

The American Chestnut Foundation NEWSLETTER



In the heart of American chestnut's natural range

March 2023

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Chestnut Breeding Obstacles

Breeding chestnut trees is very complicated. In the very beginning stages of TACF in 1983, Dr. Charles Burnham speculated that the backcross breeding method he utilized in his corn breeding research at the University of Minnesota, would work in chesntut if there were only 2-3 genes responsible for resistance. Just like in chestnut, genes control everything around us, including ourselves. Whether we have brown or blue eyes, red or black hair, all our features are controlled by genes. Our garden vegetables are contolled by genes, as in pole beans or bush bean, golden or white kerneled corn or red versus white potatoes. Genes are very complex and Dr. Jared Westbrook recently wrote about why the American chestnut backcross method has not produced trees that are 94% American and 6% Chinese as hypothesized by Dr. Burnham 40 years ago. In the last few years, scientists have been able to actually determine the percentage of American versus Chinese DNA in our backcross trees. Trees that we thought were 94% American are actually only 80-85% American and 15-20% Chinese. Below are Jared's comments about this issue.

The main reason for low blight resistance in the backcross program is the large number of genes involved in blight resistance. These genes are distributed on all 12 of the chestnut chromosomes. As we backcrossed and made the genomes more "American" we diluted out some of these resistance genes from Chinese chestnut or brought in more genes for susceptibility from American chestnut.

Blight resistance is strongly negatively correlated (r = -0.88) with American chestnut ancestry. Chromsome size is not tightly correlated with blight resistance, which implies the blight resistance is not strictly polygenic – or controlled by most all genes in the genome. Most chromosomes explain at least some of the variation in resistance and no chromosome explains more than 20% of the variation. Hence the foundational hypothesis on which the backcross program was pursued, namely that there are two to three large effect genes on three of chestnut's 12 chromosomes is not supported. The number genes that control blight resistance is less than all 27,000 of genes in the chestnut genome, but more than 3. There are probably hundreds of genes that contribute to resistance and susceptibility.

I now see the primary value of the backcross program is in conserv-

ing genetic diversity from the American chestnut populations in orchards that we can easily access. The backcross program likely represents more than 99% of the adaptive diversity of the wild *Castanea den-tata* population. Most backcross trees have low percentages of Chinese chestnut ancestry (i.e. less than 10%). We can cross backcross trees with 100% *C. dentata* 'Darling 58' pollen and select progeny with 95% to 100% *C. dentata* ancestry.

I also see a value of the backcross program in combining blight resistance (via 'Darling' 58) and Phytophthora resistance (via backcross trees).

Chestnuts on Strip Mines in Boone County

Dr Jeff Skousen, Professor of Soils and Reclamation Specialist at West Virginia University, has planted chestnut seedlings on strip mines sites in WV as part of his research. One of his chestnut sites is at the West Cazy Mine site in Boone County. The seedlings were started in 2008 in blue tree tubes as seen in the left photo. After 14 years, some of the chestnut trees actually look like trees! Thanks to Jeff Skousen for sending the photos.



Dr. Skousen stated that most of the trees with more American DNA died. The trees that have survived are Chinese chestnut and some of the early hybrids with more Chinese genes.

January Chestnut Chat

One positive aspect of the Covid-19 pandemic was the development of TACF's **Chestnut Chats**, a series of Zoom meetings that cover a variety of topics. The January 2023 chat was attended by nearly 300 people and it focused on deregulation of the transgenic tree, 'Darling 58'. Some of the highlights of the meeting were:

Lisa Thomson, TACF President and CEO.

- The Chestnut Chat series begain in April 2020, and Lisa is grateful for the number of people touched by this series.
- TACF has hired 7 new people in the last year.

- This year, 2023, marks the 40th anniversaty of TACF, as it began in 1983.
- Many members are working a new strategic plan as many new developments have occurred since the last plan in 2016. The new strategic plan will focus on: Science; Technology; Restoration; and Promotion and Outreach.
- The documentary film produced by TACF will be debuted on Earth Day 2023. The documentary began in 2016 but was sidelined by Covid.
- TACF is working on new branding and a new logo. To that end, the firm BlueIon has been hired to assist with new visuals. A new website has been launched, not only to make the website more user-friendly, but also to support the increased traffic that is anticipated if 'Darling 58' is deregulated.
- New memorandums of understanding have been developed with the U.S. Forest Service and National Resources Convervation Service.
- As stated earlier, Lisa is stepping down as President and CEO in 2023. A search committe has been hired, but it will be several more months before a replacement is hired. Lisa plans to work closely with the new hire to ensure a smooth transition. Lisa feels new leadership is needed in organizations every 6-8 years to infuse new ideas.

Dr. Andy Newhouse, State University of New York, Environmental Sciences and Forestry (SUNY-ESF)

- Andy is now the director as former director, William Powell retired.
- Andy's focus is currently on the regulatory review process.
- Briefly, the transgenic tree, 'Darling 58', is an American chesnut from New York State into which a gene from wheat, oxidase oxidase (OXO), was inserted into the tree's DNA. This gene breaks down oxalic acid (the material used by the chestnut blight fungus to kill tissue in American chestnut's vascular cambium) into hydrogen peroxide, carbon dioxide ande water. Some questions were posed about the amount of hydrogen peroxide that is produce and Andy indicated the amount is minimal. Hydrogen peroxide breaks down quickly (within an hour), so it poses no problems.
- 'Darling 58' is being evaluated by three organizations: USDA; EPA; and FDA. The USDA is the major agency as it holds the permits for the 'Darling 58' trees that are planted (at Syracuse,

Meadowview and Penn State). The USDA is interested in the impact of the OXO gene on the ecosystem. The scientists at SUNY-ESF have looked closely at the effect of the OXO gene on other fungi. The OXO gene is not antifungal. It does not kill the chestnut blight fungus; it simply stops it from growing as the oxalic acid is neutralized. As for beneficial mycorrhizal fungi, no differences between native American chestnut and 'Darling 58' have been detected. It appears that 'Darling 58' does not interup the relationship with other fungi. They looked at leaf degredation and again found no differences between native leaves and those from 'Darling 58'.

- The next step with the USDA is to file a petition for deregulation status. There have been not threats to agriculture or to human health, so the feeling is that USDA will issue such a permit, based on the results of the third open comment period (that ended on 25 Jan 2023).
- The EPA regulates pesticides. This organization is interested in the interaction between the oxalic acid produced by the chestnut blight fungus and the OXO gene. They too are proceeding with their review process. SUNY-ESF has turned in two petitions: (1) a registration for tolerance exemption; and (2) a petition for examption to allow for large-scale plantings and distribution of seedlings and pollen.
- The FDA is specifically looking at health impacts of humans and livestock.
- It was reported that SUNY-ESF hopes to hear from all three agencies sometime in 2023.
- Do all three agencies have to approve? This is a tricky question and it is unclear if all three have to approve prior to deregulaton. First and foremost, the USDA does have to approve. EPA may approve with provisional regulations (they may limit the number of planting locations). The FDA does not have to give approval, but TACF does not anticipate bagging and selling transgenic chestnut in supermakets.

Sara Fitzsimmons, TACF's Chief Conservation Officer

- There have been three open comment periods conducted by the USDA. Collectively, there have been 28,000 comments. The USDA wants only unique comments. Those comments from form letters are classified as a single comment.
- Within the 16 state chapters in TACF, there are about 500 chestnut orchards.
- Sara encouraged all state chapters to enter their chestnut trees, whether planted orchards or native, wild trees into Dentatabase, a program that tracks

chestnut trees.

- If 'Darling 58' is deregulated, any tree receiving transgenic pollen must be in the database as EPA still considers transgenic pollen to be a pesticide.
- Prior to using transgenic pollen, TACF will provide workshops and participants will be provided with pollination kits.

Dr. Jared Westbrook, TACF's Chief Science Officer

- TACF has been breeding chestnuts for 40 years.
- Curently, they are crossing trees that have the most American chestnut DNA with 'Darling 58' trees. Jared stated that he has been using these trees to see if there is an added effect of combining the OXO gene with genes from Chinese chestnut. They have found no additive effect.
- About 400 American chestnut trees have been genotyped (all the DNA has been mapped), and he believes through 40 years of breeding, 99% of the diversity of American chestnut has been captured in the backcross trees.
- Thus, even if 'Darling 58' is deregulated, there is still a need for the backcross trees since they contain most of the diversity of American chestnut.
- About 5,500 backcross trees have been genotyped. The data from these trees will be used to reduce the genetic bottleneck (this limits the genetic diversity of a species because only a small part of the original population survives). The plan is to eliminate Chinese chestnut DNA to maximize American DNA.
- Resistance to the chestnut blight fungus is a spectum. The goal is to have trees that have 80% American DNA and have as much resistance as an F1 (a cross between and American and Chinsese chestnut).
- Stacking genes to further increase resistance is part of future research.

Dr. Adriana Del Groso, SUNY-ESF's Distribution Manager

- She is relatively new, having been hired last year.
- TACF and SUNY-ESF have the same distribution plan for 'Darling 58'.
- She is building partnerships and identifying small scale planting sites.
- She indicated that there will not be much 'Darling 58' material for the first few years after deregulation. It may be limited to research, education and the public (with New York TACF members getting some of the first material since they supported SUNY-ESF over the decades).
- Nuts will have to be tested to see if they are OXO

positive. They are hoping to find a more efficient method of testing.

• Commercial nurseries will have to be involved to gear up large-scale production.

Some of the discussion indicated that while every chestnut restored to the wild may live for decades or longer, it will be impossible to annually restore chestnut to more than a very tiny percentage of the potential range. Restoration will be a very long-term process – a learning process, in effect – and the trees used for restoration will be improved continually during that time. Thus, 'Darling 58' is not the final product, but it is the first step in the generations-long process of restoration.

There are already other genetic constructs in the pipeline that are improved versions of 'Darling 58'. These improved options may be utilized in a few years if approved by the Federal agencies. Who knows what technology will come along in the next 10 to 50 years that may be light-years better than our current options.



American chesntut in a woodland.

Upcoming Events

April 1, 2023, PA/NJ Chapter Spring Growers Meeting, University Park Campus, Penn State University, 603 Barron Innovation Hub, 123 South Burrowes Street, State College, PA (8:30 am-4:00 pm). This a grower-centric meeting, covering the what, where and how to plant chestnuts. This will be a full-day of socializing, great presentations as well as a tour of the Penn State greenhouse and orchard facilities. Lunch is \$25/person.

April 15, 2023, WV chapter spring meeting, Waco Center, Glenville State University, Glenville, WV. The meeting will begin at 1:00 pm and is open to all. An update of chapter events in 2022 and plans for 2023 will be presented.

Directions to the Waco Center:

- Take I-79 to the Burnsville Exit (Exit #79)
- If you are coming from the north, turn right at the end of the ramp and head toward Glenville on Route 5.
- If you are coming from the south, turn left at the end of the ramp on Route 5.
- Go about 15 miles until you come to a "T" at Highway 33/119. There is a McDonald's restaurant on the right at the intersection.
- Turn left on Highway 33/119.
- Proceed to the top of the hill and take a sharp right onto Mineral Road. Continue about 1/2 mile. The Waco Center and Morris Stadium will be on the left.
- Proceed behind the Waco Center on the left-hand side of the building and park in spaces available in the rear of the building. An open door will lead to the second-floor classroom.

April 22, 2023, Summit Bechtel Reserve spring work day.

The goal is to weed around chestnut trees in the orchards and apply fertilizer, in conjunction with Oak Hill Scout Troop 1885. For further information contact Kimberly Bennett (troop1885oakhillwv@gmail.com).

Update from the Kentucky Chapter

Presidents of TACF's 16 state chapters continued their monthly Zoom meeting in 2023. **Ken Darnell** of the KY chapter provided an update on their chapter activities. Ken listed their top concerns as well as their successes.

Top Concerns for KY-TACF:

- Their hybrid breeding orchards are scattered around the state, and they have problems with a lack of maintenance.
- Their hybrid chestnuts are showing weak blight resistance.
- There is a lack of members to enter trees into TACF's Dentatabase.
- They have no use for grafting trees.
- They have a lack of time available from board members/volunteers.

Top Successes:

- They are building the KY board of directors to multiply skills and resources.
- They are fortunate to have a large donor who supports the KY chapter.
- They have 462 Facebook followers.
- The KY chapter is purchasing membership for young,

dynamic volunteers. These are generally college students who have volunteered.

- They have two commercial pilots who are flying to scout for flowering American chestnut in KY forests.
- They have a wonderful hybrid seed orchard in conjunction with Eastern Kentucky University.
- They have located and documented 1,100 wild American chestnuts on TreeSnap.
- They employed two part-time interns through the University of Kentucky Forestry Technical Pool. The interns are hired 3-6 months on an as-needed basis.

The KY chapter has added seven new board members over the last three years. Some of the board members include: a supervisor from the USFS Daniel Boone National Forest; a member of the KY Department of Forestry; a professor at Eastern Kentucky University; a professor from the University of Kentucky; a member of the Berea College faculty; and a retired commerical pilot.

There are six surviving hybrid orchards in the Commonwealth of Kentucky They also have several failed orchards, so they need to 'catch up' with their maintenance. There is one seed orchard and a lot of demonstation orchards.

The TACF Regional Seed Orchard is located on the campus of Eastern Kentucky University. The trees were planted in 2016 on 2.5 acres, and a 10' deer fence was installed. The 3,650 trees were challenged by artificial inoculations with the chestnut blight fungus as well as natural infections. As of 2022, the weakest trees and those that died were culled, leaving 1,214 remaining.

Ken Darnell scouts American chestnut in area forests and he cuts trees for hiking sticks. He cleans them up, and uses them for awards. One of the most famous trees in Ketucky is the Adair tree, a large, surviving American chestnut. The tree is 48" dbh and it has been used as a pollen source for many other chestnut trees.

Using TreeSnap, Ken has found 3,500 American chestnuts. He continues to follow the best 100 trees.

Mid-Atlantic RSC Steps Down

Brianna Heath, TACF's Mid-Atlantic Regional Science Coordinator, stepped down after only 7 months on the job. Apparently, she was too overwhelmed with the assigned tasks. Regarding re-hiring, TACF will be posting the position across several platforms, leaving it open for 3-4 weeks. They hope to start interviews later in March. The goal is to get someone hired and started by early-mid-May. In the interim, the Mid-Atlantic Region (MD, KY, WV and VA) will be divided up between **Sara Fitzsimmons** and **Kendra Collins** (the RSC for New England). Sara will assist MD and WV while Kendra will handle KY and VA.

A Look Back in Time

The Forest Pathology Laboratory at West Virginia University has a collection of journal articles relating to American chestnut and chestnut blight. Some of the articles date back to the early 1900s when the chestnut blight pandemic was in its early stages. Sometimes it's nice to look back and read about scientists' understanding more than a century ago.

In a Cornell University Bulletin from June, 1914, P.J. Anderson and W.H. Rankin published "Endothia Canker of Chestnut". (Note: The current name of the chestnut blight fungus is *Cryphonectria parasitica*. Prior to that, the fungus was known as *Endothia parasitica*. The name was changed after debate over morphological characteristics of the fungus). The bulletin is 89 pages, so I will duplicate just a very small portion of their writing.

"The Endothia canker was first discovered by Merkel (1906), on the American chestnut (*Castanea dentata*) in the New York Zoological Park during the summer of 1904. Observations made by the writer during the past year indicate that all varieties and species of the genus *Castanea* are subject to the disease, except Japanese varieties (*Castanea crenata*). In the same year, Metcalf (1908) states that the Japanese varieties are in general resistant, and Murrill (1908) reports having found the canker on a Japanese chestnut and on chinquapin (*Castanea pumila*) in the New York Botanical Gardens.

Van Fleet (1914) records many observations concerning the spread of the canker in the breeding plots at Washington, DC where he has been breeding chestnuts experimentally since 1894. Nearly all the trees having *C. americana* in any combination have disappeared.



Journal articles from 1914.

Van Fleet reports that seedlings of Paragon chestnut, the best variety of the European type, pollinated with native species, attained an average height of 25 feet and were bearing excellent nuts when attacked in 1910, but all have succumed. The crosses of Asiatic and native chestnut, fewer in number, showed greater resistance, but all have been seriously affected.

In brief, it may be said that there is no species of *Castanea* which is wholly immune. Some varieties show marked resistance, especially Asiatic varieties, and Metcalf (1914) claims apparent immunity for certain strains. On the other hand, no species outside the genus *Castanea* is know to be seriously affected".

The present disease of chestnut has become known by several names. Metcalf (1908) applied the name "bark disease" and since that time, this name as well as "blight" has been used by most writers. Murrill (1908) used the name "canker", but unfortunately this name has not found preference with but few writers.

In the first published accounts of the *Endothia* canker, Merkel (1906) says, "This disease was first noticed in the New York Zoological Park in a few scattered cases which occurrd during the summer of 1904." Merkel adds, "No indication of the chestnut-tree disease was noticed by me previous to the year 1904. In 1904, however, toward the latter part of the season, I noticed that certain very old chestnut trees were suffering in certain portions of their tops, but no investigation was made of the cause. Early during the following year, in fact on June 17, I became thoroughly alarmed and sent to the Bureau of Forestry a specimen of the disease on a young tree and a letter asking for information."

Metcalf and Collins (1909) state, "Even at that time [referring to the discovery by Merkel in 1904] it is certain that it had spread over Nassau County and Greater New York and had found lodgement in the adjacent counties of Connecticut and New Jersey. No earler observation than this (1904) by Merkel is recorded, but it is evident that the disease, which would of necessity have made slow advance at first, must have been in this general locality for a number of years in order to have gained such a foothold in 1904. Conspicuous as it is, it is strange that the fungus causing this disease was not observed or collected by any mycologist until May 1905.

This was a starting-point of the numerous investigations conducted by a large number of workers. Metcalf and Collins (1911) state: "There is reliable evidence, however, that it was present on Long Island at least as early as 1893."

In his first publication, Murrill (1906) advances the theory that "it is possible that the conspicuous ravages of the disease about New York city are largely due to the severe and prolonged winter of 1903-04, during which many trees of various kinds were killed or injured. Clinton (1909) did not believe a new and dangerous pathogen was being dealt with, but, as he states

later, he believed that a native obscure fungus disease had suddenly sprung into prominence, due more to the condition of the host than to the potentialities of the organism. These two guite divergent opinions were each based on circumstantial evidence which will be more fully treated under etiology and ecologic relations. As a solution of the problem, pathologists welcomed the finding of the disease in China in 1913, for this furnished a satisfactory basis for explaning many factores concerning the epiphytotic. It therefore seems certain that Metcalf's assumption as to the origin of the outbreak has been proved correct. The opposing views of others, which no longer have any significance in accounting for the origin of the disease, are nevertheless important points to be considered under the influence of ecologic factors on the fungus, and the susceptibility of the host.

The rapidity of spread has been phenomenal, and the completeness of destruction is without parallel in the annals of plant pathology. Merkel (1906) wrote in November 1905, "Since that time (1904), however, it has spread to such an extent that today it is no exaggeration to say that ninety-eight percent of all the chestnut trees in the parks of this borough (Bronx) are infected."

Metcalf (1908) states that the bark disease is now reported from Connecticut, Massachusetts, Vermont, New York (as far north as Poughkeepsie), New Jersey, Pennsylvania and possibly Delaware. In the same year, Metcalf reports to add Maryland to the list. Metcalf states in 1910 that "At present time it has spread from Saratoga County, New York and Suffolk County, Massachusetts, on the north and east to Bedford County, Virginia, on the south, and Greenbrier and Preston Counties, West Virginia, and Westmoreland County, Pennsylvania on the west."

Even though the disease was imported from the Orient, as now seems certain to have been the case, it is resonable to suppose that there were centers of infection started at distant places by the shipment of nursery stock.

As stated at the beginning of this section, the Anderson

and Rankin article is quite lengthy. It is interesting that some scientist attributed the dieback in chestnut to the drought of 1903-04 and not to a new plant pathogen. This small snipet gives us an insight into the struggles of trying to understand the cause and effect of the newly discovered disease of chestnut.

National TACF Update

On Friday, February 24, 95 members from across all 16 state chapters met via Zoom to hear about updates from the national office. Since **Sara Fitzsimmons** was promoted to Chief Conservation Office last year, she has been doing double-duty as the Regional Science Coordinator (RSC) for the North-Central region (PA/NJ, Indiana, Ohio and New York) as well as her focus on species restoration across the range of American chestnut. TACF has hired a replacement for Sara freeing her up to concentrate on the larger picture of restoration. **Stephen Hoy**, long-time forest manager at Penn State, has been named the new North-Central RSC. Stephen is not the only new hire for TACF.

A search has been underway for a new outreach coordinator to help all 16 state chapters. After interviewing candidates, two individuals rose to the top. Rather than choose only one, both candidates have been offered positions. One of the individuals will be housed in Asheville, and the other in Albany, NY. Since neither has accepted officially, more information will be forthcoming. Both new hires will begin in late March and help with defining chapter goals, networking, social media, membership involvement, etc.

WV Chapter Seed Distribution

The WV chapter began distributing seeds for direct seeding in early March to members who ordered seeds last fall. Packages of seeds were bagged with moist paper towels and mailed to members. The WV Division of Forestry has agreed to plant 100 chestnut seeds on each of three state forests, Greenbrier, Seneca and Calvin Price State Forests. The WV chapter supplied 300 tree shelters and rebar stakes for this project.



Packages of chestnuts ready to mail.



Lewis Cook and Travis Miller picked up supplies in Weston.

The WVU greenhouse has offered the WV chapter 4-5 tables in their plastic house, free or charge, for many years. This year, WVU is greatly expanding their flowers and potted plants. As such the WV chapter can only have 1 greenhouse table. To offset our limited space in Morgantown, three other greenhouses also are growing chestnut seedlings this year: Fayetteville; Shepherd University; and Potomac State. Picture above, **Dr. Lewis Cook**, WV-TACF board member(left) and **Travis Miller**, Assistant State Forester for the WV Division of Forestry, met Mark Double in Weston to pick up supplies. Dr. Cook picked up potting mix and chestnut seeds while Travis picked up tree shelters, rebar and chestnut seed. Sadly, all the chestnut material for 2023 has been spoken for. If you are interested in chestnut seed/seedlings for 2024, contact Mark Double at **WVachestnut@acf.org** to be added to the list for next year. The WV chapter hopes to plant more than 1,300 seedlings in 2023 all across the state.