The American Chestnut Foundation
Spring Board and Committee Meetings
Southwest Virginia Higher Education Center

Science Committee Meeting 4/8/2022

Attendance: Deborah Delmer (Chair), Kendra Collins (Staff Liaison), Tom Saielli (recorder), John Scrivani, Rex Mann, Barb Tormoehlem, Betty Allison, Evan Fox, Kim Steiner, Bill Powell, Becky Carter, Brian McCarthy, Eric Evans, John French, John Hempel, Tom Klak, Bruce Levine, Greg Miller, Dana Nelson, Allen Nichols, Fred Paillet, Brad Stanback, Don Wilike, Mark Double, Doug Gillis, Andy Newhouse, Dennis Liu, Yvonne Federowicz, Susanna Kerio, Caitlin Conn, Kathy Patrick, Loren Hostetter, Jim Searing, Steve Rist, Jack Swatt, Linda McGuigan, Jared Westbrook, Lisa Thomson, Paul Wingenfield, Jammie Van Clief, Jules Smith, Shana Zimnoch, Lily Kingsolver, Vasiliy Lakoba, Dan McKinnon, Eric Jenkins, Jim Talton, Sara Fitzsimmons, Tamia Dame and Judy Antaramian

Debbie Delmer: Intro…We will have a tight agenda, so be succinct. Many Zoom attendees can post questions to chat.

First order, minutes approved.

Jared Westbrook on current TACF Science:

As breeding work continues and resistance improves, we are poised to work with mined land restorations. Mine operators want to assess carbon, need tens of thousands or more chestnuts planted.

QTLs have shown that Blight resistance is controlled by potentially hundreds of genes with very small effects. Population means indicate no variance in loci among Graves, Clappers, etc.

Current breeding program: *Best x Best* trees likely inherit good resistance. Jared's selection model incorporates genotyped trees ancestry. We have selected the best (blight 1) and next best (Blight 2), etc. hybrids that don't make the cut can be used to cross with Darling 58. Non-selected trees can be culled (or just don't use).

Alex Sandercock's work at VT has shown the diversity of chestnut germplasm is regional, so, chapters are now collaborating to diversify selections geographically into regional seed orchards.

Best x Best breeding program: as much as possible (based on chapter resources and flower development) diallel crosses, with geographic subpopulations will be made, maximize resistance and avoid in-breeding, Aim to get 100+ seeds, collect OP seeds from same trees to progeny test, assess gains.

Jared ran computer simulations to predict gains in blight resistance among Best x Best – jumps from near American level resistance to better than F1 resistance.

Time to retire old methods: no more collecting OP seeds from *all* BC trees and planting *en mass* into seed orchards at tight spacing.

History of hybridization of Castenea: over eons there has been significant hybridization among various Asian and European species of chestnut, and some mixing with North American species.

Chuck Cannon from Morten Arboretum wants to mix the different species. We want to look at this – how can we produce resistant trees that are resilient and adaptable – mixing trees with various backgrounds makes sense. A la, hybrid chestnuts

Transgenic chestnut: We see stressed OxO seedlings in various studies. Maybe "always on" is costly to seedlings Now experimenting with 'wound-induced OxO'.

Stacked resistance can produce an American-type chestnut with potentially greater blight resistance (from transgenic x hybrid sources of resistance). To test if hybrid genes add more resistance to OxO program test are currently underway: looking at wild tree x OxO, stacked resistance (OxO x hybrids) and controls in several replicated studies.

Hypovirulence: we now have a permit to use super donor strain under permit restrictions. We can't release super donor into wild, so it must be controlled. Involves crossing SD strain with wild-type strain in the lab and then deploy the WT strain now containing the hypovirus.

PRR resistance: combine hybrid PRR resistant crosses with OxO. Test seeds for OxO, select for PRR resistance, intercross.

Other ongoing work::

- understanding cell death in the presence of Oxalic acid.
- Proteins in Chinese chestnut bark inhibits growth of fungus.
- Understanding the process of lignification to block mycelial growth? (Maybe easier to just study the trees).
- Trying to understand all (or as many as possible) mechanisms of resistance, among different species of chestnut
- Gene discovery for blight and PRR resistance – looking for genes using "selective sweeps" = focusing on regions associated with resistance, may lead to ID of specific genes.

- Getting to large scale seed production: need D58 deregulation. Optimize pollen production (growth chambers, getting it figured out). Need to improve OxO testing techniques. Establish seed orchards, produce millions of seeds, propagate in nurseries.

Questions:

Kim asks: Regarding QTL analysis, if hundreds of genes involved, to what degree does our imprecise rating of blight resistance confound our ability to ID *any* genes let alone hundreds? How much error will be in that model?

A. No particular trait seems especially significant, so we go with overall correlations, then reconstruct the pedigrees of all chapter trees – gives us resistance of the tree and the parents, then we see more heritability.

Debbie wonders if the populations are too small to reveal enough detail related to observable resistance traits.

A. Jared thinks we have enough data to get good answers.

Tom Klak: regarding LSAs - if so resistant, why cross with hybrids?

A. There is some LSA resistance, but not enough for a population and crossing improves resistance, genetic diversity and regional diversity.

Bruce: what about SSAs vs genotyping? (which is better?)

A. Jared would prefer genomic selection vs SSAs because it provides long term understanding better than SSAs do

Dana: can you calculate the Chinese genetic component not associated with resistance?

A. It would be difficult to do because resistance genes are all over.

Vasily on – 2022 Meadowview spring report

High light pollen production – going well, producing some pollen

OxO vs OxO-stress experiments: At Meadowview and Purdue, there was significant stress on OxO seedlings (not observed on non-OxO seedlings). May be due to over-watering, but why only with OxO seedlings? Currently under study at various locations. Exp design: includes variations in watering routine & variations in growing media (peat vs pine bark)

Future needs at Meadowview: better high light lights, biocontainment equipment Also need to fix or replace old equipment

Other goings-on:

Vegetative propagation, including rooted cuttings, Etiolated shoot grafts (grow root stock in total dark and graft with scions); and the " Steiner slice" (slice seed in half, through embryo, and see if you get two clones)

Germplasm conservation continues, including LSAs

Super donor strain at Meadowview: Involves collection of wild-type C. Parasitica strains from orchard trees, infect with super donor, reinoculated trees with infected wild-type strain.

Drone-based phenotyping: Virginia Tech experimenting with remote sensing using drones, to assess trees resistance via thousands of points of data and maybe ID revealing traits, perhaps can indicate levels of resistance?

Questions?

Bruce Levine: High light growth – are we selecting trees for early pollen production?

A. Not sure, but maybe

Sara Fitzsimmons: 2022 Regional Goals & chapter network

Regional Programs:

TACF is a hub of people coming together. We have plantings up and down the chestnut range – breeding orchards, demos, etc.

We have many different types of partnerships: non-profit, government, corporate/private, education and park/forest: grand total 236 different partners big and small

Chapter involvement:

GCO's, Best x Best program, OxO program, reintroduction/restoration projects, also story telling & outreach to legislators, etc.

Projects:

Looking at natural regen sites: is there an effect of founder population's distance from parents? (how related are kids from parents & how far do they move?

The southern part of chestnut range is a hotbed of diversity, but chestnuts are far and few between in the south. Need to find and capture germplasm.

PRR resistance in south: ID hybrids with resistance to PRR.

GCOs are going in all over. Sure, they die from blight, but usually re-sprout. You also have a good chance of some getting big and may produce flowers.

LSAs can get very big and can have higher levels of resistance. Probably need to combine with hybrids.

NATGEO filming w/ Sara to see the Highbridge Park, Harlem chestnut planting. Intersection of restoring species with restoring land. 300 American chestnuts planted there – it was an awesome piece

Long-term phenotyping ongoing throughout the chapters. LT assessments provide much better measurement of resistance.

In MAR pre-screening w/ SSAs at new greenhouse, picking the best of the *best x best*.

We are doing outreach, making it on TV, engaging volunteers, students and engaged citizens.

PSU arboretum – we see kids, and the kids of kids of the Ort tree at the arboretum and see first hand how LSA resistance is heritable.

BxB and OxO crosses require controlled pollinations. Takes longer but needs to be controlled.

Beyond seed orchard production: OxO & BxB through 2030-2040, next level crosses, stacked resistance crosses, final selections, etc. by 2060? How many acres restored in that time? Maybe 50,000 acres?

Bill Powel: Update on D58 and wound-inducible response

DarWin is an event line: Same as D58, produces OxO at a low background level, increases with wound, even greater increase in presence of C. parasitica.

Test of regulatory system: USDA test may exempt completely, or exempt for 180 days

Breeding work with TACF – trying to get to T5 generation (currently working on T4 generation) goal is to get greater genetic *and* regional diversity.

Also stacking resistance and PRR resistance is ongoing.

SUNY also stacking trans genes: OxO w/Lacase-like gene may improve resistance (makes a better lignin barrier. And OxO RPH1

GNK event: ginko-like antimicrobial peptide – maybe PRR resistance?

GLP event – germin-like protein (CC and AC) similar to OxO, basically the OxO isn't totally new – there are other similar proteins already present.

New Technologies: CRISPR/Cas9: new lines express Cas9, targets specific genes and knocks them out. Helps us understand what happens when we knock out genes…up next: "knock ins".

Host mediated RNAi: basically, small RNAs that inhibit the expression of genes, get the host to make the RNAis that go into the pathogen and knock out genes in the fungus, decrease the expression of oxalic acid.

Other species: Chinquapins with OxO? American elm, transformations in elms

Distribution of D58 once deregulated: workshop 2023 (TWCF sponsor):: invite a small group to discuss scaling to hundreds of thousands/year?

Regulatory timeline:

EPA: two routes,

1) registration and tolerance exemption: regulatory review, address deficiencies, lots of steps, anticipated registration by spring 2023, could be restricted to X acreage (250k? 50K? Whatever, it should be big enough)
2) Or FIFRA 25: review rule-making (exemption from registration is not a typical part of biotech crop reviews) no timeline
3) Should know the path forward by this coming winter

FDA: Easier because EPA does most of the work, FDA reviews EPA outcome and decides if it is safe for food. In this case, OxO is from wheat and so must be labelled as a wheat product?

USDA: petition has been submitted (2 yrs. ago), also recently submitted POPRA and EIS documents, prepare decision in (maybe) 1-6 months, final decision August 2023

All subject to further extensions.

Questions?

Debbie: What is EIS?

A. Environmental Impact Statement – they must write it, accounts for a lot of the time it's going to take

Yvonne: is the DarWin deregulatory status tied to D58?

A. Yes, upon finishing D58, they will have all the info they need, so DarWin can easily be added.

Evan Fox: do we know who writes the PCPs and reviews the comments

A. Yes, we get to see who made what comment. TACF tracked all comments. USDA says they will read every comment and take them into consideration.

John Hempel: wonders about the WesternBlots?

A. a western blot is a gel that extracts all the protein from tissues, separated by size as they move through gel, and sorted into lines based on what the protein is and how big it is. In the SUNY work on germin-like proteins, we see the map of those proteins in a Western Blot vs OxO. We see similar bands between both, indicating similarities.

Tom Klak: likely biggest roadblock to deregulation?

A. Bill cannot see any roadblocks. We know everything about this gene, so hard to predict what a roadblock could look like.
A. Debbie sees budget and people/resources at USDA as a roadblock

Jared wonders about the PakBio data,

A. Yes, the insert was where it was expected, there was a small inversion next to the genes, that happens, has no open reading frame, so doesn't do anything. A benign transformation.

Bruce Levine: does OxO also carry the neomycin resistance gene?

A. Yes, MB2 gene is common in many things. It's in there, but it's everywhere.

Susanna Kerio, CAES: cell death in chestnuts

Ongoing chestnut research: nanoparticles and drought & somatic embryogenesis:

Experiments: looking at CuONPs (copper oxide nanoparticles, reduce drought stress in many species, can we detect these in chestnuts? Can they help reduce drought in chestnuts?

~200 seedlings, 10 genotypes, control, drought, nano & non-nano treatments

Effects of CuONPs likely due to genotype, some genotypes responded more positively to CuONPs than other genotypes

May influence blight susceptibility? CuONPs had lower mortality post inoculation, higher water potential (synergistic effect of canker prohibiting good water transport in seedling).

May also influence density of stomata on trees and increased ectomycorrhizal

Limitations: slow growth, seedling size, etc.

This research is important because of climate change considerations, worth further study.

Also…

Somatic embryogenesis in Chestnut

TACF external grant, collaboration w/ Merkle

Currently cell lines in culture (Nanking, Vanuxem) testing the impact of media

Cell death in chestnuts:

Programed cell death in normal development and in response to stimuli such as pathogens

Climate change may exacerbate PCD b/c of abiotic stress

PCD Has been studied in Chinese chestnut (Tima zhenzhu), in which fruiting buds on auxiliary branches die

More likely this phenomena is in more susceptible trees/hybrids

Q? is PCD different among Chinese vs American vs hybrids? Can we ID markers to assist in selections?

Experiment: inoculate seedlings, harvest tissue, analyze with spectrophotometer – or extract DNA, run gel

Detected as a laddering pattern in gel,

TUNEL assay, more specific but more expensive

Debby Delmer: Revising the strategic plan for science

(For details, please see the plan Debby shared with all committee members)

What is OUR plan?

A business plan vs a Science Plan:

- Vision and mission executive summary of science plan,
- the detailed science plan,
- a list of proposed future projects (out 3-5 years?)
- who leads? Estimated timeline? Estimated cost? Expected outcomes?

Questions for the group to consider:

- How much science do you want to know? Share interesting papers, start a mailing list?
- Does plan include clear role for volunteers?
- Are breeding plans clear?
- TACF role in Darling 58? Field testing, make crosses now or wait for deregulation?
- Conservation…uses of GCOs? Climate change?
- Is gene discovery science clear? Are you on board?

Suggested Path forward

- Review science plan, future projects, proposals
    - Science leadership meetings
    - Present to new science advisory council
    - Finalize strategic plan
- Coordinate with restoration Committee
- Integrate plans with overall strategic plan
- Submit for approval at fall meeting (or at least have a draft)

Thoughts/Suggestions?

Bruce Levine: all good, but a structural flaw in TACF, there is a scientific program that answers to a board of non-scientists. Bruce wonders if there is not enough oversite regulating the science program, we need an element of governance to assess and approve scientific proposals, so that there is transparency and oversight.

Jared: we also need to engage stakeholders on what we are doing and what they want. We need a flexible program. We also need to have a strategy to navigate all the various checks, financial implications, research opportunities, needs of members, etc. we can't get too bogged down, we need to be somewhat fluid.

Barb mentions that some of that is already there: we work on projects by detailing, creating budgets, seeking funds, providing reports to stakeholders and committees, but we can definitely benefit from a science oversight committee.

Debby would like to see a committee of qualified reviewers (getting together annually?) to review our plans.

Vasily: How do we see advisory committee being a sounding board for other committees involved.

Let's take the summer for all the groups to weigh in and collaborate

Kim, when he was science chair, same idea to advise the breeding program, science minded folks willing to help. They weren't members of the board and not necessarily invested in our work, just good scientists. Now we wonder about who helps us and brings connection, enthusiasm.

John Scrivani thinks the science review from 5 years ago was very helpful. He also thinks we need expert restoration folks helping.

Jay mentions we need goals, high level goals, but then we have mid-level goals, goals we work on over the next few years. All part of a big process

Sara: *vision and mission*…can be revised, more inspirational. Food for thought: we are trying to restore the "function" of the chestnut. Allows us a framework to make science decisions. Is our work replacing the former niche of American chestnut?

Tom Klak: why plan is no longer called 3BUR?

Because these things (breeding, biocontrol & biotech) are all aspects of science. They are important, but there is so much more science out there, even 3BUR is variable and encompassing of disciplines, so Debby just calls it *science* – and we are working with <u>lots</u> of science.

Originally it seemed like three very different technologies that need to be put into one toolbox, but we are not necessarily wedded to that, and now, all the science is interconnected.

To be strategic, we need to think about where we are going and how we get there, using all technology