The West Virginia Chapter
of
The American Chestnut Foundation

NEWSLETTER

In the heart of American chestnut's natural range

August 2020

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North-Central Drought

The summer of 2020 in north-central WV was extremely dry. Most of the rain seemed to miss the northern portion of the state and, as a result, there has been a moderate drought. Chestnut seedlings planted in the spring of the year are very susceptible to summer drought as they had not developed sufficient root systems to combat a lack of water. One of the many backcross plantings in WV is located at the University Forest in Bruceton Mills (Preston County). The chestnut planting is near Coopers Rock for those familiar with the state park. The planting has no water outlet nearby, so water has to be transported in 5-gallon jugs, making it difficult to water nearly 300 trees. With the aid of Amy Metheny from West Virginia University and Rob Eckenrode of AllStar Ecology in Fairmont, WV who used AllStar's truck with a 300-gallon tank, the trees were watered thoroughly.

Rob Eckenrode waters chestnut trees at the University Forest.

Rob and the 300-gallon tank, courtesy of AllStar Ecolong in Fairmont, WV.

Chestnut Festival

The Chestnut Festival is generally held each October in Rowlesburg, but the festival was canceled in 2020 due to the coronavirus pandemic. The chestnut festival is generally one of the largest fundraisers for the town of Rowlesburg, bringing in around $7,000 each year. With the cancelation of most fairs and festivals in WV, Governor Jim Justice used the Governor's Contingency Fund to offset the loss of revenue for many towns in WV. Governor Justice said, "Fairs and festivals are the fabric of our state, they're our identity in so many ways". The Governor allocated $1.347M to help many localities. The Governor said that this money won't cover all the losses, but it may bridge them over. The Rowlesburg Chestnut festival was given $684 from the contingency fund. The Rowlesburg Labor Day Festival was also given $684, making the total to Rowlesburg of $1368.

Bolgiano Property

A number of years ago, about 40 acres of property in Randolph County (northwest of Valley Head) was donated to TACF by Chris Bolgiano with the intent of planting chestnuts on the property. To date, no chestnuts have been planted as the property is very remote and as absentee landowners, there is no one to oversee the property. WV-TACF board member Jimmy Jenkins surveyed the property and determined the value to the timber to be approximately $40,000. Some of the terrain of the property is very steep and, if any logging is conducted, it will be challenging. TACF Vice President, Betsy Gamber, requested information regarding the property, as a Randolph County

hunt club was interested in leasing the property for hunting. Betsy's reply is as follows:

We have decided that we do not want to lease it out for hunting. Since we are not familiar with the legalities of hunting rights and do not want to expose ourselves to any potential liability issues, we feel this decision makes sense for TACF. In terms of the property itself, we are considering putting it up for sale, but have not made a firm decision at this time. I am currently reviewing all TACF assets and we plan to make a decision soon.

National TACF News

Most of the information in the WV chapter newsletter is related to state issues. The national board of TACF met on Zoom on 17 Aug 2020 and below are some snippets of information from the national organization.

Treasury. Despite the downturn in the financial market as a result of the Covid-19 pandemic, accounts are nearly back to pre-pandemic levels. The Merrill Lynch fund, part of the Will Group (Dick Will from WV), has done an outstanding job of managing TACF's funds. No more than 5% of the account are in anyone holding, so the portfolio is well balanced. The PPP (payroll protection plan) loan from the federal government will be forgiven in its entirety.

President's report. Lisa Thomson reported that TACF's staff has been fully employed during the pandemic, even though the national office in Asheville, NC has been closed.

The regional science coordinator (RSC) for the southern region, Ben Jarrett, resigned and that position is still open. TACF hopes to advertise the position beginning the first of October. In the meantime, our RSC, Tom Saielli, has been doing double duty, covering both the mid-Atlantic and the southern regions. There was worry at the national office that with the downturn in the nation's economy, TACF's membership would decline. The exact opposite has occurred and TACF has exceeded 5,000 members nation-wide, the highest number ever. Lisa recognized two staff members, **Judy Antaramian** and **Shanah Zimnoch** for their attention to membership. Some states such as NY and PA have more than 800 members each. The WV chapter currently has 150 members.

Chestnut Chats (on-line conversation) that occur on most Fridays have been a huge success. More than 100 individuals participate each week with more than 250 for the Chestnut Chat conducted by Dr. William Powell of the State University of New York (SUNY), who spoke on the transgenic tree. Lisa also spoke about **Rex Mann** from the Kentucky chapter. Rex was the keynote speaker at the 2019 chestnut festival in Rowlesburg. Rex is a master storyteller who enthralls any audience with his colorful stories.

Rex had a severe stroke earlier this year. As part of his therapy, Rex and his son, Scott, have reached out to the Eastern Band of the Cherokee Nation, relative to planting backcross chestnut trees on tribal lands. Rex and Scott met recently with elders from the Cherokee Nation and the tribal leaders were very enthusiastic about participating with TACF. Lisa commented that it is important to have good communication with indigenous people, as they were the first landowners.

Science. Dr. Jared Westbrook,

Director of Science, stated that molecular mapping has been conducted on 100 of the best backcross trees. Of special interest are trees with intermediate resistance to the chestnut blight fungus. Data is being collected on architectural traits (apical dominance, branch diameter, etc) to see if tall, American-type trees correlate with resistance levels. Transgenic trees (oxalate oxidase) trees, produced at SUNY are being tested at five locations, where permits were obtained (Vermont, Maine, New York, Virginia and Indiana). The goal is to screen trees for both resistance to chestnut blight and *Phytophthora* (ink disease). For germplasm conservation and the

assessment of genome mapping of wild-type trees across the native range, 500 samples were collected, some from WV. To date, genotyping of 190 of the 500 trees has been completed. **Hudson Alpha Institute for Biotechnology** in Birmingham, AL has partnered with TACF to conduct computational biology, also known as bioinformatics. This is a field that involves the use of computational technology to gather, store, analyze and integrate biological and genetic information. Hudson Alpha is working the genomes of both Chinese and America chestnut and they hope to develop maps that delineate the location of the resistance genes.

Details About the Chestnut Blight Fungus

Most members of The American Chestnut Foundation know that the loss of American chestnut from forests of eastern North American was caused by a fungus. The Latin binomial of the fungus is *Cryphonectria parasitica* (pronounced: cry-pho-neck-tree-a pear-a-sit-ick-a). It is believed that the fungus was imported on root stock of Japanese chestnut that arrived in the port of New York City at the turn of the 20th Century. At that time, there were many nursery and seed catalogues available that offered plants and seeds from across the world. It was well-known that Chinese and Japanese chestnuts produced larger nuts than their North American cousin and, as the adage goes, the bigger the better. Thus, it is not surprising that Americans wanted larger chestnuts and that meant ordering root stock and/or seeds from Asia.

H.W. Merkel was a forester at the New York Zoological Park in the Bronx, and he is credited with recognizing the seriousness of the disease which was first known as chestnut bark disease. From reports in New York, it appeared that the disease was present on Long Island as early as 1893 (Metcalf and Collins, 1911). Merkel noticed leaves on several branches of mature American chestnuts were brown in the middle of the summer of 1904. This browning of leaves is known as "flagging". According to Metcalf and Collins (1909), by 1905, the disease was present in Merrimack County, NH, Warren County, NY and as far south as Albermarle, VA. By 1911, it was estimated that the loss of timber by the chestnut blight fungus was conservatively $25M. It was surmised that the loss was insignificant compared to the loss if the disease attacked the fine chestnut timber of the southern Appalachians.

How did the fungus, growing on Asian chestnut rootstock infect American chestnuts so quickly? The fungus spreads pre-dominantly through spores that enter wounds on a chestnut tree. The fungus found a tree that had no resistance, and over 50 years killed 4 billion trees in eastern North America. The spores germinate (much like seeds) and the spores produce filaments that grow in and under the bark. The filaments grown collectively as "fans", as seen in the photo below in Figure 1.

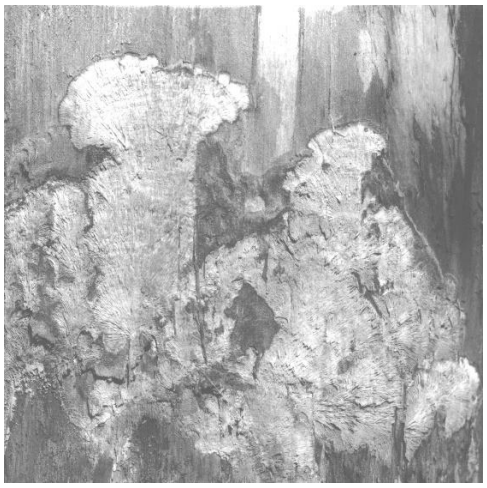


Figure 1. Fans of the chestnut blight fungus that grow in the vascular cambium of an American chestnut tree. The fans of the fungus exude organic acids, most notably oxalic acid.

The fungus produces many organic acids, namely oxalic acid, and the acid kills the cambium tissue. Once the fungus grows around a branch or stem, everything distal to the canker is killed. Thus, the flagging on a branch is indicative of the fungus girdling the branch, thereby starving leaves of water resulting in browning.

How do spores of the chestnut blight fungus travel? The spores can travel in a multitude of ways—blown around by wind, carried by rainwater splash, moved around by birds, insects, mammals, invertebrates and humans. Once a spore lands on a chestnut tree, conditions must be right before the spore can germinate. If there is not enough moisture, too cold or hot, or rendered ineffective due to UV light from the sun, the spore will die. Each canker on an American chestnut tree can produce millions of spores. Thus, infection by chestnut blight spores are the exception, not the rule. While the

fungus was effective at killing American chestnut trees throughout the trees' native range, only a very small percentage of spores actually produce cankers.

The chestnut blight fungus produces two types of spores: sexual and asexual. Both spore types are produced in the orange-red bumps seen on cankers, known as stoma (strō-mă). The asexual spores, produced in a pycnidium (pick-nid-e-um), are produced by mitosis and, therefore are all identical genetically. When pycnidia become wet, they swell and the asexual spores (conidia) can be exuded on long tendrils called cyrrhi (sear-eye). Asexual spores are spread predominately by rain splash and are commonly referred to localized spores. Sexual spores on the other hand are produced by meiosis and they differ genetically. Sexual spores (ascospores) are produced in a pear-shaped structure called a perithecium (pear-ĭ-thee-see-um). Inside the perithecium are sacs called asci (ass-sigh) and each ascus contains 8 two-celled ascospores. Each perithecium has a cream-colored body and a thin black neck. When you look closely at the stroma on a canker (maybe with a magnifying lens), you will notice black dots. The dots correspond to the top of the black necks. Both sexual and asexual spores are produced within the stroma on a canker. The spores are microscopic, as they are only a few micrometers long. A cartoon of a pycnidium and perithecia are shown in Figure 2.

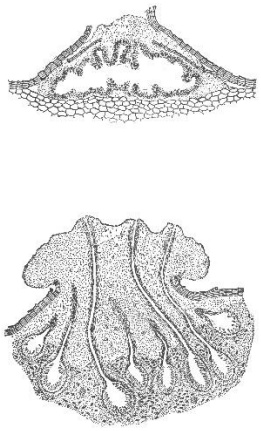


Figure 2. A cartoon of the fruiting bodies of the fungus. A pycnidium is depicted in the top panel where one-celled asexual spores are produced. The bottom panel depicts sexual fruiting bodies, perithecia. The long necks protrude to the top of the structure and the sexual two-celled ascospores are shot out in packets and picked up by the wind.

A close-up picture of the fruiting bodies (stroma) is seen below in Figure 3.

Figure 3. Close-up of stroma of the chestnut blight fungus. Notice the dark dots. Each stroma has 3-12 dark dots corresponding to the necks of sexual fruiting bodies (perithecia) that are below the surface.

Pycnidia and perithecia will be discussed in more detail. The asexual one-celled spores in pycnidia (**conidia**) can often be extruded in orange tendrils formed when the humidity is high (Figure 4). There are tens of thousands of conidia produced in the sticky tendrils and the spores are often washed down the tree during rain events. The sticky spores also are easily transported by insects that crawl across a canker. Insects carry the spores on their bodies and can be piggybacked to other trees.

Figure 4. Sticky asexual spore tendrils (cyrrhi) that exude one-celled conidia.

Sexual spores (**ascospores**)are produced in cream-colored, flask-shaped structures, perithecia. In each perithecium, packets of 8 two-celled ascospores are produced in a thin membrane called an ascus. Thousands of asci are produced in each perithecium and when conditions are favorable in the later summer/early fall, the body of the perithecium can pump out the asci via the black neck. The spore packets are often picked up by the wind and transported overland. As a result, ascospores are referred to as long-distance spores.

Figure 5 shows a picture of several perithecia that have been teased from the canker. By wetting a small piece of bark, perithecia can be teased out of the stroma by holding the bark with tweezer while a needle is used to probe under the fruiting bodies and remove them for examination under examination with a stereomicroscope. Notice the clump of perithecia on the left

that have a lot of orange material at the top. This offers a good representation of how far under the bark the perithecia are formed.

Figure 5. Sexual fruiting bodies (perithecia) that have been teased from the stroma on a canker. The cream-colored base is where the packets of two-celled ascospores are produced. The long black necks protrude to the surface of the stroma. Spores are expelled through the black necks into the wind.

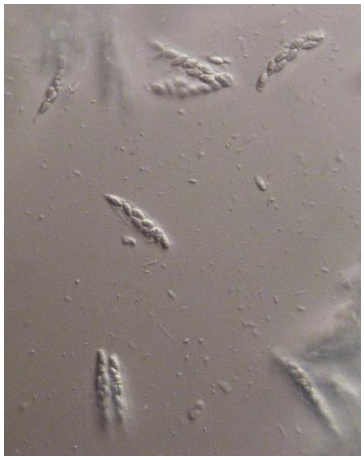


Figure 6. A microscopic picture of packets of 8 two-celled ascospores in asci.

The two cells types differ in size. The two-celled sexual spores are much larger than the one-celled asexual spores as seen in Figure 7.

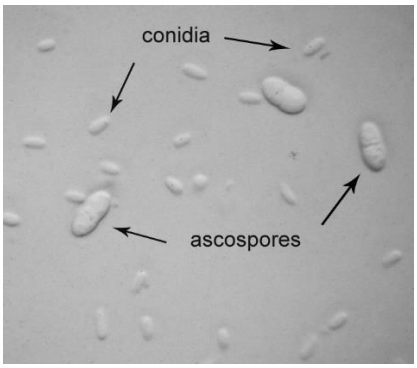


Figure 7. Microscopic picture showing the size difference between asexual spores (conidia) and sexual spores (ascospores).

Regardless if either a sexual or asexual spore land on a susceptible American chestnut, either can germinate and produce a canker. Most American chestnut trees harbor multiple cankers and, in concert, the cankers girdle the stem, thereby killing the tree.

Literature Cited:

Metcalf, Haven and Collins, J. Franklin. 1909. The Present Status of the Chestnut Bark Disease. USDA Bureau of Plant Industry, Bulletin No. 141, Part V.

Metcalf, Haven and Collins, J. Franklin. 1911. The Control of the Chestnut Bark Disease. USDA Farmers' Bulletin 467.

Open Comment Period by USDA on Transgenic Tree

If you have been a member of TACF for any length of time, you are aware of the genetically-engineered American chestnut produced by scientists at the State University of New York (SUNY). For those unaware, a group of scientists led by **Dr. William Powell,** post-doctoral and graduate students have worked for decades to insert a gene from a wheat plant into the DNA of an American chestnut.

Dr. William Powell, SUNY team leader

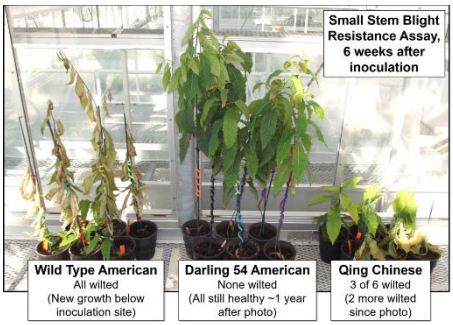
TACF supports this work as biotechnology is one of the three prongs needed to restore American chestnut into our eastern North American forests (breeding, biotechnology and biocontrol). It has taken many years to produce a tree that performs equal to or better than Chinese chestnut when inoculated with the chestnut blight fungus.

Dr. Powell and his group have tested more than 30 genes from different plant species that could potentially enhance blight resistance. To date, a gene from bread wheat has proven most effective at protecting the tree from the fungus-caused blight. This wheat gene produces an enzyme called **oxalate oxidase** (OxO), which detoxifies the oxalate that the fungus uses to form deadly cankers on the stems. The gene breaks down oxalic acid into hydrogen peroxide and carbon dioxide. The effectiveness of the gene can be seen in the photos below.

Two chestnut stems were inoculated with the chestnut blight fungus. The stem on the left is transgenic, produced by SUNY. The tree has produced significant callus tissue, warding off the fungus. This stem will survive. Inoculation of the American chestnut stem on the right produced a large, killing canker.

In an inoculation study comparing Chinese, American and transgenic trees, researchers at SUNY were able to show that the transgenic

tree performs better than Chinese when inoculated with the fungus.



Wild-type American (left), transgenic (middle) and Chinese chestnut (right) six weeks post inoculation with the chestnut blight fungus. The transgenic trees show less symptoms than the resistant Chinese chestnut seedlings.

The oxalate oxidase defense enzyme is found in all grain crops (i.e. wheat) as well as in bananas, strawberries, peanuts and other familiar foods consumed daily by billions of humans and animals, and it's unrelated to gluten proteins.

The blight resistant American chestnut trees have undergone extensive field trials and ecological studies to ensure there are no non-target effects or harmful changes to the environment. With the help of several collaborators, they have evaluated potential impacts of transgenic chestnut on soil fungi, aquatic insects, terrestrial insects, other wildlife, persistence in the environment, tree growth rates, pollen flow, and surrounding plant communities. Even more ecological studies are planned. These tests are being completed to prepare for Federal regulatory review by the USDA, EPA, and FDA. So far, all the data collected from these ecological studies indicates that transgenic chestnuts do not have any harmful effect on the environment, and that they are functionally no different than trees produced by traditional breeding.

A successful restoration program will need all the tools in the toolbox. Biotechnology is an important tool, and we should support the tireless efforts of the SUNY group. **How can you help?** For those members who have an internet connection, go to: https://www.federalregister.gov/documents/2020/08/19/2020-18135/state-university-of-new-york-college-of-environmental-science-and-forestry-petition-for#open-comment For those without internet service, you can submit written comments to:

Docket No. APHIS-2020-0030, Regulatory Analysis and Development

PPD, APHIS

Station 3A-03.8

4700 River Road Unit 118

Riverdale, MD 20737-1238

The 60-day public comment period is now open and will remain open until **Monday, October 19, 2020**.

Now is your opportunity to support TACF and help restore the former King of the Forest. You don't need to have data or know scientific terms. You simply need to express your opinion in layman's terms. Write your thoughts on the importance if this tree—what do recall about this iconic tree or your hopes for what our future forests might look like with restored American chestnut as a component. Include your name and address with your comments.

Please do your part and respond to this open comment period. Each and every comment counts.

WV-TACF Virtual Fall Chapter Meeting

The WV-TACF has held its fall general meeting in conjunction with the Rowlesburg Chestnut Festival. Since that festival was cancelled due to the coronavirus pandemic, an on-line meeting will be held at 10:00 am Saturday, October 10. **Cherin Marmon-Saxe**, TACF's office and business systems manager at the Asheville office will assist with the meeting. More information will be emailed in advance of the meeting.

WV-TACF Officers

(Elected in October 2019)

President—Mark Double
Vice President—Dr. Don Kines
Treasurer—Sam Muncy
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