

SCIENCE AND TECHNOLOGY COMMITTEE MEETING – [LINK TO ALL SEPTEMBER MEETING MATERIALS HERE](#)

Date: Wednesday, September 22, 2021

Time: 10:00 AM EST

Zoom Recorded Call

<b>S&amp;T COMMITTEES MEMBERS IN ATTENDANCE:</b>	Steve Barilovits, Chair, Dr. Jared Westbrook, Staff Liaison, Sara Fitzsimmons, Recorder, Dr. Gary Carver, Jay Cude, Nathan Cude, Mandy Cromwell, Dr. Deborah Delmer, John Dougherty, Dr. John French, Dr. John Hempel, Laurence Grossman, Dr. Thomas Klak, Bruce Levine, Dr. Gregory Miller, David Morris, Rose Marie Muzika, Dr. C. Dana Nelson, Allen Nichols, Dr. Jeanne Romero-Severson, Jim Searing, Brad Stanback, Ross Whetten, Don Willeke
<b>NOT IN ATTENDANCE:</b>	Tracey Coulter, Dr. Hill Craddock, Eric Evans, Patrick Flaherty, Hugh Irwin, Dr. Douglass Jacobs, Dr. Joe James, Dr. Carolyn Howes Keiffer, Dr. Brian McCarthy, Dr. William McDonald, Dr. Fredrick Paillet, Dr. Ronald Phillips, Vicki Pierson, Phillip Rutter, Dr. Kim Steiner

CALLED TO ORDER AT 10:00 AM

OPENING COMMENTS / APPROVAL OF PREVIOUS MINUTES

- Roll Call - via zoom
- Minutes from [04/08/2021 meeting](#) unanimously approved by committee
  - Bruce Levine - moved
  - John Hempel – second
- Additional S&T Meeting will be held on Wednesday, October 27, 2021 10:00 – 1:00 PM EST

HIGH LIGHT GROWTH OF OXO SEEDLINGS – THOMAS KLAK

- Field pollinations at University of New England
- Pollen production under highlights
  - 800 vials of pollen produced with highlight chamber with LED lights
  - Checking to see if pollen is viable with sugar agar incubation
  - Were able to tweak chamber enough to have tree produce chestnut pollen in one year
    - Look out for article in the fall Chestnut magazine!
    - Is a process their team is still tweaking?
  - Have found 0 drift in the OXO controlled pollinations.
- Testing the nuts to see if they are OXO positive
  - Test turns black with presence of Hydrogen peroxide (by-product of oxalic acid)
- 550 trees planted under USDA permit in Cape May ME orchard. Mix of OXO, F1s, B3F3s, AM and CH
  - Mice have been girdling some of the trees in the orchard
  - Some of the OXO plants were under the highlight producing pollen. Now planted out in Maine. Will be curious to see how it survives in the different climate

*Question*

- Jared “Is there more space” Yes.
- Jeanne “Is there a mix of trees from the range” Yes. “Great planting, but remember OXO is not the end all be all.”
- Sara “Can we get more data to prove that there is no OXO pollination drift.” Yes, we need to pool data from across the range.
- Bruce “How is pollen frozen? How long is it good for?” Pollen good for ~ a year at -80 C and dry.

**PUBLIC COMMENT PERIOD AND REGULATORY UPDATE- ANDY NEWHOUSE**

- Syracuse has another large mixed OXO and breeding program planted
- Open comment received many large name environmental groups support
- USDA: Received NOI in August, and recently completed last comment period. Should have results in Aug 2023 as stated in NOI.
- EPA: FIFRA registration and tolerance exemption. First part of registration submitted. Estimated completion 2023. Exemption is being requested 25b for larger scale restoration. No timeline as is relatively new.
- FDA: Draft application completed. Should be the shortest approval of the agencies. Estimated completion Fall 2022.
- Canada: Hoping to submit next year

*Next steps:* Looking at combining OXO strategies.

- Inducible expression of Oxo
- Looking at other gene in genome that target expression
- Enchaining Phytophthora resistance.

*Questions:*

- Rose Marie “Phytophthora strategies?” Mixing backcross program with Oxo
- Jim “Any conflicts with Canada?” We need to be talking with both, but one’s decision should not stop another.
- Nathan “Do we have to get state approval” Depends, but most states follow the EPA. NY may add more. We are talking about that
- Tom K + John French “Joe James farm has some great resistance and are excited about stacking the OXO that.” Talk about 25b another time

**RESMAP PROJECT: SMALL STEM VS FIELD ASSAYS FOR BLIGHT - BRUCE LEVINE**

- Our old backcross program assumed resistance was more simple than it is. However resistant genes are on more loci and throughout the genome. Therefore, we need to test more trees, possibly thousand (Jared is looking into what phenotypes are good indicators for resistance). Bruce and team are looking into prescreening with the RESMAP trials.
- Currently we have 16 families that were tested, SSA are being done in four locations this year. Lots of data to be collected and then all trees will be tested. 3-4 years from now they will be field inoculated to compare the methods.

**RESTORATION SCIENCE OUR SECOND SCIENCE. - JIM SEARING**

- Restoration is KEY to TACFs mission and our public relations. How do we switch from tree breeding and science to restoration and out planting?
- There are professional societies for restoration. International organizations that have been around since the 80s. Society Ecological Restoration (SER) a prime example and has set standards. One being not bringing back a past ecology, but helping improve the ability for native species to thrive. There are eight principles underpinning ecological restoration (see presentation)

- Another organization with a background with relevant restoration is from the Native Seed Supply. Which almost mimics TACFs seed plan. Which is great for the restoration effort and committee. Jim recommends we and ESF do more to align and define ourselves with these efforts and organizations.

#### Questions – clms

- Rose Marie Muzika: Prior restoration in prairies, refer best to those ecological systems where there were not trees that lived for hundreds of years
  - Balance of approaching our reference system, philosophically, creating novel ecosystems, balance the effort in terms of reference vs future conditions; important to factor climate change which will affect what and where we plant
- Debby: Important to get our minds thinking about the reality of restoration; A lot of people that come new to the game thinking the effort was simply planting a few seedlings in the forest where they were before;
  - Practice larger scale to gain data from prior efforts; Elevating what we are doing and recognizing eco-restoration as a science
- Jeanne Romero-Severson: There is extensive forestry literature out there, but not read. Close tie between why TACF exists and how we raise our money is Restoration. We must have a dynamic plan; do need to work on a plan. Good or bad we have a few years to make it; Having a solid plan also opens other funding sources.
- John Scrivani: We are not the only people thinking about restoring the eastern forest, USFS, TNC and many state and wild life agencies, many are interested in our efforts, who do we cooperate with and how do we work together with them; Our trees fit in well with the larger effort, and we will need partners to do it, we cannot do it all.; Lisa – just signed another version of MOU with NRCS, very important partnership. Jay/Lisa announcing/reminding everyone that John Scrivani is TACF's new restoration Chair.
- Dana Nelson: Bruce – Do you cut disease off of the assays before you put them in the field – how does to work from GH to field after inoculation; Jared – Yes and offers a picture
  - For Jim: 3 things come to mind with respect to ongoing research which would be relevant – case studies; modelling work introduced Northern Research Station, adding Darling 58 into this approach; Stacy Clark planting research at the Southern Research Station, ongoing plantings of backcross Ac's that have been planted and observing over time. Pull all of the work together to help TACF forward.
- Dennis Liu: Challenge could be getting too global and expansive; framework is really important. Dealing with a single species and its role in the forest, we are in a powerful position to show how these principles work and ask really good questions about the difference between a chestnut dominant forest vs other dominance? Fundamental contribution to ecological thinking and science as well as having social resonance. View of the Eastern US look like from the sky – verdant beautiful paradise, green that is for sure, but it is not. What does that really mean? [Braiding Sweetgrass - Robin Wall Kimmer](#) deep thread of thinking, scientist; indigenous background, interesting voice to keep in mind.
- Tom Klak: Experiment in ME, heavy forest extraction, working with sustainable harvest, no clearcuts, planting wild type seedlings and letting them go – no more TLC, growing nicely. We need wood, we need cardboard, we need to work with sustainable harvesting of wood products and opening up sunny spaces, rebalancing the forest and bringing in keystone species.; Great chapter knowledge to share.
- Debby Delmer: Overstory – clear cut planting of monocultures does not do as well as mixed population placement – do we have what combinations of trees work best mycorrhizal connections and roots interactions? Interesting project for a chapter or two to take on.

- Jay Cude: All restoration is local, great opportunity for chapter engagement; practical side – allot of hardwood forests in his part of the world have been cut as many as three time, by high grading – cutting out the good stuff and leaving the bad, evolution in reverse, foresters feel it is important to cut it all down to replant the trees that you want. Real applications for restoration strategies that includes the chestnut to be integrated into upgrading the degraded parts of the forest.

#### SCIENCE REPORT – JARED WESTBROOK - [Link to meeting video and presentation](#)

- Infographics – Vasiliy Lakoba, Director of Research has created to demonstrates new ideas and approaches
  - What does success look like in terms of R&D for our program
    - Disease resistance
    - Ecosystem Services
    - Genetic Diversity
  - Combine and Deploy
    - Darling 58 crosses showing the results and what the resulting characteristics are based on cross
    - Pollinations
      - Tom Klak, main supplier of pollen
      - In 8 states/ 12 orchard locations/ 50+ trees
      - Crossed with exceptional backcrossed blight resistance with Darling 58
        - Diversity
        - Disease Resistance
      - Doing 5 generations of outcrossing to wild trees
        - Minimize in-breeding – Single tree from NY
        - Dilute out founder tree and bring in more wild type tree genomes
        - Diversity from across AC range, adaptive capacity for climate change and for environments from North to South
        - Third Generation completed
      - High Light Growth Chamber
        - Built by Meadowview team
        - Lily Kingsolver, Nursery Manager working hard through the inception and through the kinks in development
        - 30 or so trees
        - First pollen from growth chamber has been produced – Chinese
        - Looks good for being able to supply pollen for the fourth generation
- Genotyping with Virginia Tech – Meadowview and have expanded out to the chapters
  - 3000+ - Meadowview
  - 1800+ - chapters
  - Gaining AC ancestry information
- Adaptive Diversity – Landscape Genomics Project – Virginia Tech – Alex Sandercock
  - How much of the wild population that we have captured are in the orchards?
    - Divide AC range into adaptive provenances
  - Sequenced 384 AC across the rang
    - 23,000,000+ snips
    - Genome environment association analysis
    - Next to do genome sequencing on backcross trees and compare
    - Working smarter, targeted efforts
- Resistance evaluation of Darling 58 progeny
  - 3 common gardens planted, ESF, Maine, Meadowview

- Look at growth and long term resistance of these trees
- Optimizing propagation of wild trees for germplasm conservation
  - Bring from the forest into orchard environments
  - Increasing diversity in breeding program
  - Traditional grafting proving difficult
  - Researching new methods
    - Dragon Galic
      - Graft onto juvenile sprouts and grow in very dark conditions
    - Grafting onto hypocotyl proto root
    - Painting girdled stump sprouts with rooting hormones
- Scott Merkle – put OxO into additional founder lines
  - Cloning immature embryos
  - Transformations within months
  - ESF helping confirmation of expressions levels
  - Does this require additional regulatory review or an extension of our existing conditions
- Dual Resistance / Rapid Genomics Partnership
  - Cost effective genotyping assay to predict PRR resistance
  - Markers in resistance regions targeted and tested for effectiveness
  - Selecting against founder genome for wild type genomes in Darling 58 outcrosses
    - Markers help to select for more diversity and wild type genome
    - Data in October from first 100 trees
- Chapter orchards visits with Jamie and Israel and did a massive phenotype effort that will be housed in dentataBase
  - Over 100 orchards phenotype for long term blight resistance traits
  - Found trees from each chapter with exceptional blight resistance
    - Best by best crosses to create regional orchards
    - Dual purposes for the trees
      - Offer traditionally bred material in addition to the transgenic bred material
      - OxO for stacked blight resistance
- The Marty Method – nondestructive early screening method for long term blight resistance
  - Cut off tip of a robust seedling and put fungus on tip
    - Let fungus grow
    - Measure resistance and see traits forming
      - What combination of these traits is correlated best with long term resistance?
    - Cut off tip and plant trees in the field
- RESMAP – Map of all of the ways that blight resistance has been measured
  - Ideal is long term resistance (2+ years post inoculation)
  - Need an early screening method that is heritable and correlates with the long term resistance measuring
  - How predictable
- Gene-Edited Future: Open up new pathways; Long term project; Always an unknown – Vasily Infographic
  - Develop a CRISPR pipeline to do gene knockouts in Chinese chestnut
    - Will knocking out candidate genes make the tree more susceptible
    - Inherently easier to do then knowing exactly what DNA changes to make to the American chestnut have the resistance of the Chinese chestnut
    - What about the Chinese chestnut genome is different?
- Gene Editing: Precision Editor
  - Target enzymes to do a “find and replace”

## Q&amp;A Session

- Bruce Levine: Small stem assay standard method is also non-destructive, and comparing both methods. Concerned about how much we are leaning on Darling 58 in the future. Might present a PR problem if it appears that we are “giving up” on breeding. Has anyone tried floral dipping for transforming chestnut? JW: Prior presentations have been heavy breeding. Propose is to select the very best backcross trees and combine the chapter efforts and regionalize crossing your best material. Plant out in regional seed orchards. Use the rest in the darling program for the diversity. Work smarter.
- Debby Delmer: Jared has a really good balance and is not ignoring the value of breeding. When doing the RNA seek, look for compounds that can be biomarkers and new assay for resistance.
- Tom Klak: Evidence found in LSA's? What is the evidence? Can we see and to understand better what's going on in the other methods; Confirmed that they are 100% American, they do have a resistance phenotype, need to look at the backcross traits with non LSA trees.
- Greg Miller: Product TACF is developing is not just a single kind of tree, and we should use “series of prototypes” keeps options open; Spend too much time and attention of where the DNA comes from, we need to spend more time what the DNA does, and how the tree performs.

FIELD AND ORCHARD PRESENTATION – SARA FITZSIMMONS [Link to meeting video and presentation](#)

- Seed and Seedling Production – Going from tree to restoration is going to be an iterative process, we will find something better in the time span it will take an ecological impact with this species and get it restored decades, if not centuries. Near term, getting seed and seedlings so that we can get them into the forest.
  - 5-7 years out to get to 100,000 transgenic out
  - Improving out production capabilities
    - Chapters play a big role
    - Planting material
    - Nursery partnerships
    - Citizen Scientists
- A lot of what we can do is restricted by what we have out there, and the quality of the material
- External Grants – generally small grants meant to jump start larger projects, jumpstart grad students, jumpstart collaborating labs to investigate something that may become a bigger project.
  - Please get reviews in as soon as possible
  - Budgeted for \$50,000
    - In 20 years upwards of \$1,000,000 has been spent
    - 164 Awards
    - 44 Unique Institutions
  - Review committees' reviews and recommend to the Board
  - Resources: <https://www.acf.org/our-work/external-grants/>
  - TACF Magazine: <https://www.acf.org/our-community/magazine-archives/>
- Phenotyping backcross modelling that Jared has done has been phenomenal job
  - Solidifies and galvanizes chapter work
  - 5 – 10 years more work to do
- dentataBase
  - Has come such a long way – so much more useable and will continue to become more useable
  - So important in tracking the future of tracking of the tree and all the work that is being done
    - 800 daily page views
    - ~150 users
    - 370,00 + trees

- 629,000+ tree observations
- Ability to track plantation plantings
- Success measurements
- Metrics
- Engagement at all levels is so important

## Q&A Session

- Nathan Cude: Specific technologies that could help speed up our progress to produce seedlings, can we put out a call for funding? SFF: yes – for example clonal production; JW: Segregation of the gene, and testing every single for gene inheritance. Current processes are very labor intensive. Talking to a company to use antibody strips
- John Hempel: Bartlett Truck in slide are they collaborating? They have, doing a lot of work in CT, CEO is a Penn State alum, so he sends in bucket trucks to help collect material. Had a great meeting with TACF/ESF/Bartlett – what else can they do? Long standing collaboration.
- Greg Miller: More prototype discussion; Recall ability; Modify what we tell the public.
- John Hempel: Greatest finger through the forest is the interstate highway system. Already have rugged fences along the highways.

## MEETING ADJOURNED AT 12:37 PM

*Minutes respectfully submitted by Jamie Van Clief and Cherin Marmon-Saxe - recorder*

DRAFT