The West Virginia Chapter of The American Chestnut Foundation NEWSLETTER

In the heart of American chestnut's natural range

February 2024

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Future of TACF's Science Programming

The 19 January 2024 ***Chestnut Chat*** featured a number of individuals who presented data on various aspects of TACF's science program. **Sara Fitzsimmons**, TACF's Director of Restoration, oversaw the meeting, and she reiterated that restoration is a decades-long process. The work that currently is being conducted rests on the laurels of work that has been conducted over the last 100 years. The current work is just a point on a continuum.

A brief history of chestnut research is listed below:
-In the 1900s, there was an attempt to replace American chestnut trees with trees from China.
-In the 1920s, breeding was conducted using all chestnut material available at the time (American, Chinese, Japanese).
-In the 1950s, gamma irradiation for mutational breeding was conducted.
-In the 1970s, hypovirulence was tested for the first time in the U.S.
-In the 1980s, traditional plant breeding was conducted, both backcross breeding an the use of Large Surviving American (LSA) chestnuts.
-In the 1990s, genetically-modified trees were initiated.
-In the 2020s, new molecular tools (CRISPR and RNAi) are being utilized.

The problem of restoring American chestnut is a very complex problem that requires complex approaches. There is no silver bullet, therefore a number of approaches are necessary. In assessing any of the approaches, we need to look at the stability of resistance, and the answers come on 'tree-time' not human time. Humans have a tendency to rush things along to witness success, but we are dealing with trees, not an annual crop.

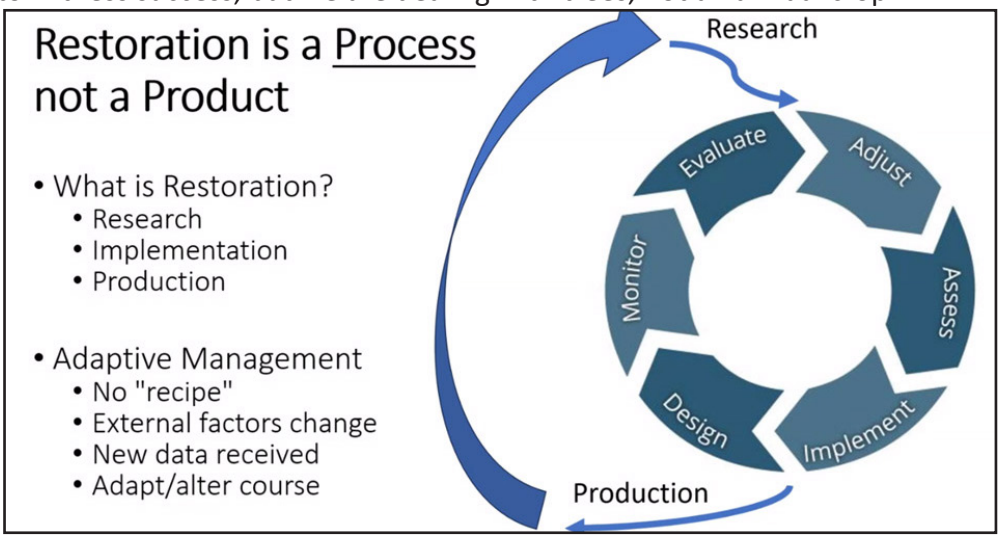
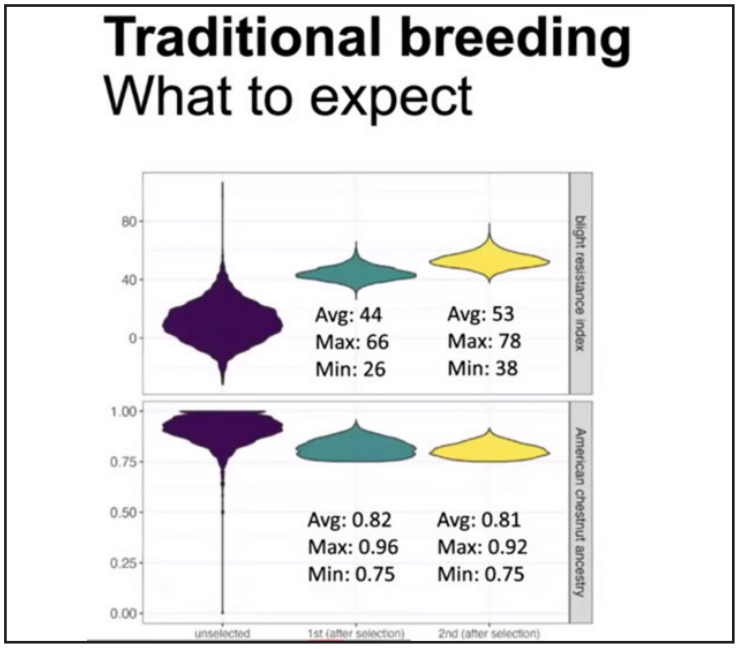


Photo courtesy of Sara Fitzsimmons

Current research is focusing on Best X Best. What does that mean? Historically, when the backcross breeding technique was initiated by **Dr. Charles Burnham** at the University of Minnesota in the early 1980s, crosses were made using American and Chinese chestnuts. Each generation of trees was backcrossed until we reached the B3F2 generation (third backcross, first intercross). At that point, all trees were open pollinated. Thus, we knew the mother, but the father was unknown. It was a mother X whomever. With the Best X Best approach, both the mother and father trees are known. We are selecting the best mothers and the best fathers. That is a big difference compared to open-pollinated trees.

Many state chapters have a tree that can be classified as 'Best', and that may translate to only 1 tree in an entire orchard. TACF has been collecting pollen from the very best trees and freezing it to use the following year. **Jared Westbrook** TACF's Director of Science, utilizes a scale of 0 to 100 where American chestnut resistance is 0 while Chinese chestnut is 100. TACF's current trees have a score of greater than 40 for resistance and 60 for American form. The average score of all the trees in TACF's breeding orchards (thousand of trees in all stages of breeding) is 10. Some of the best trees have a score as high as 66. A second generation of Best X Best trees should have trees with scores between 50-80. This is shown the following diagram from Jared.



The top panel is a blight resistance index while the lower panel shows the average American ancestry. The purple forms on the left represent unselected trees. The green forms in the middle represent the first Best X Best selections (average resistance score of 44 with a high of 66) and average of 82 for American ancestry. The yellow forms represent the second selection of Best X Best where the average blight resistance of 53 with American an ancestry score of 81. These are promising numbers.

Jared pointed out that we are not increasing Chinese ancestry, and there will be no reduction of American ancestry using this procedure. To make sure we do not repeat the problem with Darling 58, all the trees are being genotyped (examination of each individual trees' DNA sequence). Small samples of leaves are collected from trees and send to 'Diversity Arrays', a company at the University of Canberra in Australia. They charge $20 for each assay. By using these data, trees can be selected that have greater resistance than their parents.

We need trees to be competitive in natural settings. The goal is to plant the very best 10% in optimal spacing and then wait 7-10 years for them to produce seeds. Those seeds should be competitive with good resistance and American form. Trees are selected based on the following criteria:

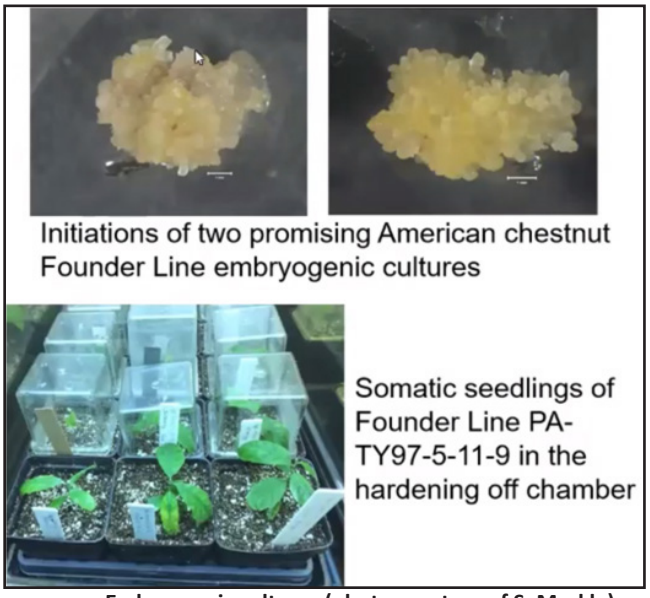
- Main stem alive
- Canker size (are they larger than 15 cm)
- Are cankers sunken
- % Dead canopy
- Exposed wood
- Sporulation of the fungus
- Presence of stump sprouts

Scott Merkle of the University of Georgia spoke about new transgenic blight resistance approaches using American chestnut founder lines. New regionally-adapted American chestnut lines (Founder Lines) are used as transformation targets. New gene promoter Oxalate Oxidase (OXO) combinations are used to modulate the expression of the OXO gene. Merkle also touched on new transgenic approaches to protect chestnut trees from the chestnut blight fungus.

Founder Lines are mostly new American chestnut embyogenic culture lines from source trees (mainly Large Surviving Americans or LSAs) representing different parts of the American chestnut range. They have already screened the new lines and picked the best ones to transform to produce new transgenic trees. Pictures of embryos and the plants that develop from the embryos are show on Page 3. It takes 2 years to go from the embryo stage to a plant that is large enough to pot.

Merkle talked about gene promoters, a short DNA sequence just upstream of a gene that turns that gene on. Four different promoters were discussed:

- Constitutive promoter: a gene promoter that keeps a



Embryogenic cultures (photo courtesy of S. Merkle)

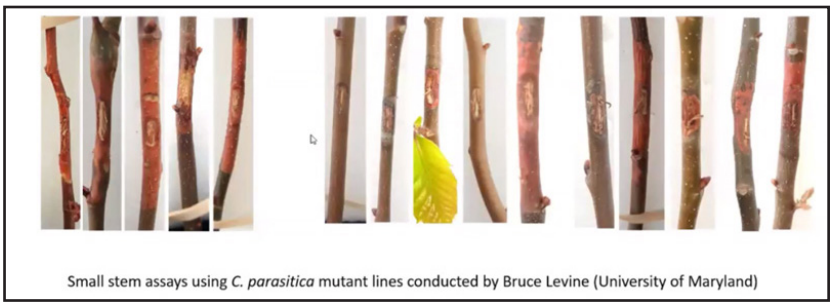
gene turned on all the time in all tissues of a plant;

- Inducible promoter: a gene promoter that only switches a gene on with a certain stimulus (e.g. wound in the bark);
- Tissue-specific promoters: a gene promoter that only turns a gene on in specific tissues of a plant;
- CaMV35S promoter: a promoter for a certain gene in the cauliflower mosaic virus: this constitutive promoter turns on the OXO gene in the Darling 58 tree.

To date, Merkle and his team have been experimenting with a constitutive promoter from a plant gene (*Arabidopsis ubiquitin*). The rationale is that a constitutive promoter from a plant gene to drive OXO may cause fewer undesirable effects than one from a viral gene (CaMV35S). They have successfully produced transgenic events in 9 Founder Lines representing all regions of the American chestnut range using the ubiquitin promoter. The first somatic seedlings are now germinating.

Merkle also is working on wound-inducible promoters. These promoter strongly produce OXO when induced by inoculations with the chestnut blight fungus. Similarly, tissue-specific promoters are being tested. These promoter produce OXO only in the trees' inner bark where blight infection occurs.

One interesting find by Merkle is that OXO alone is unlikely to provide full resistance to the chestnut blight fungus. In cooperation with **Bruce Levine** at the University of Maryland, chestnut stems were inoculated with virulent chestnut blight isolates along with a mutant chestnut blight fungus that has no oxalic acid production. Those non-OA isolates still produce killing cankers. This is seen in the following photo from Bruce Levine.



Small stem assays using *C. parasitica* mutant lines conducted by Bruce Levine (University of Maryland)

The five stems in the left panel were inoculated with wild-type chestnut blight isolates. The 10 stems in the right panel were inoculated with chestnut blight isolates that were unable to produce oxalic acid. Many of the stems were killed by the fungus, thereby proving that oxalic acid is not the sole killing organic acid produced by the chestnut blight fungus.

Jason Holliday from Virginia Tech explained the use of cis-genes (genes that come from either American or Chinese chestnut) rather than a gene from wheat, as used in Darling 58. Holliday raised the question, 'what makes Asian chestnut species resistant to chestnut blight?" He is working to identify candidate resistance genes from Asian chestnut species. How does he go about doing this? He uses large populations of American chestnut to look for parts of the genome (the complete set of genetic material) that are driving resistance. He then maps those regions that are associated with blight resistance. These regions are scattered across all 12 chromosomes in American chestnut. That process yields thousands of genes. He then looks for what genes are turned on during infection with the chestnut blight fungus. That narrows the list to hundreds of genes that are expressed in Chinese chestnut but not in American chestnut. Holliday has about 50 candidate genes. These genes have roles in pathogen perception, signaling, cell death, defensive metabolites and cell wall strengthening. Holliday then silences the genes to validate their role in resistance by using viruses as silencing agents. He is silencing genes in Chinese chestnut to see if they become susceptible when inoculated with the chestnut blight fungus.

Bruce Levine from the University of Maryland is researching what the chestnut blight fungus does to American vs Chinese chestnuts. Levine went back to historic literature and found some 1980s publications that showed when the chestnut blight fungus was virus-infected, it stopped producing oxalic acid. Levine began using DK-80, a strain that

allows for efficient targeted disruption of certain genes in the fungus. Levine even inoculated another fungus, *Sclerotinia* into chestnut stems. *Sclerotinia* is a plant pathogen but it does not produce oxalic acid. Even *Sclerotinia* was able to produce cankers in American chestnut, thus verifying that oxalic acid is not the only factor in pathogenicity of the chestnut blight fungus. A number of organic acids are produced by the chestnut blight fungus, oxalic acid included, but if a mutant strain of the fungus that does not produce oxalic acid can still infect and girdle chestnut stems, there are other issues to investigate.

Levine is looking at 'effector proteins'. These are small proteins that selectively bind to a protein to regulate its biological activity. Effector proteins have several activities. They can help a pathogen invade host tissue; suppress certain systems; or otherwise help a pathogen to survive. They are generally critical to the virulence of a fungus. Levine believes that some effector proteins are interfering with the tree's defense system. Levine has three effector proteins that he is investigating, NLP-1 and two polygalacturonase genes. Levine hopes to knock out these genes to see what unique effects they have on the ability of the fungus to invade chestnut tissues.

Kendra Collins, TACF's New England Regional Science Coordinator, concluded the Chestnut Chat. She noted that a lot of the investigations reported by the previous speakers are still in early phases. While the average member of WV-TACF cannot participate in some of the experiments noted previously, Citizen Science can help with the process of moving forward. Our Regional Science Coordinator (**Cassie Stark**) and our Regional Science Outreach staff (**Catherine Martini**) are developing templates that prioritize hands-on field activities. We can add new Germplasm Conservation Orchards (GCOs) and fill in voids of existing GCOs with new seedlings. For American chestnut trees that are not flowering, scion wood can be taken and used for grafts. WV-TACF member, **Scott Burnworth,** in Morgantown has agree to assist with any grafting. Grafted trees can then be planted in our existing GCOs. Collins is looking to establish reintroduction trials with State and Federal partners. These trials would be a minimum of 300 trees but a better trial would include 500-100 trees.

There was some discussion at the meeting about Large Surviving American chestnuts (LSAs). On a 0-100 scale with 0=fully susceptible American chestnut and 100=fully resistant Chinese chestnut, LSAs are about 15. While LSAs can offer some resistance, it is limited. Resistance is a sliding scale, generally never 0 nor 100. In addition to resistance genes in trees, the environment also plays a role in the success of an orchard. Poor soil, drought, invasive pests, etc. all play roles.

The take-home message of this Chestnut Chat is that there are a lot of moving parts in American chestnut restoration. Despite the setback with Darling 58, there is much hope on the horizon.

Potential Burnsville Dam Planting

An email to **Brian Perkins** at Glenville State University was forwarded to Mark Double. The email was from **Brian Carson** with the Army Corps of Engineers at the Burnsville Dam. Carson was asking about planting chestnut seedlings. One of the objectives in the WV Chapter's not-yet-finalized new strategic plan is to establish a fully-planted 100-tree GCO. A 100-tree planting consists of 10 trees from each of 10 different mother trees. Carson indicated that the Army Corps has about 13,000 acres available at the Burnsville Dam, and he is sure that he can find an open area with acidic soils, full sunlight and good drainage. Carson indicated that a chestnut planting would be a joint project between the Army Corps and the WV Department of Natural Resources.

Since it is still winter, none of the details have been worked out. The WV chapter does not have 10 lines, so we will be unable to plant all 100 trees in 2024. However, we should be able to plant 70 to 80 seedlings, so this will be a good opportunity for some WV members to assist in this planting. More details will be forthcoming.

WV Chapter Spring Meeting

The 2024 spring chapter meeting will be held on **Saturday, April 6** at the Waco Center on the campus of Glenville State University, Glenville, WV. The meeting will begin at 1:00 pm in a classroom on the second floor.

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. A Zoom link will be available for those unable to attend in person.

Spring Workday at Summit Bechtel Reserve

The spring workday at Summit Bechtel Reserve (SBR) will include the Buckskin Council Order of the Arrow. The Order of the Arrow is a group of scouts within the Boy Scouts that works and volunteers to make scouting and scouting facilities better. Members are scouts that have been voted in by their individual troops, and it is an honor to be a member.

The work day at SBR is **April 27, 2024**. The camp is a National Boy Scout High Adventure camp located at Glenn Jean, WV just north of Beckley on the New River Gorge.

The event is Friday April 26 to Sunday April 28th with the work day on Saturday April 27. All participates are invited to camp with the scouts, or volunteers can just come on Saturday. This will mark 10 years since American chestnuts were introduced to SBR. Native American chestnuts also have been found at the camp which encompasses 11,400 acres, mostly West Virginia wilderness.

Work will involve pulling weeds and checking tree cages. Some seedling planting may occur. WV-TACF, along with local scout troops near the camp, maintain six orchard locations at SBR.

- Fire Ring North
- Antoline Conservation Orchard on the Freedom Trail
- Robert E. Murray Drive Orchard
- Wood Lot
- Consol Bridge
- Conservation Trail.

TACF members **Lewis Cook, Jerry Legg, Steve Swank**, and **Joseph Golden** have worked with **Sam Muncy** since 2014 to plant and care for the American chestnuts at SBR.

2019 chestnut planting at SBR's Consol Bridge

To attend this event, an individual must log in with the Boy Scouts and have taken the Boy Scout Youth Protection Training. Login is free. The training is necessary as participants' training must be verified to obtain entry to SBR. You do not have to join the scouts.

This is an excellent way to see behind the scenes at SBR and teach scouts about the goals of TACF. To log in to the Boy Scouts and arrange to take the Youth Protection Training, use the following link:

https://my.scouting.org/

If you have questions contact:
Sam Muncy 304-669-9659
Sam@samandsharonenterprises.com

Tacf.org--It's Official

If you have visited The American Chestnut Foundation's website lately, you may have noticed a small change. We have officially switched our website domain name from acf.org to tacf.org!

We are pleased to announce that after 27 years as acf.org, our web address now aligns with the organization's acronym, The American Chestnut Foundation (TACF). Don't worry, any existing links to acf.org will still work in perpetuity.

You also will notice that all TACF email addresses now end in tacf.org, so please update your contact list and browser bookmarks. Acf.org email addresses will still function throughout 2024, but will be deactivated after next year.

Chapter's Committee Meeting Highlights

A zoom meeting of chapter presidents and administrators was held on 26 January 2024. Some of the highlights from the meeting were:

- New domains for all the chapters have been changed. The new WV chapter domain has been changed to: **tacf.org/wv.** All of the WV chapter newsletters from 2020 to present are listed on our web-page.
- New chapter email addresses will be forthcoming. As of now, when someone submits an email to a specific chapter, they see the address as @tacf.org. The email is then converted to an @gmail.com account for the chapter administrators. The problems with converting accounts is the amount of spam that follows the conversion. The national office is sorting out the best way to address this problem.
- All chapters are encouraged to work on chapter insurance.
- Volunteers can now be tracked on-line. When volunteering for any chapter activity, hours can be submitted on TACF's website. Go to tacf.org, and under the 'engage' header, go to 'volunteer'. There you can fill out a 'volunteer application' that consists of your name, email, home address, etc. Once registered you can submit your volunteer hours on the 'record volunteer activity' button. Having an accurate account of volunteer hours across all 16 state chapters will assist the national office with grant applications.
- The national office is working on a holistic planning document. This plan will highlight the efforts that can be made by each chapter, as each chapter will have an individual science plan. The plans will include a resources page that will cover soup-to-nuts issues from how to collect pollen to review of bylaws.
- The national office is looking to install a common garden study to assess the Best X Best trees. A common garden study utilizes the same trees and the same design at various areas in chestnut's native range. Each common garden study will consist of 1,000 trees. As of now, two sites have been identified, but collaborators will be needed to identify the other two sites (one each in the South and Northeast regions).

Chestnut Weevils

Every March, the WV chapter pots nuts that will grow into seedlings that are then distributed across the State of West Virginia where they are planted in either May or October. This is the chapter's second year using D40 pots, pictured below. These pots a much deeper than the 4" square pots that were used in previous years.

After chestnuts are collected in late September, they are floated in water. Chestnuts that float are not viable, and are discarded. Nuts that sink are viable. Chestnuts require a cold period referred to as stratification. In September, nuts are packaged in a damp potting mix in zip-lock bags and stored in a refrigerator over the winter. Come late winter, the process is repeated in that the nuts are removed from the bags and floated again in water. This year, about 25% of the nuts became non-viable over the winter. If this process of checking the nuts in later winter is not conducted, many non-viable nuts will be potted, so the second floating reduces the amount of time and effort when the nuts are potted in March.

During the winter, hundreds of chestnut weevils (*Curculio sayi*) fill the refrigerator. The 1/4" weevils emerge from the ground in late May but they do not lay eggs until the fall. Eggs hatch in 10 days and larval development is completed in 2-3 weeks. Soon after the nuts hit the ground, the grubs chew a circular hole in the side of the nut to enter the soil. The emergence holes are seen in the photo below, from a nut with an emerging radicle.