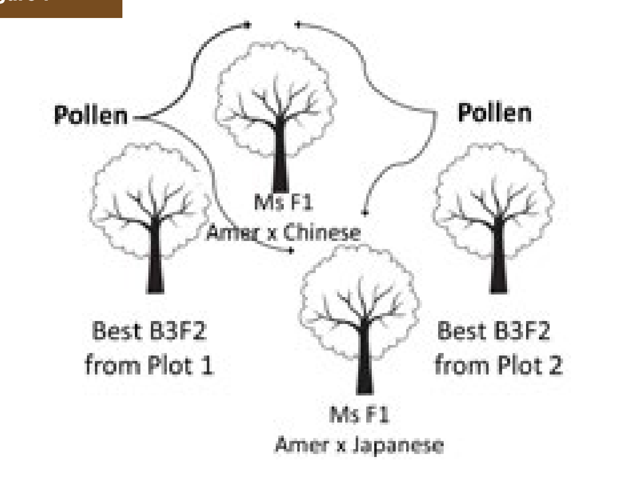
Almost all male-sterile F_1 chestnut trees never exsert stamens and thus never shed any pollen. Their male catkins remain in a green, immature state until they senesce and turn brown. The female flowers, in contrast, develop normally. **Figure 2** shows male-sterile (left) and male-fertile (right) male catkins. The female flowers in both photos are mature. Only the male-fertile catkins have stamens.

How to produce male-sterile F_1 trees

A reliable way of producing male-sterile F_1 trees is to use the American chestnut parent as female in a cross with Asian chestnut species i.e., (American chestnut female x Asian chestnut male). If the Asian chestnut tree is used as female in the cross, the F_1 progeny will be male-fertile (**Figure 3**; Sisco et al 2014).

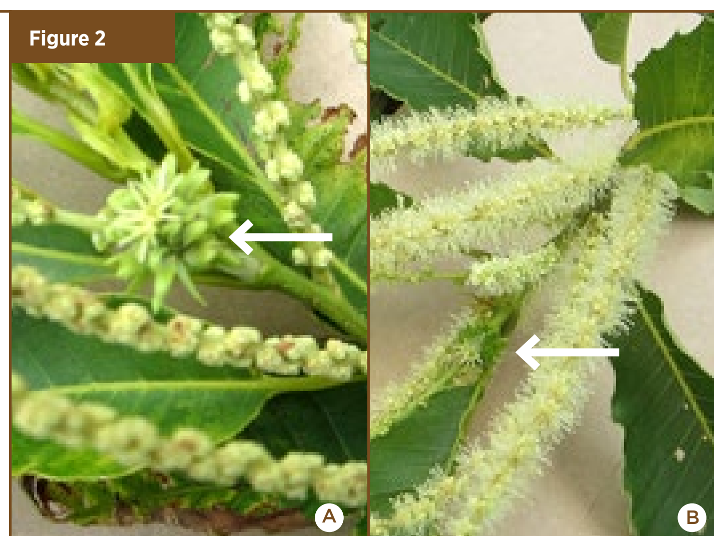
Not all American chestnut trees, when used as female parents, will produce male-sterile F_1's. Evidence so far

Figure 1



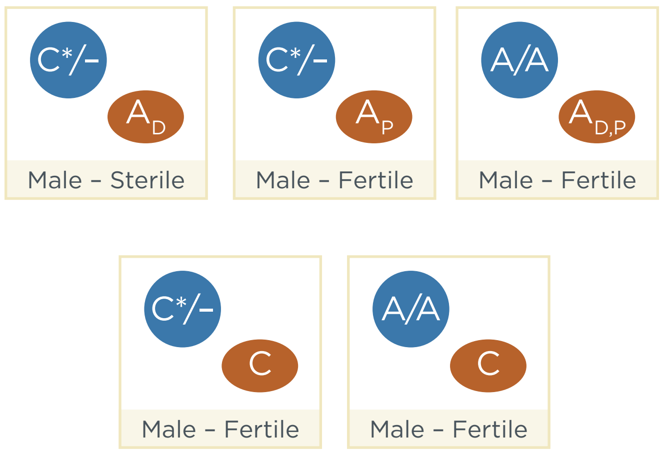
Pollen flow in a chestnut seed orchard that contains male-sterile F_1 controls along with selected B_3F_2 trees. Only the B_3F_2 trees shed pollen. Open-pollinated seed gathered from the B_3F_2 trees should result in B_3F_3 progeny. Open-pollinated seed gathered from the male-sterile F_1's should be "Better B_1's."

Figure 2



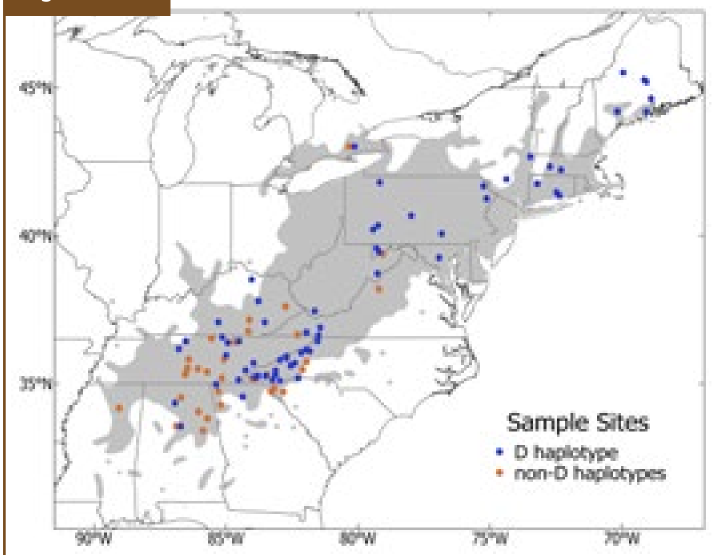
Male-sterile (A) and male-fertile (B) chestnut catkins in full bloom. Each inflorescence includes a female flower in full bloom (arrows). No stamens are exserted from the male-sterile catkins in (A), so they are functionally male-sterile. No pollen grains are released from these catkins. In contrast, thousands of pollen grains are shed from the male-fertile catkins in (B). Photos by Paul Sisco.

Figure 3



Only one combination of nuclear genes and chloroplast haplotype has so far been found to be associated with the male-sterile phenotype in crosses of American and Asian chestnut species. Male-sterility is found in trees with one or more dominant Chinese chestnut nuclear genes (C*) in combination with a single American chestnut chloroplast haplotype (the "D" chloroplast haplotype, indicated by A_D in the diagram).

Figure 4



Location of "D" and "non-D" chloroplast haplotypes in American chestnut trees (*Castanea dentata*) in published studies to date: Kubisiak and Roberds (2003), Shaw et al (2012), Sisco et al (2014), Dane and Sisco (2014), Perkins (2016), and Gailing and Nelson (2017). The dots merely show location, not the number of individuals found at any one site.

indicates the American chestnut female parent in the cross with Asian chestnut needs to have a particular chloroplast haplotype labeled A_D in **Figure 3**. [For a definition of haplotype, see the box at the right]. This single chloroplast haplotype, by far the most common haplotype in American chestnut trees, has been variously labeled "D" by Sisco et al (2014), "D2" by Shaw et al (2012) and Perkins (2016), "D1" by Dane and Sisco (2014), and "H1" by Gailing and Nelson (2017). The locations of American chestnut trees having the D chloroplast haplotype are indicated by the blue-colored dots in **Figure 4**.

Male-fertility is restored when the dominant Chinese nuclear gene(s) associated with male-sterility are eliminated.

"Better B_1" trees in D cytoplasm can be either male-sterile or male-fertile, depending on whether they inherit the dominant Chinese form (allele) of nuclear gene or genes associated with the sterile phenotype indicated by C* in **Figure 3**. Once the C* allele(s) are eliminated from the nuclear genome, all trees will be male-fertile, no matter what their chloroplast haplotype.

Male-sterility may be caused not by D chloroplasts but by associated maternally-inherited mitochondria.

Cytoplasmic male-sterility is a common phenomenon in plants, associated with an incompatibility between genes in the nucleus and genes in the cytoplasmic organelles, most likely the mitochondria (Chase et al 2010). In chestnut, chloroplasts and mitochondria are strictly maternally inherited. Male sterility in chestnut is probably caused by the incompatibility of the C* allele(s) with a particular mitochondrial haplotype that comes along with the D chloroplast haplotype.

Definition of "Haplotype"

Mitochondria and chloroplasts contain only one copy of their circular genome. The genotype of this single copy is called a "haplotype" from the Greek word for "single."

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Using Oak Silviculture

TO REINTRODUCE AMERICAN CHESTNUT

By Cornelia (Leila) Pinchot, Research Ecologist, Forest Service, Northern Research Station; Scott Schlarbaum, James R. Cox Professor of Forest Genetics, Director, UT Tree Improvement Program, University of Tennessee Department of Forestry, Wildlife & Fisheries; and Scott Tepke, Forester, Allegheny National Forest

Throughout much of American chestnut's range, the tree co-occurs with various species of oak, commonly northern red and chestnut oak. Oaks benefited from the loss of chestnut, by taking advantage of the increased light and growing space made available when its once abundant cousin was largely extirpated (Wang and Hu 2015). More recent changes in oak-dominated forests, such as increased herbivory and alteration to disturbance regimes, however, threaten the continued dominance of these species (Dey 2014). Because of this, promotion of oak regeneration is now a predominant focus of silviculture research and management, particularly on public lands, throughout the oak-hickory (formerly oak-chestnut) forest type. It would be practical, logistically and financially, then, if the silvicultural strategies used to regenerate oak can also be used to facilitate American chestnut reintroduction.

BC_3F_3 American chestnuts two years after planting in a removal harvest on the Allegheny National Forest.

In Pennsylvania, the three stage shelterwood system is often employed to promote the establishment and growth of oak regeneration (Brose et al 2008). To test the suitability of this system for hybrid American chestnuts, we installed a study on the Allegheny National Forest (ANF) in NW Pennsylvania in 2017 with the goals of comparing hybrid American chestnut survival, growth, and competitive ability across the three silvicultural treatments used in the three stage shelterwood system; and to compare success of chestnuts planted as high-quality seedlings with direct-seeded chestnuts.

Methods

The three-stage shelterwood system involves three harvests over the course of 15-20 years, each removing a percentage of the overstory and midstory trees, with the goal of progressively increasing light availability for oak seedlings as they establish, while limiting light for fast growing shade-intolerant species, like tulip poplar. These three treatments; preparatory cut (prep-cut), shelterwood seed cut (shelterwood) and removal cut, create a gradient of light availability and competition from sprouts and seedlings of other hardwood species. Correspondingly, they offer an opportunity to test the ability of planted hybrid chestnuts and direct-seeded chestnuts to thrive across varying levels of light from above, and competition from below.

With the help of Northern Research Station and ANF personnel and Tidioute Charter School students, we planted 757 high-quality hybrid backcross chestnut seedlings and 617 seeds across nine sites (three replicates of each of the three harvest treatments) in the Coalbed Run project area of the ANF in April, 2017 (**Figures 1 and 2**). Eight BC_3F_3 hybrid chestnut families were sourced from TACF and two BC_3F_2 families from the Connecticut Agricultural Experiment Station. The chestnut seedlings were just over 2½′ tall and 1/3″ thick (at the root collar) on average at the time of planting. Chestnuts were planted on a 12′ x 12′ grid, and chestnut type (seedling vs. seed) and family were arranged in incomplete blocks within each of the nine planting sites. Five-foot tall Plantra© tree shelters were installed on all chestnuts to protect them from herbivory. We recorded survival and height of the chestnuts and height and species of the tallest competing woody stem within 4 ¼′ of each chestnut toward the end of the first two growing seasons.

Figure 1

Chestnuts planted in A. Prep-cut site, B. Shelterwood site, C. Removal cut, all photographed during the second growing season after planting.

Results and discussion

Two years after planting, 92% of the seedling-planted chestnuts were alive, compared with 49% of the direct-seeded chestnuts. Survival was similar across the silvicultural treatments. We suspect lower survival for direct-seeded chestnuts was due in part to predation and possibly desiccation, both of which are common challenges faced by direct seeded chestnuts. The ease and cost savings of direct seeding may justify

Figure 2

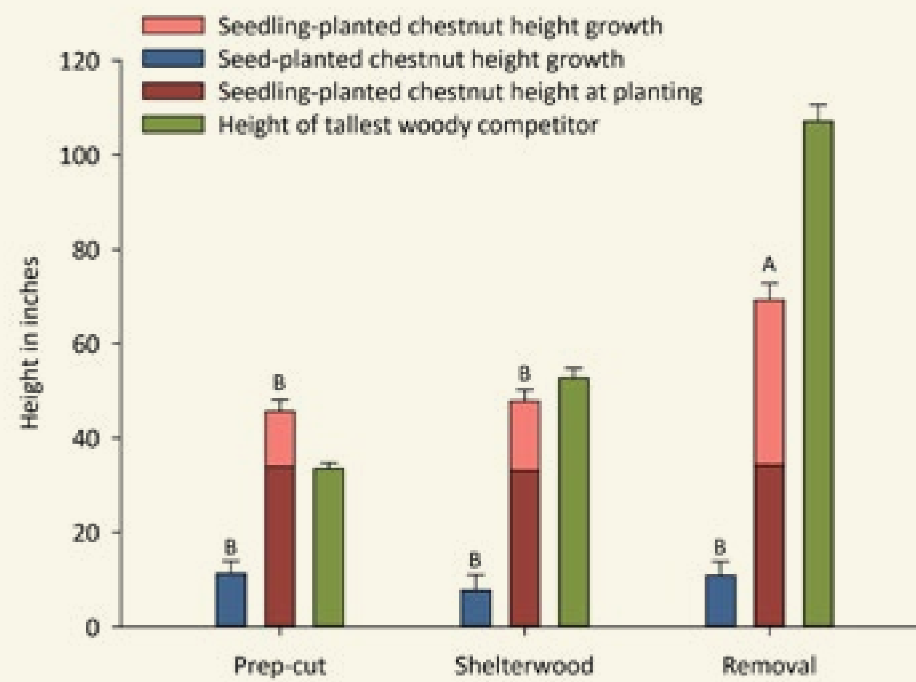
Direct seeded chestnut during its second growing season. The tree shelter was temporarily removed to take the photograph. Units on height stick are cm.

their use, even with reduced survival, though the long-term competitive ability of these direct-seeded chestnuts across the treatments is unknown. Given the substantial investment that goes into developing backcross seeds, however, it may be worthwhile to plant seedlings in order to maximize survival, particularly while its availability is limited.

Both chestnut height growth and total height over the first two years were statistically similar between planting types (seedling-planted vs direct seeded) in the prep-cut and shelterwood treatments (**Figure 3**). Growth and total height were greater, however, for seedling-planted chestnuts in the removal treatment. Basal area of residual overstory trees and percent canopy openness were similar between the prep cut and shelterwood treatments (99 ft/ac^2 and 24%, 95 ft/ac^2 and 25%, respectively), indicating light availability was comparable between these sites, which likely explains the similarity in height growth. The increased harvest intensity in the removal treatment (10 ft/ac^2 residual basal area and 65% canopy openness) provided more light to the chestnuts (and competing vegetation). The seedling-planted chestnuts responded to this increased light availability by growing over twice as much in height compared with the two other treatments (**Figure 3**). The direct seeded chestnuts, however, did not differ in their growth among the silvicultural treatments. This was likely caused in part to the robust sprout, sapling, and herbaceous competition in this treatment; the average height of which was ten times the height of the direct-seeded chestnuts, while only 1 ½ times the height of the seedling-planted chestnuts. Furthermore the stored carbohydrates in the root systems of the seedling-planted chestnuts presumably contributed to their increased competitive ability compared with the direct-seeded chestnuts.

The prep-cut and shelterwood treatments appear to be most efficacious for planting due to their reduced competition response, whereas the removal cut will probably require competition control, particularly if planting direct-seeded chestnuts. We will continue to monitor these chestnuts in future years to evaluate their survival and their growth relative to competing vegetation. Patterns we have found may change over time, particularly as the stands progress through the harvest sequence for the prep-cut and shelterwood treatments. Stay tuned!

Figure 3



Height and height growth of direct-seeded and seedling-planted chestnuts and height of tallest competitor two growing seasons after planting.

ACKNOWLEDGEMENTS

The American Chestnut Foundation provided chestnuts and generous funding through their External Grants program that made this work possible. Sandra Anagnostakis also provided chestnuts for this study. We thank Andrea Hille, Ecosystem Management Staff Officer, the Allegheny National Forest (ANF) for supporting this work, and Greg Sanford, Forester, ANF for assisting with site selection and study establishment.

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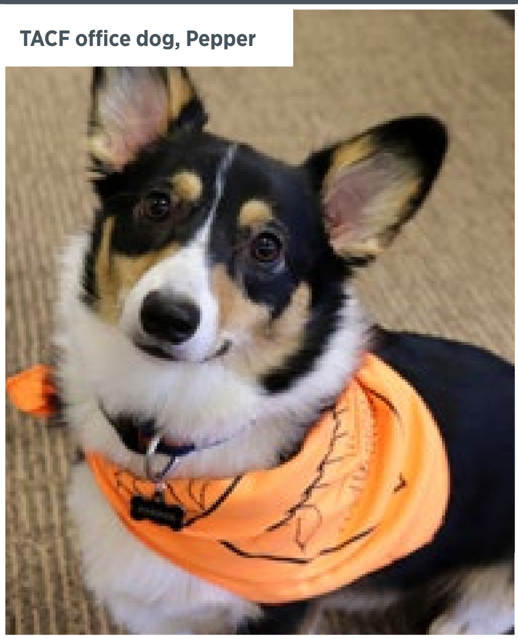
TACF office dog, Scooter



TACF office dog, Pepper

Meadowview Farm Maintenance Technician, Jim Tolton

Young chestnutters, Segi and Simi Smith-Pariola (unisex, sizes S-XXL)

"A Chesnut [sic] Burr for an Eyestone:" A Revolutionary War Toast

By Larry Brasher, AL Chapter

I was born on the Fourth of July in historic Morristown, New Jersey, where Washington and his troops suffered the worst winter of the Eighteenth Century in 1779-1780. Several summers ago, on July 4, I returned to the Morristown Green to celebrate my birthday. One of the highlights of my visit was listening to Thomas Winslow, Park Ranger at Morristown National Historic Park, read the Declaration of Independence.

Winslow also recited his favorite patriot toasts from the Revolutionary War including this one:

"Sore eyes to all Tories and a Chesnut burr for an eye stone."

What's an "eye stone?" In the eighteenth century, it was a smooth, lens-shaped device for removing foreign substances from the eye. A chestnut bur? Ouch!

A Massachusetts patriot first offered the sharp toast in Worcester to a people well-acquainted with chestnuts. On July 22, 1776, the town gathered at the liberty pole on the green: "The bells were set a-ringing and the drums a-beating. The Declaration was read to a large body who testified their approbation by repeated huzzas, firing of musquetry and cannon. After repairing to a tavern, the following toasts were drank:

Prosperity and Perpetuity to the United States of America.

His Excellency George Washington.

The Patriots of America.

George rejected and Liberty protected.

Sore Eyes to all Tories, and a Chesnut Burr for an Eye Stone.

Perpetual Itching without the benefit of Scratching to the Enemies of America.

May the Freedom and Independency of America endure till the Sun grows dim with age."

And may the American chestnut, also, endure as long!

Savory Chestnut Hummus

By Cherin Marmon-Saxe, TACF Office and Business Systems Manager

In good *humour*, I submit this recipe with the measurements of origin and my honest attempt at converting it to American units. Of the 16 years I lived in England, I easily spent the first full year trying to learn the metric system! Once I figured it out, I was converted. For whatever measurement you choose, I do hope you enjoy this savory treat.

Hummus Ingredients

English	American	
500g	2 ¼ cups	Vacuum Packed Whole Chestnuts
250g	1 ¼ cups	Water
10g	2 tsp	Sugar
5g	1 tsp	Salt
1	1	Garlic Clove
To Taste	To Taste	Lemon Juice
100g	½ cup	Olive Oil

Pita Bread Ingredients

English	American	
200g	1 ¾ cups	Strong Flour
5g	1 tsp	Salt
100g	½ cup	Rosemary Water
10g	2 tsp	Chopped Parsley

Method

The day before, boil a bunch of rosemary with half a liter (just over a quart) of water and leave to infuse overnight (this is the rosemary water), or use plain water with chopped rosemary.

Hummus: Boil the chestnuts, sugar, salt, garlic and lemon together until the chestnuts are soft. Blend smooth with the olive oil, and check for seasoning, possibly adding a bit more water to make the right consistency, then lemon and salt to taste. Put straight in a piping bag.

Pita: Put all dry ingredients into the mixer and turn on. Add the rosemary water until you get a dough consistency. Add the chopped parsley and mix again. Weigh the dough into 75g portions and roll out (not too flat). Cook in a dry pan until coloured (depends on which side of the pond) on both sides, and then puff over an open flame until light and 'pottery' (English term for earthy brown).

To Plate: Put a large splodge (technical English term!) of hummus on the plate and cover it in finely chopped celery and some fresh parsley leaves (garnishment of your choice). Drizzle with olive oil and grate remaining chestnuts over the hummus to finish. Be sure you heat the pita bread for full effect.

A TRIBUTE TO DAVE ARMSTRONG, PA/NJ CHAPTER

By Sara Fitzsimmons, TACF Director of Restoration

With a truly aspirational goal, it is the grassroots activism of its members, citizen scientists, and partners that sets TACF apart from other organizations. Their dedication allows for the long-term sustainability necessary for such a large-scale mission of forest species restoration.

I've been working with the remarkable people of The American Chestnut Foundation (TACF) for 20 years. During that time, we are bound to suffer loss. This past winter we lost some true legends. In the last issue, we paid tribute to Dennis Fulbright, Bill Lord, and Ann Leffel. In this issue, we say goodbye to Dave Armstrong.

Dave grew up in Clearfield, PA, and earned a Masters Degree at Long Island University. He served in Vietnam then retired from the Army as a Major. Dave loved nature and he is a major reason the PA/NJ Chapter is as large and active as it is today. He opened the first chapter office in York, volunteering at least 40 hours a week developing newsletters, coordinating plantings, and writing grants. Dave took on every imaginable task and nearly every role one can within a TACF chapter.

I had the privilege of attending Dave's memorial service in February. During a remembrance of their father, his daughters said, "You didn't want Dad to start talking about chestnuts, because then you knew he wouldn't stop talking about chestnuts!" I'm sure that's more than true of many of you!

Keep doing what you're doing. You contribute to this ambitious and hopeful mission in your own way. Thank you for being a member of TACF; for planting trees; for offering a spare room and meal to TACF staff and volunteers; for renting a tow-behind lift to harvest chestnuts; and for sharing your story to anyone who will listen, so the history of this species can be both remembered, and created anew.

IN MEMORY OF OUR TACF MEMBERS

DECEMBER 11, 2019 – MARCH 26, 2020

Brad Alexander
From:
Jane E. Brown

Dave Armstrong
From:
John E. Fuge

Roosevelt and Carlene Blankenship
From:
Delia and John Olson

James Ely Bradfield
From:
John G. and Amy Bradfield

Col. I. D'Arcy Brent II
From:
Audrey Brent

Rita and Joseph Camardo
From:
Joseph Camardo

Tina Lorentzen Carlson
From:
Eri Lorentzen

Nancy Crim
From:
Rebecca Jones

Emilie Crown
From:
Ronald Kuipers

Chuck and Brady Ebersole
From:
Michael Ebersole

Gary J. Flinner
From:
Donna Graney Hutchko

George "Jim" Freytag
From:
Matthew G. Freytag

Dennis W. Fulbright
From:
Dr. William and Nora MacDonald

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From:
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Vic Geibel
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From:
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Ryan Hiller
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From:
Denise Gehring

Lowell Edwin Lingo
From:
Deborah W. Loverd

Gale Link
From:
Eddie H. Doss

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From:
Edward A. Klammer
Raleigh E. Puckett and Shelby Inscore-Puckett

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From:
Mark Lu

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From:
Mr. and Mrs. Hartwell Davis, Jr.

Angus M. MacLean
From:
The MacLean Foundation

Michael Meyerhoff
From:
Sandra Meyerhoff

Bob G. Moore
From:
Eric W. Moore

Herman Schoeb
From:
Maureen Binetti

Christopher H. Sener and Martha H. Platt.
From:
John Sener

Eleanor and Jack Thorsen
From:
Lisa and Walter Thomson

Kathy Thorsen
From:
Robert L. Burns

Janice Rae Warne
From:
Cary Rorke Warne

Gray Wexelblat
From:
Paul M. Wexelblat

In Memory of Your Childhood Chestnut Tree
From:
John Bridges

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

IN HONOR OF OUR TACF MEMBERS

DECEMBER 11, 2019 – MARCH 26, 2020

John and Maryann Adamski
From:
Eileen S. Moore

George and Ann Bass
From:
Jennifer Bass

Steve Bloomfield
From:
Sarah Baldwin

Rodney Byam
From:
Emily A. Ball
Jennifer Byam

Tony Case
From:
Laura Triplett

Mary Cassell
From:
Craig DuBose

Cataloochee Ranch
From:
Sima R. Cooperman

William Henry Chapin of Oberlin, OH
From:
David L. Chapin

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Blanche Montesi

In Honor of Your Family
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In Honor of All of Us Who Love Trees
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Helen M. Ingersoll

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