The West Virginia Chapter of The American Chestnut Foundation NEWSLETTER

In the heart of American chestnut's natural range

September 2021

WV-TACF Officers
President--Mark Double
Vice President--Dr. Don Kines
Treasurer--Sam Muncy
Secretary--Jeff Kochenderfer

Board of Directors
Dr. Joe Golden
Jimmy Jenkins
Jerry Legg
Dr. William MacDonald
Dr. Brian Perkins
Rick Sypolt
Robert Sypolt
Dr. Melissa Thomas-VanGundy

Find us on Facebook: @WVTACF
National Office: 50 N. Merrimon Street, Asheville, NC 28804
Phone: 828-281-0047
chestnut@acf.org

Newsletter Editor: Mark Double
mdouble122@gmail.com

Table of Contents

Storing Chestnuts After Collection

Now is the time of year for chestnut collection. Depending on the species, and other factors (elevation, temperature, rainfall, etc.), chestnuts will drop anywhere from mid-September to mid-October.

Once chestnuts are collected, they need to be prepared for storage over winter. Chestnuts need a period of cold storage, termed stratification. This is a process of treating seeds (chestnuts) to simulate natural conditions that the seeds must experience before germination can occur. When chestnuts are collected in the fall, wash them in a solution of water and household bleach. Add nine parts water to one part bleach. (The nuts that float are generally not viable as they are already colonized by fungi. Throw out the floating nuts). Allow the chestnuts to soak for 10 minutes, remove to paper towels and allow them to dry. Do not rinse them as the residual chlorox will aid in reducing fungal contamination.

The nuts then need to be added to a moist medium--they key is moist, not wet. A wet medium will promote fungal growth. The goal is just moist, so the medium is damp enough so the nuts do not dry out in storage over the winter. Once the nuts are cloroxed and dried, add them to 1-gallon plastic bags with the moist medium. Many media are suitable such as sand. Make sure the ratio is 9 parts of a dry medium to 1 part water. The mixture used at Meadowview is:

3 parts vermiculite
3 parts peat moss
3 parts perlite
1 part water

Mix well and add chestnuts to the mixture. Add a layer of medium followed by a layer of nuts and repeat until the bag is full. Poke holes in the plastic bag to allow for respiration. Store in your refrigerator crisper (not the freezer) and the nuts should begin to germinate in March.

An American chestnut with burs.

Daikon Radishes

An article on daikon radishes may be more suited for Master Gardeners, but these radishes are very applicable to chestnut plantings. Many soils in West Virginia have a high clay content, a difficult medium for chestnut roots to manage. This is where daikon radishes can help. Since chestnuts do not compete well with grasses and weeds, a common practice is to spray herbicide around the perimiter of chestnut seedlings and trees. It is this zone where daikon radishes can be planted. This radish produces a very large tuber as seen in the photo below.

Photo from New Hampshire National Public Radio.

The daikon radish can improve soil health, break up soil hardpans (due to high clay content) and control weeds. This radish is also known as the tillage radish. According to the University of Maryland's Steve Groff and Dr. Ray Weil, the "super carrot" taproot drills down 2'-4' into the soil, forming channels in the soil after they desiccate and decay over the winter. These channels reduce compaction which improved water filtration and surface drainage. The channels also allow the soil to warm up quicker in the spring. When the taproots hit the hardpan, fine roots are sent out to find a crack in the hardpan. Eventually, the roots crack open the hardpan. For tillage purposed daikon radishes are best planted in late summer, 40-60 days before the first frost. These radishes do not grow well in low nitrogen soil. They are heavy nitrogen feeders, so fertilize as needed.

I first heard of daikon radishes from Tom Saielli, TACF's Mid-Atlantic Regional Science Coordinator. Tom suggested I could find these radishes in a number of seed catalogs. I found daikons in one particular seed catalog for $9.00 per 1/4 pound. This came with a shipping cost of $9.00. With tax, the total was more than $21. I was ready to place the order when I called a local farm and feed store in Morgantown. They sold a 3 pound bag for $15, so it's worth checking locally before ordering from seed companies. The $5 per pound from a local dealer is much less than the $40 per pound from a seed catalog--not to mention the shipping costs.

I planted some daikons in my orchard. Maybe I will have some news to present in 2022.

USDA Multi-State Project on Chestnut

Back in the late 1970s, there was a great deal of interest in American chestnut due partly to a 1975 article in ***Science*** magazine from researchers at the Connecticut Agricultural Experiment Station. The article hinted that a return of American chestnut might be possible due to a finding by a French researcher who detailed a debilitated strain of the chestnut blight fungus that was able to control blight cankers on European chestnut trees. Discussions followed and eventually a multistate project began in 1982 with the first meeting held in Frankfort, Michigan. The mission of multistate projects is to enable research on high-priority topics among State Agricultural Experiments Stations (SAES). In this way, technological opportunities and complex problem-solving activities which are beyond the scope of one SAES can be approached in a more effiecient and comprehensive way.

Multistate projects are awarded on a 5-year basis. At the conclusion of each project, a termination report is written and a new report is written for the subsequent 5 years. The USDA project on chestnut is one of the longest running projects, having been renewed continuously since 1982.

An annual meeting is held each year and hosted by one of the project members. As a result, the meeting has been held in many states over the course of the last 39 years. Generally 30-40 members attend the meeting and many detail their research. There is an exchange of ideas and many institutions assist others by conducting complementary research and sharing materials. Due to the Covid-19 pandemic, the meeting was held virutally in 2020 and 2021. This year's all-day meeting was held virtually on 10 September, hosted by TACF's Tom Saielli.

The following is a brief summary of some of the presentations.

Soum Kundu, Ph.D. student, Mississippi State University. Soum talked about the role of the fungal Arv1 protein in sterol metabolism and pathogenicity of the chestnut blight fungus. Sterols are a major

component of eukaryotic cells. They are necessary for fluidity, permeability and protein function. Unlike mammals that have cholesterol as a preferred membrane sterol, fungi synthesize ergosterol. Soum stated that about 75% of sterol content in filamentous fungi consists of ergosterol. This important fungal product is synthesized in the endoplasmic reticulum and transported to the plasma membrane. Soum showed data that a standard virulent isolate of *Cryphonectria parasitica* has a high content of ergosterol, while a debilitated hypovirulent fungal isolate has a greatly reduced amount. Soum is conducting transmission electron microscopy to check the integrity of the endoplasmic reticulum in both virulent and hypovirulent isolates.

Sara Fitsimmons, TACF's Director of Restoration. Sara talked about the need for germplasm conservation orchards and the need for finding new sources of American chesntut throughout the range. Additional lines of American chestnut are needed to break the genetic bottlenecks. She reported that planes and drones are being used to detect flowering American chestnut in remote areas of the eastern U.S. It was detection via an airplane that led to the discovery of the largest Americn chestnut in the State of Maine.

In terms of the genetically engineered tree (Darling 58), she is working with a company to develop an easy and inexpensive test to determine if nuts/trees contain the oxalic oxidase (OXO) gene.

Phytophthora (ink disease) screening is being conducted at the regional screening center at the Austin Flint Orchard in Georgia. About 3,400 seedlinng were screened for Phytophthora resistance at the nursery in 2020.

Another project of Sara's is the determination of the long-term phenotype of backcross trees. What do backcross trees look like at 15-20 years? Is the main stem living or dead; are there cankers and if so, do they girdle the stem. She is looking at a number of traits to ascertain the health of backcross trees. So far, she has assessed 1,735 trees. The goal is to cross the **Best X Best**. The progeny of these crosses will be planted in backcross conservation orchards (BCOs). She has 150-200 American chestnut lines in BCOs and some have good resistance to the chestnut blight fungus and also good growth habit.

As a whole, TACF has planted about 2,000 acres and about 500,000 trees. About 35% of the land is on private property.

Sara also mentioned dentataBase, a computer program that tracks both native and planted chestnut trees. There is a new module and it is easily updated and it can track small plantings. She has about 150 users that have entered 13,000 crosses and 370,000 total trees. The goal is to engage more people to use the database.

Chestnut Chats is a monthly on-line talk. The 1.5-hour sessions cover a wide variety of topics. TACF has had more than 2,100 TACF members and 1,050 non-members viewers attend. In addition, 14 there have been international viewers.

Sara closed by talking about the Lesesne State Forest in Virginia--the hopeful site of the 2022 meeting. This forest is fascinating and showcases more than 10,000 hybrid chestnut trees that were planted in the 1960/1970 era. Also planted in the 1970s were some seedlings from large, surviving American chestnuts collected by The American Chestnut Cooperator's Foundation (Gary and Lucille Griffin, Blacksburg, VA).

Laurel Rodgers, Shenandoah University. She provided an update on microbiomes and chestnut trees. A microbiome is a characteristic microbial community that occupies a well-defined habitat on a plant. The term refers not only to the microorganisms, but also their activity on the plant. Laurel stated that some microorganisms assist plants in maintaining plant health. She reported that there is a great amount of diversity of microorganisms on plants; there are 84 species of fungi on Norway spruce needles. Laurel is interested if some fungi can be used a biological control agents. Do they produce antimicrobial chemicals? Do these microorganisms increase as a result of a plant pathogen invasion? Can they outcompete harmful microorganisms? Do they reduce leaf damage and loss?

Laurel is trying to address if there are any differences between the microbiomes of American and Chinese chestnut. To answer that question, she is comparing American, Chinese and hybrids growing in two orchards in Virginia (Culpepper and Aldie). Laurel and her students collected bark tissue after surface sterilizing the bark. The bark pieces were placed in sterile water and fungal hyphae were removed as they grew out of the bark. The hyphae were cultured on nutrient agar on Petri plate and the DNA was extracted for fungal identification. She

found there is some overalap of microorganisms between tree species. She identified some fungi that have been reported as components of microbiomes on other plants: *Fimatariella rebenhorstii; Hypoxylon* sp.*; and Albifimbria* sp. Laurel stated that they are in the process of identifying unknown fungi, so they can have a complete microbiome comparison.

Monica Sakalidis, Michigan State Universtiy. Her report was on brown rot of chestnut fruit, caused by the fungus, *Gnomoniopsis smithogilvyi*. This fungus is an emerging disease in Michigan, causing cankers on trees and rot in the nuts. To date, the fungus has been found in Italy, Switzerland, France, India, Australia and New Zealand.

Top panel: Impact of brown rot on nut fruit (photo from M. Sakadikis); Bottom panel, *G. smithogilvyi* in culture. (photo from iForest [2013] DOI: 10.3832/ifor0952-006)

A survey conducted of Michigan chestnut orchards in 2006-2007 detected no brown rot. In 2016, there were some symptomatic trees. By 2017, 80% of orchards had brown rot. The fungus tends to rot the nut and eventually the entire nutmeat turns brown. The fungus overwinters in debris on the forest floor. Fungal spores are released in the spring during flower receptivity. Secondary infection occurs via rain splash. All *Castanea* species are susceptible to brown rot.

Monica checked the incidence of brown rot of three cultivars at the Clarksville Research Staton: 'Labor Day' (Korean X Japanese); 'Colossal' (European X Japanese); and 'Benton Harbor' (Chinese). She rated the trees on a 0-4 scale with 0=healthy and 4=infected. 'Benton Harbor' was about 1% infected followed by 'Labor Day' at 4%. The highest infected cultivar was 'Colossal' at 10%. She then sampled 1200 nuts and found 16 species of fungi from those nuts. *Siricoccus castanea* was the most commonly isolated fungus. Monica concluded that there may be more than one fungus responsible for rot in Michigan orchards.

Chestnut blight is still the #1 concern for orchardists in Michigan. The contribution of *G. smithogilvyi* is sitll unknown, so a survey of two chestnut orchards and four chestnut stands was undertaken. The following was data from 2021. Bark plugs were taken from trees and the data included the number of virulent and hypovirulent *Cryphonectria parasitica* isolates that were recovered, along with the number of *G. smithogilvyi* isolates.

Stand	Virulent	Hypovirulent	G. smithog.
NW Orchard	65	104	2
Central Orch.	34	20	5
Central Orch.	80	47	7
County Line	14	27	1
Frankfort	30	44	0
Leelanau	7	25	0
Total	230	267	15

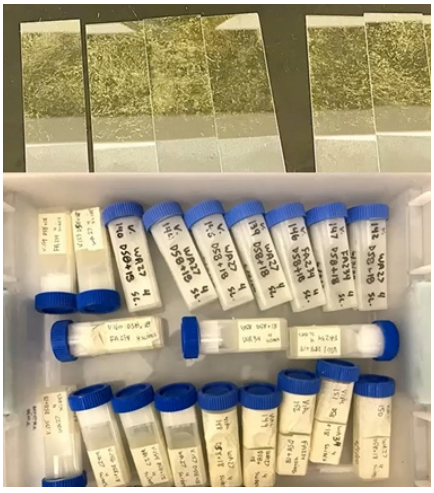
Note that County Line is a NW stand. Frankfort is a coastal stand and Leelanau is a western stand. One of the central orchards had the most virulent *C. parasitica* and the most *G. smithogilvyi* while Frankfort and Leelanau did not detect any *G. smithogilvyi.* Sampling of chestnut orchards and stands will continue.

Thomas Klak, University of New England. Tom and his students at UNE are working on producing pollen under high light from the State University of New York's transgenic trees. Tom reported having success producing pollen from 1.5-year-old transgenic chestnut seedlings. Once male catkins are initiated, pollen is collected on microscope slides. Tom puts 3-4 microscope slides containing pollen into plastic vials that are then stored at -80 C. The pollen-filled slides are then shared with cooperators who pollinate fertile female flowers in their transgenic orchards. There is sufficient pollen on each microscope slide to pollinate about 15 female flowers. To date, Tom has about 800 vials containing pollen-filled microscope slides.

Catkins produce from a young chestnut seedling under high light conditions (Photo from T. Klak).

Tom reported that not all of the transgenic seedlings produce pollen. In 2021, 51% of the pollen collected came from only 2 of 24 seedlings. Eleven seedlings produced some pollen while 13 seedlings failed to produce any pollen.

How fast can pollen be produced until high light conditions? After four months on May 1, catkins began to appear. By June 4, pollen was beginning to be produced and by July, there was sufficient pollen to be used in the field. In Cape Elizabeth, ME, southeast of Portland, ME, they are pollinating trees in germplasm conservation orchards that are only 4-years-old.



Microscope slides containing transgenic pollen that are stored at -80 C in plastic vials (Photo from T. Klak).

In 2020, transgenic pollen produced at UNE was used to pollinate 5,500 female chestnut flowers in transgenic orchards at the University of Vermont, Meadowview, VA, the Smithsonian orchard (northern VA), Purdue University, Maine, Allen Nichols' orchard in NY at at SUNY in Syracuse. There should be a much greater number of transgenic nuts produced in 2021 as there was more pollen produced this year. Note: transgenic orchards in the various states have been approved by USDA-APHIS and strict guidelines must be met.

Erik Carlson, Ph.D. student, State University of New York. Erik is working on transgene expression in American chestnut that is modulated by a pathogen-inducible promoter from poplar. In short, an inducible promoter is used to either switch ON or OFF the expression of a gene of interest. In this instance, Erik is using a promoter from a poplar tree in an attempt to increase the expression of the oxalate oxidase (OXO) gene in transgenic chestnut trees.

Erik noted that OXO is not a pesticide as the enzyme does not kill the fungus; it does not possess 'cidal' activity. The OXO gene changes the fungal lifestyle of *C. parasitica* from a pathogen to a saprophyte.

Agrobacterium tumifaciens is a bacterium that is commonly used for the production of transgenic plants in a wide variety of plant species. *Agrobacterium* has the ability to transfer DNA between itself and plant, and for this reason it has become an important tool for genetic engineering.

Erik noted that there are three types of promoters: (1) constitutive; (2) tissue-specific; and (3) inducible. Constutitive promote gene expression in all cell types at all times in high levels. As the name implies, tissue-specific promote expression in certain cell types such as leaves, roots, shoots, etc. An inducible promoter is turned off most of the time, but is turned on in response to certain conditions such as drought, herbivory, disease, etc.

The first promoter used in the SUNY transgenic trees was a vascular-specific promoter (Vsp) from soybean. This promoter was used to produce the Darling 4 transgenic tree. When used with OXO, it significantly increased tolerance to chestnut blight, but not to the level of Chinese chestnut. To increase the level of resistance, they used a stronger constitutive promoter from cauliflower mosaic virus. It is expressed in all cells at all times at high levels. This is the most common promoter used in plant genetic engineering because it gives a larger amount of gene product. This was the promoter used in Darling 58. In this instance, OXO was similar or even exceeded that of Chinese chestnut. In the photo below are trees using the Vsp promoter in Darling 4 (on the left) and the cauliflower mosaic virus promoter in Darling 58 (on the right). While the Darling 4 produces a large swollen canker, Darling 58 has a barely percivable canker. *(photos from Erik Carlson)*

The next step is the use of the wound-inducible promoter from poplar. The specific promoter is a protease inhibitor that inhibits cell damage by inhibiting compounds produced by insects and pathogens. In a variety of tests, this wound-inducible promoter (win3.12) was shown to have a strong reponse to wounding and infection by insects and plant pathogens. This was tested in inoculated chestnut seedlings whereby American chestnut showed wilt in 100% of the leaves, 'Qing', a Chinese chestnut cultivar, had wilt in 10% of its leaves and the transgenic chestnut with the win3.12 promoter did not have a single leaf with wilt symptoms. Trees with the win3.12 promoter have been outplanted in orchards.

Erik concluded his talk by stating that the transgenic chesntut trees with the

win3.12 promoter could potentially play a complementary role in restoration along side the Darling 58 line. Crossing transgenic lines with different promoters may reduce the risk of gene silencing due to a higher copy number. This research culminates decades of research in American chestnut and poplar.

Andy Newhouse, State University of New York (SUNY). Andy reported on the current regulatory status of the transgenic Darling 58 tree.

USDA-APHIS regulates movement, planting, etc. of transgenic plants and microorganisms. To accommodate the regulations of APHIS, SUNY has:

- enhanced diversity of their transgenic chestnut lines;
- conducted long-term field tests;
- garnered extensive public support and demand for its trees;
- garnered many positive comments during the USDA open-comment period; and,
- garnered qualified support from major environmental groups

The petition for nonregulated status to USDA-APHIS was accepted in January 2020 and is currently being reviewed by the agency. A recently posted **Notice of Intent** to write an **Environmental Impact Statement** was issued in August 2021. They expect ot have a decision on the degegulation of the transgenic tree by August 2023 (subject to change).

EPA--He will submit this month a registration and tolerance expemption document (the same process used to register pesticides). EPA encourgaged SUNY to submit a 25 (b) exemption form. The reason is that SUNY hopes that the transgenic tree is a long-term release while pesticide exemptions are more on a short-term basis. Thus, the 25 (b) exemption will allow EPA to take a closer look at the ramifications of transgenic release. They hope to submit in the Fall, 2021.

FDA--Andy hopes to submit the applicaiton to this agency in the Fall, 2021. The FDA regulates food and feed. They want information on nutrition, allergy issues, etc. to show that transgenic chesnuts are equivalant to non-transgenic nuts. The FDA process is much shorter than the others; this may take a year or so for a decision.

Canada (Environmental and Livestock Feed Assessments (CFIA) and Novel Food Assessment (Health Canada). SUNY has had preliminary discussions with these agencies and they hope to submit documents in 2022.

Next Steps for SUNY:

- combine OXO with other breeding and biocontrol strategies;
- included inducible expression of OXO using the win3.12 promoter from poplar;
- identify and incorporate genes from related chestnuts (some genes in American chestnut might be present, but they are not at sufficient levels to be effective and might be considered--this could benefit a decision by USDA-APHIS if foreign genes are not used);
- enhance resistance to *Phytophthora* root rot.

Steve Jeffers and Linus Schmitz, Clemson University. The team at Clemson University is collaborating with TACF and the US Forest Service to study Phytophthora root rot (PRR). They have evaluated the virulence of different populations of *Phytophthora cinnamomi* on hybrid chestnuts. They are using bating methods to detect *Phytophthora* species in soils where chestnuts are growing or might be planted. One of the soils tested came from WV chapter member, Sam Muncy, who sent samples from the Summit Bechtel Reserve. Jeffers found an isolate of *Phytophthora cryptogea*. This isolate has an unknown virulence on chestnut, so further studies are warranted. As a result of this finding, Sam has moved his germplasm conservation orchard sites.

In a study of 39 soil samples sent to Clemson, 33% were positive for *Phytophthora* species. The five samples from WV were all positive for *P. cryptogea*. Of the 39 soil samples examined, seven were from the Mark Twain National Forest in Missouri. Two of seven samples were positive for *Phytophthora*, making this the first reported cases of *Phytophthora* in Missouri.

In their field trials testing hybrid chesntuts, the Clemson group had been using the same *Phytophthora cinnamomi i*solates every year, but they became concerned that those isolates might be less effective over time. Thus, in 2019, they decided to use different, fresh isolates each year.

They are screening oomycete-specific fungicides for PRR. (Oomycetes, known as water molds, are a group of several hundred microorganisms that include some of the most devastating plant pathogens, like the Irish potato famine and sudden oak death). Steve feels that some fungicides could be used to control chesntut blight in GCOs or where valuable trees have been planted where *P. cinnamomi* is present. In a 2019 field trial, they had promisting results. Trial 2 in 2020 was not as consistent with

Trial 1, so a third trial is scheduled.

They are also designing experiments to determine when the roots of American chestnut seedlings become infected by the zoospores of *P. cinnamomi.* They are inoculating roots in aqueous solutions to try and determine the timeline of infection. So far, they have determined that 100% of the plants are infected by 12 hours post inoculation. They hope to repeat this study.

Hill Craddock, The University of Tennessee at Chattanooga. Hill has been doing backcross breeding for 25 years, in collaboration with TACF. They are at the point that they are producing F2s which are intercrosses from selected backcross hybrids. They are growing these F2s for seed orchards (large numbers of trees that are densely planted). They will be culling all but the best 2 or 3 trees out of 100 trees planted.

They are evaluating common genotypes in a common garden study using the F2s he has produced. This involves trees from Pennsylvania, Georgia, Tennessee, Maryland and Virginia that are all planted at each site. Each tree will be labeled for long-term studies. Eventually, there will be a DNA sequence for every tree.

They are planting GCOs, but they are having problems due to PRR. They are looking at populations of backcross hybrids as reservoirs for genetic diversity. They have a lot of American germplasm captured already in the GCOs. Some hybrids have adequate resistance since they flower and will make good candidates for advancing with the transgenic trees if deregulated.

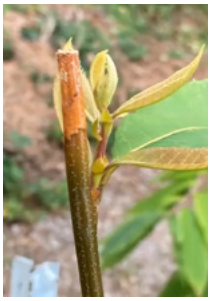
Hill also is conducting grafting propagation work. With the help of TACF staff, they are conducting long-term phenotyping of trees, combining phenotype data with genomic data.

They are still working with breeding and selection for PRR resistance.

In collaboration with Jared Westbrook of TACF, Hill is working on whole genome resequencing of *Castanea* recent collections from Alabama, Arkansas and Georgia.

Hill is also working on preparing herbarium vouchers that will complement genomic studies. The value of genetic information is enhanced if there are museum specimens. At some point, it is nice to know the genomic data, but it is also important to have the plants that you can look at morphology under a microscope.

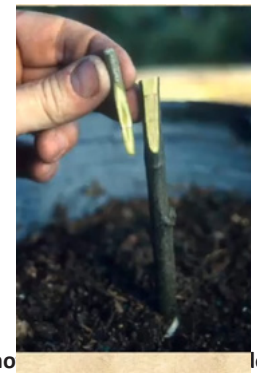
With student effort and colleagues from Berry College in Georgia, Hill conducted a workshop this summer where 700 seedlings were inoculated using the technique developed by Dr. Martin Cipollini at Berry College. In the photo below, the technique is to remove the end of a branch and inoculate with the chestnut blight fungus. The measurements that are taken are linear canker growth in one direction.



Cipollini stem inoculation technique (Photo from Hill Craddock).

The measurements that are taken are taken at 90-days and Hill believes this method will be able to separate the most resistant from the most susceptible trees. At the end of 90 days, they will cut off the infected end and sterilize the remaining tissue. The plant regrows virgorously beneath the cut, so the trees will continue to grow. Since this is a common garden study, trees from Pennsylvania to Georgia can be compared. These tress will be followed long-term in the field.

Conservation of southern chestnut germplasm is important. Discovery and mapping of chestnut trees near the southermost and westernmost fringes of the range is where genetic diversity is greatest. Hill propagates rare plants by seed or by grafting. We can keep these trees alive by making blight-resistant or PRR-resistant hybrids, because it is nearly impossible to grow pure American chestnut in the south due to *Phytophthora*. American chestnuts simply don't live long enough to flower. Hill can graft an American chestnut onto blight-resistant rootstock, as shown by the photo below.



Pho lock

Hill collects scionwood from wild trees and grafts onto resistant rootstock. He has been able to collect pollen from the grafted trees. Grafting is quite beneficial as some American chestnuts are in

real wilderness areas and difficult to access.

Hill continues conducting backcross breeding. The following photo is from Kate's Bluff in White Pine, TN where the hybrid trees are six-years-old and growing quite well.

Photo courtesy of Hill Craddock

These trees have not been challenged (inoculated with the chestnut blight fungus); that may happen in 2022.

Hill concluded his talk with a discusiosn about chinquapins, *the other chestnut t*hat occurs in North America. Chinquapins are extremely diverse in habit, tree form, leaf shape and size. He had a graduate student who conducted a literature search and discovered 23 different taxa of *Castanea* that had been named historically in North America. In 2019, Hill sampled chinquapins in northwest Arkansas and the Quachita National Forest, northwest of Hot Springs.

Chinquapin photo from Hill Craddock

In 2021, Hill sampled southern Arkansas. In the area between the Ozark National Forest and the Quachita National Forsest is the transition zone between *Castanea pumila* (Allegheny chinquapin) and *Castanea ozarkensis* (Ozark chinquapin). South of Chattanooga is an area where Alabama chinquapins (*Castanea alabamensis*) can be found, and they are distinct from the other chinquapin species. Hill noted that many of the Allegheny chinquapins has been decimated by chestnut blight in the southern U.S.

Taylor Perkins, University of Tennessee at Chattanooga. Taylor talked about the evolutionary genetics of chinquapin and American chestnut. Allegheny chinqaupin (*C. pumila*) is normally a very short plant (<1 m tall). This is in contrast to Ozark chinquapin (*C. ozarkensis*) that can grow up to 30' tall. Alabama chinquapin is endemic to northern Alabama and northwest Georgia. It is a single stem and a tall tree. *Castane*a species vary in leaf shape. *C. pumila* has wide, somewhat rounded leaves while *C. ozarkensis* leaves look much like American chestnut.

Allegheny chinquapin (pumila) Ozark chinquapin (ozarkensis)

Photo courtesy of Taylor Perkins

In the above photo, Allegheny chinquapin (left panel) is distinctly different from Ozark chinquapin (right panel). What is not visible in the photo is both chinquapins species have heavy layers of leaf hairs on the leaf undersurface. American chesntut has no leaf hairs on the leaf undersurface. Taylor wanted to know if hybridization between American chestnut and *C. pumila* and *C. dentata* led to *C. alabamensis,* as *C. alabamensis* displays signs of an intermediary between the two. *C. alabamenis* was described first in 1925 and thought to have been driven into extinction by chestnut blight. This tree has leaves similar to American chestnut but it only produces one nut/bur, as do chinquapins. Taylor did phylogenetic testing and principle component analsysis and *C. alabamensis* appears to be a separate clade.

Waddell Orchard

by Robert Sypolt

An inventory in mid-September found 90 trees. My big surprise was I found two trees that died in the last week and a few more that are looking poorly. One of the trees showing yellowing leaves is the Cathedral State Park tree from which bloom was taken for the Randolph County state record chestnut tree. It is very disappointing because those chesntut trees have native American genes that are beneficial in the backcross program. I cut off one branch with chestnut blight from the Cathedral tree; some branches did have burs. We lost a couple other trees with chestnut blight along with a few earlier this summer. As of now, we have 17 trees with burs, compared to 11 trees last year that produced 235 nuts. Two trees from the Clements nursery have a few burs. Almost all the trees have some burs. Some of the trees that are dying have chestnut blight while others have succumed to *Phytophthora* root rot. It's time to plant more trees!

At the park across from the Szilagyi Center, in Rowlesburg, potted chestnut trees will be sold at the Chestnut Festival, Sunday, October 10, 2021.