

**The American Chestnut Foundation  
Spring Board and Committee Meetings  
Southwest Virginia Higher Education Center, Abingdon, VA  
March 23-24, 2018**

**Science and Technology Committee Meeting  
March 23, 2018; 8:00 – 9:30am**

**In Attendance:**

**Committee Members** - Dr. Brian McCarthy (Chair), David Morris, Dr. Hill Craddock, Dr. Greg Miller, Brad Stanback, Dr. Carolyn Keiffer, Dr. Jared Westbrook (staff liaison), Dr. John French, Dr. Kim Steiner, Allen Nichols, Dr. Bill Powell

**Visitors** - Barb Tormoehlen, William J. Cude, Michael Doochin, Yvonne Federowicz, Cartter Patten, John Wenderoth, Larry Yozwiak, Bruce Wakeland, Robert Sypolt, Elizabeth Grabaw, Stephen Barilovits, Yurij Biuhn, Doug Gillis, Lois Breault-Melican, Denis Melican, Glenn Kotnik, Paul Sisco, Cathy Mayes, Ricky Caldwell, Rex Mann, Dr. Joe James,

**Staff** - Kendra Collins (notes), Lisa Thomson, Ben Jarrett, Jim Tolton, Dan McKinnon, Eric Jenkins, David Kaufman-Moore, Betsy Gamber, Tom Saielli, Michael French, Samantha Bowers, Sara Fitzsimmons

**Review of Minutes, Fall 2017**

Please add committee member John French, via conference call, to the meeting roster. With that addition, the minutes were approved.

**Roundtable Introductions - McCarthy**

All members, visitors, and staff briefly introduced themselves. Chair McCarthy commented on the value of developing clarity and unity in our messaging, pulled as a key point from the storytelling workshop with Scott Mann many participated in the previous day.

**Science Report and Breeding Program – Westbrook**

Dr. Jared Westbrook's PowerPoint presentation is available as a PDF, amended to these minutes.

A tale of two genomes – mapping the Chinese and American chestnut genomes. Dr. John Carlson (Penn State) and Dr. Meg Statton (U. Tennessee) are working on a Chinese chestnut reference genome, and are very close to completion after 10 years of work. Dr. Jeremy Schmutz at Hudson Alpha is working on an American chestnut reference genome, using newer, cheaper, and much faster technology. His work is also nearing completion.

Dr. Westbrook is working with Dr. Jason Holliday at VA Tech on genotyping all B3F2 parents at Meadowview (26 of 29 Clapper lines, and 21 of 25 Graves lines). This allows for testing the accuracy of progeny testing vs. genomic selection. Genomic selection is proving as good, or better, than progeny testing for blight resistance. This lends confidence to using genomic selection as a viable tool for selecting for blight resistance. Phytophthora root rot (PRR) resistance is a much more heritable trait. In

this case, progeny testing is highly accurate, but genomic selection is still very good and can be used over progeny testing.

Genotyping will be done on the remaining trees at Meadowview, and we will finish progeny test planting this year, in order to get more data and make the prediction model more robust by 2020. Then we can use the model to determine the remaining trees to cull and finish the seed orchards by 2022.

In order to enhance blight resistance, two more generations of recurrent selection are planned beyond the B3F3. Simulation of resistance through the B3F6 generation shows high average level of resistance and low variability of the population when using enhanced selection methods. The plan is to take the most blight-resistant 1% at each generation. We do not plan to remove any American lines, in order to preserve American diversity. On a related note, Westbrook made several test crosses to Nanking at various generations to see how much resistance we may be losing with each backcross. Those trees are in the ground but are not large enough to inoculate yet. The new greenhouse at Meadowview will allow for small stem assay (SSA) comparison to plantings and inoculation in the field.

Transgenic x backcross progeny were created in partnership with SUNY-ESF. The seed will be planted in pots next week, and a SSA is planned to screen for initial levels of resistance.

Chapter seed orchards are moving forward nicely. We will continue updating our progress with chapter seed orchards on an annual basis, as we work towards completion of this goal.

AIM (Ancestry Informative Marker) genotyping of chapter backcross lines is underway in partnership with Dr. Jeanne Romero-Severson at Notre Dame. All chapters have sent in samples from their B3F2 selections. This analysis will be used to quantify effective pop size, determine the amount of American vs. Chinese ancestry in the breeding population, correct pedigree errors, etc.

In addition, Graves B3F2 families from the chapters will also be screened for PRR. Twigs sent in for AIM genotyping will also be genotyped with 25 SNPs to predict PRR and identify trees we want to look at further.

Miller asked how much Chinese in our trees can we tolerate? We have assumed the less Chinese the better, but that's just an assumption. Westbrook has data from lots of B2 populations to help answer that question. He needs the full Chinese genome to compare our trees with, which will be available soon and allow for that kind of analysis.

#### **TACF Science Review - Westbrook**

We will hold a science review of our program on Aug 21-22. The review committee consists of Steve Strauss (Oregon State), John Davis (U. Florida), Nick Wheeler (NSCU and Weyerhaeuser), Richard Snieszko (USFS, Dorena), and Sally Aitkin (UBC). Science staff will be involved, as will Drs. McCarthy and Steiner. Leading questions from the S&T Committee, or Board in general, would be welcome. Anyone with

questions to consider during the science review should send those to Westbrook, McCarthy and/or Steiner.

#### **Status Update of Transgenic Chestnut – Powell**

Dr. Bill Powell's PowerPoint presentation is available as a PDF, amended to these minutes.

Dr. Powell presented regulatory review process of 'Darling' American chestnut. Many experiments were needed as part of the process that would be standard for any crop. Because chestnut is a tree some additional parameters were addressed including mycorrhizae colonization of the roots, insect/pollinator feeding on pollen, and several aspects of the leaves – leaf litter decay, insect feeding (terrestrial and aquatic), germination of seed in leaf litter, feeding leaves to tadpoles. Tadpole feeding study showed all leaves, except beech, are not statistically different in their hazard levels (sample size 30 tadpoles/leaf type). In a bee feeding study they collected pollen from bees, added OxO enzyme so had a known quantity or none in the pollen, and found no difference between American and transgenic pollen, but more difference between bumblebee populations.

Dr. Powell reviewed the regulatory review framework with USDA, FDA, EPA. All three agencies don't have to regulate, but at least one will. USDA is on the list because of the promotor used, FDA because chestnut produces a food product, and EPA because the transgenic plant may be considered a pesticide. This pipeline has been used for lots of crops but never for a tree for restoration. Dr. Powell has been working on the process for 2.5 years.

Started with EPA and have been through 3 rounds with them already. Also recently wrote a position paper suggesting EPA doesn't actually need to regulate the trees, and had a good meeting with EPA, hoping to find out by end of April if this approach will be approved. The reasoning is that OxO does not act as a pesticide, rather it protects the tree from the acid the fungus produces, but doesn't actually kill the fungus.

A case study of hypothetical GE herbicide-resistant canola would be regulated by USDA and FDA, but EPA regulates the use of the herbicide itself, not the plant. This case study encourages the argument that the EPA does not need to be involved. This case study is one of several on the EPA website to help navigate the regulatory process. If EPA agrees they do not need to regulate, SUNY-ESF is done with that pathway, and if not they will continue working with the EPA process. EPA suggested there may be an exemption from registration worth pursuing as well.

Plan on submitting the USDA proposal in the next few weeks. It will be reviewed first for completeness, then for full review of content. USDA has no time limit on this, but general time frame is 12-18 months to get through their review process. FDA review is voluntary. They haven't started yet, but expect that to be a 6-12-month review.

Miller asked what will SUNY's "regulations" be for dispersal of transgenic chestnut. Powell suggested that after a few years ideally the tree could just be out there for anyone to use. FDA and USDA would

allow for that, but if EPA needs to be involved there may be more tracking of the trees needed. It was asked if merging backcross and transgenic trees would subject them to TACF's germplasm agreement? Something to ponder. Powell would likely put a trademark on the trees to certify resistance of 'Darling' trees. Another partner in this process is the USFS, and they are in support of this work.

Registration of transgenic chestnut is significant in the larger context and there is lots of interest in how this process plays out. If EPA doesn't regulate OxO, that might set precedent for other trees with OxO. Many projects using OxO might be revived if EPA doesn't regulate SUNY's tree. If EPA exemption is pursued, that might be very narrow and not as applicable to other species or projects. The only other biotech trees currently on the landscape are eucalyptus and pine in South America, and poplar in China, but nothing else in the US.

Once through the regulatory process, any breeding with this event is covered by this regulatory review. If you add new events they can be added with a shorter, perhaps 6-month, process to tack them on to your registration.

Elizabeth Grabaw, a visitor from VA Tech, shared her experience with trying to register OxO in peanut. In her experience EPA did need to be involved, but is hopeful SUNY's approach will work and open the door for others to follow suit. Dr. John French also shared some of his discussion with a high-ranking EPA official and former colleague. Based on those discussions, French was only about 50% confident that EPA would not need to regulate and suggested continuing to prepare assuming that EPA regulation will be needed.

#### **Chapters Report – Fitzsimmons**

Sara Fitzsimmons' PowerPoint presentation is available as a PDF, amended to these minutes.

Sara Fitzsimmons presented a few updates from the chapters on their breeding programs, with a focus on several key projects and initiatives.

TACF's on-line database, dentataBase, continues to advance in development and use. Most recently we have developed functionality to include SUNY-ESF's program and general asexual reproduction, and also improved the speed and efficiency of the system. We continue to load historic data and to date approximately 95% of Meadowview plantings have been loaded and we're working on getting the observations for those plantings entered. About 70% of chapter data has been entered as well. On the to-do list: complete historical data entry, refactoring, reporting functionality, support for grafts, and integration of TreeSnap app.

Reintroduction Trials have been installed in several chapters. Many have been installed in ME since 2015, starting a fire study in GA this year, and Genetics x Environment study is going in at NC State. At PSU trials were installed in 2005 and the trees are mostly dead from blight, but data will be collected and analyzed for reporting in the fall.

All chapters have been working to collect twigs from B3F2 selections for AIM analysis discussed in Westbrook's presentation.

In PA, looking at canker length vs spacing on 1' x 3' and 3' x 3' spacing. Canker lengths were significantly statistically smaller at the wider spacing.

Mid-Atlantic region looking at stump removal treatments for rogued orchards. Flame treatment, triclopyr (glyphosate = translocation so can't be used), or some combo of treatment.

PA seed orchard selections – 90% phenotypic selection, 10% remaining should use genomic selection.

Lots of interest and work going on across chapters with SSAs. Lots of "no takes" and weird canker types in many of the experiments last year, so we're still working on methods.

Several chapters are working on germplasm conservation already. All chapters are being asked to work on tracking down new American sources and planting GCOs in the next five years. Looking at using a combo of nuts, grafts and transplants to get new sources in the ground. TreeSnap app fits in well with this effort to help get more people involved with locating sources for new germplasm. UTC is pursuing a grafting project with 9 sites in AL and TN, and 19 new sources. ME Chapter is planting lots of GCOs as well. For transplants we're aiming for 10 individuals/location to represent that location. Working with Westbrook on planning a range-wide collection of American leaf samples to give us a better idea of genetic diversity and where we should target our sampling.

#### **Additional items for the good of the order**

The meeting ran over time and no additional items were discussed. Meeting adjourned at 9:40.