Maryland Chapter

The American Chestnut Foundation

NEWSLETTER
AUGUST 2019

INSIDE THIS ISSUE

Come to the Annual Meeting on **October 5th** for the latest Chestnut updates

CLIMB THE LADDER

Sailboats cannot sail directly upwind, but can sail to within about 45 degrees of the wind direction through the combined effects of lift generated by wind on the sail and the motion of the keel trough the water. Thus a boat's track to an upwind mark is typically a zigzag course, with each turn designated a tack. Wind shifts can play an important role on the choice and timing of the tacks. A shift in one direction, known as a "lift", can allow the boat to sail more directly to the mark, while a shift in the other direction, known as a "header" has the opposite effect. These shifts are often oscillatory. A boat that can anticipate and respond properly to these shifts will shorten the total distance needed to sail to the mark. This tactic, known as "Climbing the Ladder", is used by winning skippers to make the most of their opportunities.

To a chestnut person, the term "Climbing the Ladder" most likely is assumed to have something to do with pollinating or harvesting chestnuts. But here I am using it in a sense that is analogous to the sailing definition. In this newsletter and in our upcoming Fall meeting, you will find descriptions about the progress the Chapter has made, the shifts we have responded to, and shifts we are likely to encounter in the near future. Our shifts are not the oscillatory wind shifts of sailing, but rather persistent changes in our understanding of the science of breeding a blight resistant American hybrid. The tacks are our responses to those shifts.

Fall Meeting

Plan on coming to our **Fall Meeting, October 5**, to get a much clearer understanding of the shifts in the science, as well as the recent and potential future tacks in the program in response to those shifts. The meeting is at the ***Rockville Izaak Walton League Meeting House at 18301 Waring Station Road, Germantown. The meeting starts at 11:00***. Lunch will be provided. Elections for Chapter Officer's & Board Members will be held at the meeting. If you would like to be considered for one of these positions, please contact me at **lwgrossman@icloud.com**

Our Regional Science Coordinator, Tom Saielli, will talk about the recent developments in the science, including genomic testing done on trees in our orchards, and recommendations for changes in Chapter programs based on these developments. Bruce Levine will bring us up to date on our Chapter's Small Stem Assay work underway at the Baltimore Country Agriculture Center in Cockeysville. Look for an article about this in this newsletter. But since this remains a work-in-progress, there will be more at the meeting. And it is a chance for you to ask the experts the burning questions that have formed in your mind.

National Meeting

The American Chestnut Foundation's 2019 Annual Meeting will be two weekends later, on October 19 in Gettysburg, PA. These annual meetings are usually much more distant from Maryland and represent a significant hardship for MD Chapter members to attend. This year it is an easy daytrip that for the cost of registration provides a treasure trove of chestnut science, the activities of other chapters, and chestnut lore in general. Type ACF.ORG into your browser and follow the links for the meeting registration and agenda.

Orchard Activities

Look in this newsletter for a status report on our orchards. The harvest season will be upon us soon. If you are interested in participating in harvesting and are not on the volunteers email distribution list contact Karl Mech (karlmechjr@gmail.com) to get your email address added.

Hope to see you soon.

Larry Grossman, Chapter President

Hiding in Plain Sight

By Dave Gill

There are still pure Native American chestnut trees in Maryland and we need your help to find them. Even though the chestnut blight may have killed the original tree, the roots continue to send up shoots that grow for upwards of 15 years before the blight kills the shoots. It's those survivors that we are searching for so that we can diversify our genetic sources for our breeding pool by collecting pollen and seeds.

So how can you help? If you think you have found a American Chestnut you have several avenues for verifying your find. A great tool to help you report a possible American tree is called ***Tree Snap***. You can download the application to your smartphone or tablet from either Apple Store or Google Play stores for free. Easy to follow directions in the application will guide you through the identification process.

Another approach is to identify a possible American Chestnut tree using the criteria listed at the WEB link below then send your samples to Gary Carver at the addressed listed on the WEB site. Helpful information in identifying the tree is located at the American Chestnut Foundations web site - https://www.acf.org/resources/identification/

Fall is a great time to locate the trees because you can easily spot the green burs containing the chestnut seeds. The burs generally start to open in late September to early October. If you can collect some of the seeds, it would be most helpful.

As part of this effort to capture and preserve American Chestnuts, we are looking for volunteers willing to plant and take care of pure American Chestnut trees in small germplasm conservation orchards on their property. As the trees mature we would be collecting tree pollen and nuts to aid in our breeding program. If you would like to assist in this effort, contact Ron Kuipers at m_rkuipers@yahoo.com or 240-838-9992.

ORCHARD NEWS

By Ron Kuipers

The work that we do in our orchards can be hard and sometimes frustrating. It is also rewarding when we look at our thousands of new trees, some of which will eventually contribute to ones with blight resistance.

This year, much has begun to change in the orchards because of the new greenhouse project (see below). We now plant most of our trees in the fall as seedlings rather than in the spring as seeds. Also, the current practice of inoculating three and four year old trees in June and culling the less resistant trees up to a year later is now being performed with seedlings in the greenhouse. This will eventually greatly reduce the work required in the orchards.

For the next several years, however, the two inoculation programs will overlap, creating double work until the existing seed orchards are inoculated and culled.

Planting

Our annual spring plantings at the end of March were limited to 150 seeds at the Lyles Musick orchard, and a small numbers of seeds at Sugarloaf and IWL- BCC Germplasm Preservation Orchards (GPO's). We were also involved in a couple demonstration plantings and a new small GCO established within the Savage River State Forest in Garrett County.

The Savage River GCO used locally collected seeds and was formed in partnership with the State DNR, the County Forestry Board and the University of Maryland.

Fall planting of seedlings from the greenhouse will likely take place in both seed orchards in early October.

Inoculations and Culling

Inoculating, evaluating and culling is an ongoing process, with inoculations in June, evaluations six months and a year later and culling occurring at any time. Expect major culling work days this fall and winter in both the seed orchards and other orchards that need to be rid of dead or inferior trees.

Pollination and Harvesting

We will be harvesting at the end of September from six trees that we hand-pollinated in June -- five trees in the Monocacy orchard and one pure American growing near Lake Needwood. Two of the Monocacy trees and the Needwood tree are expected to yield two lines of Musick B3 seeds for the Lyles orchard. Three Monocacy B2's were crossed with American pollen to produce seeds for Phytophthora resistance research. We also plan to harvest open pollinated seeds from several family lines in the IWL-Damascus and WMREC orchards. These seeds will be planted in the greenhouse next February.

In addition to these highlighted activities, there is a lot of work that goes on in our orchards almost every day. These include mowing, weed-whacking, hand weeding, cutting dead branches and new sprouts from culled trees, fence repair, and spraying for weeds, Ambrosia beetles and Phytophthora. You may not receive a request for help with this work, but this may not mean that help would not be appreciated. If some activity on that list is appealing or if one of the orchards is a short distance away, I encourage you to give a call to the relevant orchard steward. Info on MD orchards and stewards at: https://www.acf.org/md/orchards/

The Maryland Chapter's Pilot Program to Prescreen Orchard Trees for Blight Resistance

By Bruce Levine

The Maryland chapter of TACF is well into the process of planting the "seed orchards" which will ultimately produce the highly-resistant chestnut seeds we need to restore chestnut to the forest. What we have learned from results in relatively advanced seed orchards in other states, however, is that the best trees in those orchards still fall short of the levels of resistance of Chinese chestnut we hoped to see. There are several possible reasons for this, and TACF is working on revisions to its breeding program to enhance the resistance of our breeding population (see separate article). In the meantime, there are strategies TACF chapters can use to improve the quality of trees in seed orchards, even from existing breeding lines. Maryland is leading a pilot effort on one of these strategies, the use of small stem assays (SSAs) to pre-screen seedlings for resistance before planting.

Seed orchards are planted with B3F2 seeding's, which are intercrosses of Chinese-American hybrids that have been back-crossed over three generations with pure American trees. B3F2 seedlings have the potential to inherit the full range of Chinese resistance genes from both sides of their families, but not all of them will. That is why we plant plots of 150 seedlings, planted on a 1 ft. x 7 ft. grid, for each highly-resistant mother tree we hope to have in our seed orchards. The idea is to inoculate these seedlings with blight fungus, and over the course of several years, remove all but the most resistant tree in each plot. Unfortunately, it appears that the genetics of blight resistance is more complicated than TACF scientists originally thought, so 150 trees may not be enough to ensure that we end up with a sufficiently resistant tree. In addition, the tight spacing of our seed orchard plots is stressful on seedlings, and causes many to die prematurely for reasons other than blight. To remedy this, we need to plant and compare more trees for each breeding line, and plant them further apart. But that would take more land and labor than we have.

Instead, the Maryland Chapter decided in 2019 to plant greater numbers of seeds per breeding line, but to plant them in pots in a greenhouse, and use small stem assays (SSAs) to inoculate them with blight fungus in their first year of growth. Research concluded with other TACF chapters last year, showed that SSAs are sufficient to at least tell highly-resistant seedlings from susceptible ones. SSAs on B3F2 seedlings last year resulted in the death of 80-90 percent over the course of one season, leaving surviving trees with the kind of superficial, swollen infections we normally see on resistant Chinese chestnuts. Using SSAs will allow us to pre-select and plant only the most resistant 10% of seedlings per breeding line, and plant them further apart in our seed orchards to ensure better growing conditions. Ultimately, this will allow us to select resistant parents from among the 50 most resistant seedlings from a population of several hundred, rather than from 150 highly variable seedlings. By tracking the seedlings with ID numbers we can also simultaneously determine which mother trees are producing the most resistant seeds, which will help us exclude substandard breeding lines in our seed orchards.

Baltimore Greenhouse SSA Seedlings

We are very grateful to the Baltimore County Agricultural Center for allowing the Maryland Chapter to plant and grow our SSA seedlings at their facility in Cockeysville Maryland. We planted 4,000 seedlings in February, of which about 900 survived and were large enough to inoculate with chestnut blight fungus in early July. As of mid-August, we are starting to see the pure American control seedlings and some, presumably the most susceptible, B3F2 seedlings die from the inoculation. We are recording the date each tree dies and will use the data to compare them by breeding line, so we can select and plant only those lines with the highest survival rates. In late September, we will take the surviving (most resistant) seedlings from those best lines, clip off the infected inoculation site, and plant them in our seed orchards. Previous research shows that we can expect the vast majority of seedlings to survive the winter and re-sprout below the inoculation site in the spring. And if we have fewer than 50 seedlings per line to plant this fall, that's OK. We can harvest more seeds from the same mothers, and plant and screen them in pots next year, and repeat until the seed orchards are filled with superior seedlings. It will take several years to know for sure, but we hope this pilot will result in substantially more resistant mother trees in our seed orchards. Stay tuned.

July SSA Inoculations

A small stem inoculation will produce a long canker on both resistant and susceptible trees, but the canker on the resistant tree (see the Chinese tree on the left) will be superficial and swollen as the tree rebuilds tissue underneath. The canker on the susceptible tree(The American chestnut on the right) grows deep in the bark, killing it and giving it a sunken appearance, as well as cutting off the flow of nutrients to the rest of the stem.

Genetic Data Leads TACF to Revise its Breeding Program

By – Bruce Levine

A successful scientific program like the TACF breeding program requires regular assessment of results, and whenever necessary, adaptation of methods and protocols. Thanks to improvements in technology, TACF scientists have been able to use DNA sequence information and resistance scores from trees in its seed orchards and chapter backcross orchards, to take stock of the breeding program's progress so far. This year, TACF scientists and partners at Virginia Tech and Hudson Alpha announced that they had identified loci (physical locations in the genome) on 9 of the 12 chestnut chromosomes, which are associated with variation in blight tolerance among Clapper BC3F2 trees. Although we still have not discovered the specific genes for the resistance of Chinese chestnut, we now have a sense of where the genes are located and how many there may be. This puts TACF much closer to discovering the genes and potentially genetic markers for resistance, which would greatly improve the efficiency and effectiveness of the breeding program. That's the good news.

The disappointing news is that this means that a large number (nine or greater) of genes are responsible for resistance in the population studied, far more than the two to three genes for which the breeding program was originally designed. This helps explain the observation that the average resistance of the B3F3 trees tested so far is somewhere between that of pure American and F1 hybrids (50-50 Chinese-American), rather than highly resistant Chinese chestnut, as hoped. All or most of the resistance genes inherited from the original Asian chestnut trees may be in our chapter's breeding population, but not necessarily together in any given tree. Understanding what each gene does and how they relate, singly or in combination, to blight resistance is not yet understood. (Some may be more significant than others. Some may only work in combination with others.)...

TACF also sees a tradeoff between the proportion of American chestnut backcross trees' genomes inherited from American chestnut and chestnut blight tolerance, i.e. the most resistant trees have inherited a higher percentage of their genomes from their Chinese great-grandparent than the roughly 6 percent we would expect from a B3 (third backcross) population. This does not necessarily mean that a high percentage of Chinese ancestry is necessary for resistance, but rather

that three generations of backcrossing has not been enough to separate the genes responsible for resistance from other Chinese genes inherited along with them.

TACF has also been continuing its work with partners to identify genes for resistance to **phytophthora root rot (PRR),** another invasive disease that threatens the American chestnut, especially in the south. As is the case with chestnut blight, Chinese chestnut is resistant to PRR, and PRR resistance seems to be governed by multiple genes. Unlike with chestnut blight, however, some of those genes seem to convey high levels of resistance by themselves. TACF has developed screening methods and facilities to determine whether parent trees carry resistance to PRR, something which southern chapters may wish to take into account in the breeding program. (For Maryland, our Musick lines appear to carry PRR resistance, but the Clapper lines do not.)

Based on this new information, TACF scientists have concluded that the backcross breeding program, as originally designed, will not produce enough highly-resistant B3F3 hybrids in the foreseeable future to generate the diverse population of parent trees we need for restoration. At a science meeting in Abingdon, VA in July, TACF scientists discussed several possible changes to the breeding program that move away from a strict backcrossing approach. These include:

1. TACF would like chapters to collect DNA samples (from young leaves in the spring) from all hybrid trees each chapter has used as a parent in their breeding programs, which TACF will analyze to determine the percentage of their genomes they inherited from Chinese chestnut. TACF would like chapters to use this information to reduce the number of breeding lines, and to concentrate on cultivating those that have the highest levels of Chinese genes. This will ensure the greatest probability of capturing genes for resistance. At the same time, TACF will use the data collected to develop a set of genetic markers that can be used to predict resistance.

2. Chapters concerned about PRR (including Maryland) can provide seeds from parent trees in their breeding programs to TACF to evaluate whether those parents carry PRR-resistance.

3. TACF is reducing the number of replicates of each breeding family in seed orchards from nine to three. Where more replications have already been planted, inoculations and/or genetic tests will be used to determine which are the best, and which should be discontinued or even removed.

4. TACF is no longer asking chapters to keep trees with different sources of resistance in separate seed orchards. In addition, chapters are encouraged to plant B1 or B2 intercrosses in seed orchards if they are resistant and show sufficient American growth habit. These trees can potentially have very high levels of resistance and enough American character to be bred safely with more advanced hybrids.

5. TACF would like chapters to find more local American trees, and preserve their DNA by gathering seedlings, seeds or scion wood (for grafting) from surviving wild pure American trees, to place in germplasm conservation orchards. This will help maximize the local genetic diversity captured by each chapter in later stages of the breeding program.

6. Assuming regulatory approval, TACF would also like interested chapters to cross local pure Americans with transgenic "OxO" trees from the College of Environmental Science and Forestry (ESF) in Syracuse, New York. This is not required for the breeding program, but serves two purposes: first to preserve local American germplasm by making highly-resistant crosses, and second to create a diverse population of American trees carrying the OxO gene so those trees can be planted throughout the chestnut's natural range.

ESF researchers Dr. Charles Maynard at left and Dr. William Powelll developed the transgenic "OxO" trees

The TACF breeding program has produced thousands of trees that represent a diverse population of pure American ancestors, but which also have elevated levels of blight tolerance. As information about the genetics of blight resistance continues to improve, these trees will form the basis of a much more efficient and effective breeding program. In the meantime, we must take stock of what we have, identify the best, and prepare ourselves for what promises to be faster progress.

We Need Your Financial Support

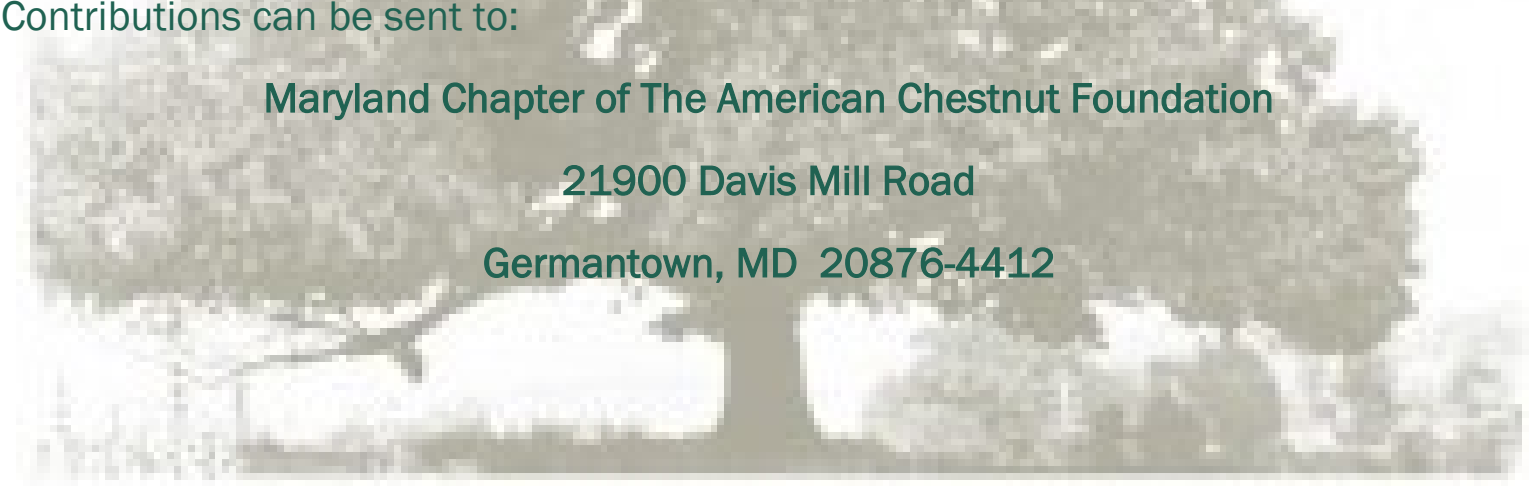
The Maryland Chapter maintains over 20 Chestnut research orchards across the State. We are fortunate to have so many dedicated volunteers to help with the many projects in this program, but it takes more than volunteer hours to continue our work. We face a continued uphill battle with storm damage to orchard fences, insect invasions and the increasing need for sophisticated test to help refine the resistance in our trees. For those who are Chapter members, of your $40 annual dues to The American Chestnut Foundation, the Maryland Chapter gets $15 toward our operational expenses. That $15 is fully applied toward yearly maintenance but falls short of our needs to meet the ever changing scientific requirements of the program. We have been fortunate in some prior years to have received limited grant money, which greatly helped, and we will continue to pursue those funding avenues. Unfortunately there are no grants in our immediate future and we need to seek other funding opportunities. Additional funding would allow us to continue with the Small Stem Assay project and to start some initial genetic testing of our hybrid trees to determine which ones carry sufficient blight resistant genes to use in reforestation efforts.

Your tax-deductible contributions to the Maryland Chapter are greatly appreciated. Contributions can be sent to:

Maryland Chapter of The American Chestnut Foundation

21900 Davis Mill Road

Germantown, MD 20876-4412



Save the dates – Upcoming Chapter Events

- ✓ Harvest Chestnut seeds – approximately last two weeks of September
- ✓ Move SSA Seedlings from Baltimore Greenhouse to Seed Orchards – First two weeks of October
- ✓ Annual Chapter Meeting – October 5th
- ✓ Planting SSA Seedlings at WSSC & CMREC seed orchards – approximately third week of October
- ✓ Annual meeting of The American Chestnut Foundation in Gettysburg – October 19th
- ✓ MD Chapter Board Meeting – November 16th
- ✓ Inoculation evaluations at WSSC & CMREC – mid November
- ✓ Tree culling work parties at WSSC & CMREC – early December

We are looking for enthusiastic candidates to run for Officer and Board Member positions in the Chapter.

If you have an interest in putting your name into consideration, either send an e-mail to the nomination committee at: bobpeggymac@gmail.com or nominate yourself at the annual Chapter meeting on October 5th.

If those positions don't interest you, we are in great need for orchard stewards at the following chestnut orchards. Contact Ron Kuipers at m_rkuipers@yahoo.com to get more info about these steward positions.

- Southern MD Orchard – Waldorf, MD
- Thorpewood Orchard – Thurmont, MD
- WSSC II – Sunshine, MD

There is a need for secondary orchard stewards to help the primary steward keep the orchard running at the following locations:

- Central MD Research & Education Center – Ellicott City, MD
- WSSC III Seed Orchard – Sunshine, MD
- Monocacy Orchard – Dickerson, MD
- MD State Highway Admin Orchard – Hampstead, MD