Progress Report

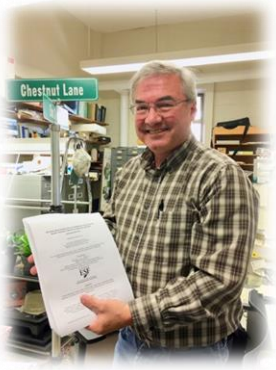
Moving Closer to 10,000 Trees

Activities undertaken between November 1, 2018 and October 31, 2019

As the years go by, we get closer towards our goals. The following is a partial list of highlighted accomplishments in the past year.

- **Big news!** Submitted a revised, 286 page, petition to the USDA APHIS BRS for nonregulated status (**please read first section below on how you can help**).
- Planted 407 T2 (3rd outcrossed generation from original T0 trees) seedlings from last year's harvest. Propagated in tissue culture and then planted 650 T1 transgenic Darling 58 American chestnut trees. Total of 1057 new blight tolerant American chestnut trees planted in 2019 in SUNY-ESF regulated field plots.
- USDA BRAG plots planted - this is a program to start long-term field trials comparing growth and environmental impacts between our transgenic Darling American chestnut and the backcross American chestnut, hybrid chestnut, radiation treated American chestnut (Stronghold program), and wild-type American chestnut trees, in collaboration with TACF, Penn State, and VA Tech universities.
- Expanded diversification to include VA and IN plantings. Cross-pollinated 27 Mother trees with transgenic American chestnut pollen. We harvested 2130 nuts from these crosses with the expectation approximately half will inherit the OxO gene for blight tolerance.
- Planted our expanded seed orchard and holding plot.
- A great new video was made by Templeton World Charity Foundation (who has provided grant funding to support our work) entitled The Chestnut Tree: Bringing Back an American Icon, see it at *https://www.youtube.com/watch?v=-mhMdUryolU&feature=youtu.be*
- Working with collaborators at Hudson Alpha Institute for Biotechnology and TACF, Ellis 1 American chestnut genome has been sequenced and the Darling 58 genome sequencing is in progress.

USDA Submission - major milestone!



Last September, we submitted a first draft of our 188 page petition for non-regulated status of the blight-tolerant American chestnut for a completeness review by the USDA. We received useful feedback in two responses with 15 and 19 pages of questions and format changes. We addressed all the responses and added new data that resulted in our October 2019 286 page submission. It is now in another completeness review, which we expect to pass. Next milestone will be an important open comment period. This is a critical part of the review process and if you want to plant our blight tolerant American chestnut trees, this is another place where we can really use your help. We are asking that you, along with your frends and family, send in your individual comments to the USDA during this period. *We will notify you with another email when we know the dates of the open comment period, and it will also be noted on our project website at* https://www.esf.edu/chestnut/.

Jim Searing from TACF has some suggestions on writing comments. The key is to write what you know and feel about the American chestnut in your own words. Below are Jim's suggestions…

In your own words, consider including these topics in your letter. Start with an opening statement of support (something like we offer here)

> *I support the SUNY-ESF application for releasing blight-tolerant American chestnut for restoration purposes due to…* [then we suggest focusing on the ideas from the list below that best fit your personal experience, as they have shown to be useful in other regulatory reviews. This can be as short or long as you like.]

i. Your historical memory or family experience with the tree
ii. Your knowledge of horticulture or growing hybrid chestnuts
iii. Your knowledge from farming or growing plants
iv. Your concern about the destruction of the forests and what the loss of the tree meant to forest conditions
v. Your desire to encourage or save wildlife, including bird populations
vi. Your desire to hunt
vii. Your experience with wood and building product usage
viii. Your experience with forests, plants, and other scientific knowledge
ix. Your desire to serve as stewards on the land
x. Your concern and desire to help mitigate climate change and promote natural carbon storage
xi. Your desire to save an important species from extinction
xii. Your interests in going above and beyond the backcross program
xiii. Your belief that we should right an environmental wrong caused by human intervention (chestnut blight)
xiv. Your desire to use the American chestnut as a model to save other trees from the threats attacking the forests across the US

Then include a closing statement; e.g., [*I urge the USDA to rule in favor of the Darling 58 blight-tolerant American chestnut to allow the restoration of the American chestnut to the forests of the eastern United States and save the tree from extinction.*]

Thank you for your help. Now that the USDA petition has been written and submitted, we will reformat this document and all its data for submissions to the EPA and FDA.

Outcrossing transgenic Darling 58 American chestnut to wild-type "mother" trees

Graduate student, Hannah Pilkey, has developed an efficient way to collect, store, and distribute the pollen from the blight-tolerant, Darling 58 American chestnut trees. This was tested by sending pollen to TACF orchards in Meadowview, VA and to the IN chapter TACF orchard. These methods were very successful, producing 514 nuts in VA, 525 nuts in IN, and 1091 nuts at ESF in NY, for a total of 2130 nuts from 27 "mother" trees with the expectation approximately half will inherit the OxO gene for blight tolerance. James McKenna wrote, "This pollen, on slides,

was excellent – **the best chestnut pollen that I have ever seen**." These methods will aid the diversification plan outlined in the Westbrook et al., 2019, article listed at the end of this report.

Six USDA BRAG grant plots planted starting long- term comparisons of transgenic and conventionally produced chestnut trees

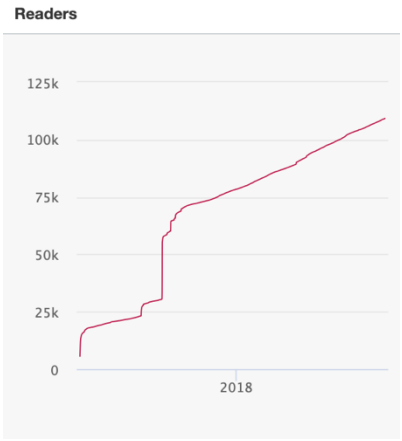
The 10,000 Chestnut Challenge donations are producing blight-tolerant American chestnut trees to establish production orchards and holding plots so we will be ready to distribute seedlings, nuts, pollen, and scions to the public once we have federal regulatory approval. But the production of the trees also supports research and allows us to leverage additional research grants. One such grant we received was from the USDA Biotechnology Risk Assessment Grant (BRAG) program. This grant starts long-term field trials comparing growth and environmental impacts between our transgenic Darling American chestnut and the backcross American chestnut, hybrid chestnuts on the market, radiation treated American chestnut (Stronghold program), and wild-type American chestnut trees, in collaboration with TACF, Penn State, and VA Tech universities. This year open field and shelterwood plots were initiated in three states, NY, VA, and PA with the trees.

Expanding our seed orchard

Last year we increased the size of the Tully orchard by 2/3rds by expanding the fence, irrigation, and removing unwanted rocks. This field site includes a holding plot to plant seedlings waiting for distribution and a seed orchard to produce transgenic nuts. When originally established, it was big enough to plant 50 transgenic American chestnuts and 50 Mother trees. We have doubled the seed orchard area and starting planting second (T1) and third (T2) generation blight-tolerant seedlings and plantlets in the holding plot in preparation for distribution.

American Chestnut Research and Restoration Project public outreach and education

One of the most useful short articles to explain ESF's American Chestnut Research and Restoration Project is still attracting new readers with over 100,000 to date (figure to right). Thank you for sharing and please continue to do so. It can be read at: https://theconversation.com/new-genetically-engineered-american-chestnut-will-help-restore-the-decimated-iconic-tree-52191




THE POST-STANDARD

ESF RESEARCHERS FIND A WAY TO BEAT THE BLIGHT

A **new video** was also made this year by the Templeton World Charity Fund in support of the chestnut restoration. It can be viewed at: https://www.youtube.com/watch?v=-mhMdUryolU&feature=youtu.be

We also continue to get good news coverage. Please keep spreading the word about the American chestnut tree!

Sequenced the Darling 58 blight-tolerant American chestnut genome

Last year we announced that Hudson Alpha Institute for Biotechnology (https://hudsonalpha.org) in collaboration with TACF (https://www.acf.org) and ESF, sequenced the entire Ellis 1 American chestnut genome. Ellis 1 is the cell line established from nuts donated by John Ellis and Jim Donowick (Jim is on the left of the Pond1 tree, the mother of Ellis1, with Allen Nichols, Andy Newhouse, and Linda McGuigan on right). This year they finished the Darling 58 genome, the first blight tolerant American chestnut tree. Now that they have been sequenced, they are going through a process called annotation, where all the genes are identified in the genomes, which should be completed over the next year for Ellis 1, and soon after for Darling 58.

New Publications

Peer reviewed journals:

Brown, A.J., Newhouse, A.E., Powell, W.A. and Parry, D. 2019 Comparative Efficacy of Gypsy Moth (*Lepidoptera: Erebidae*) Entomopathogens on Transgenic Blight-tolerant and Wild-type American, Chinese, and Hybrid Chestnuts (*Fagales: Fagaceae*), *Insect Science* DOI 10.1111/1744-7917.12713

Westbrook, J., Holliday, J., Newhouse, A., and Powell, W. 2019. A plan to diversify a transgenic blight-tolerant American chestnut population using citizen science. Plants, People, Planet https://doi.org/10.1002/ppp3.10061

Book chapter:

Powell WA, Newhouse AE, Coffey V. 2019. Developing blight-tolerant American chestnut trees., In Perspectives on Engineering Plant for Agriculture, P. Arnold Editor, Cold Spring Harb Perspect Biol, doi:10.1101/cshperspect.a034587