Progress Report

Moving Closer to 10,000 Trees
Thank you for your support!

Activities undertaken between November 1, 2017 and October 31, 2018

Here are some highlights of our activities this year. More details follow in the accompanying stories.

- Over 600 T1 transgenic Darling 58 American chestnut trees were propagated in tissue culture and then planted in our regulated field plots.
- We hosted a public Pollination Workshop to instruct attendees how to pollinate American chestnut female flowers with transgenic pollen.
- T1 transgenic Darling 58 American chestnut trees were inoculated with the blight fungus in order to confirm inheritance of blight tolerance.
- A new grant was received from the USDA BRAG program to start long-term restoration 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.
- In September, we submitted the first draft of the documentation for a completeness check as the first step in seeking regulatory approval by the USDA.
- We cross-pollinated 14 Mother trees with transgenic American chestnut pollen. We harvested over 1700 nuts from these crosses with the expectation approximately half will inherit the OxO gene for blight tolerance.
- These nuts were in a tannin study suggested by the FDA preparing for their review.
- We expanded our seed orchard by increasing the deer fencing and irrigation by approximately two-thirds. Planting this new area will begin in the spring 2019.
- Working with collaborators at HudsonAlpha Institute for Biotechnology and TACF, we have sequenced the entire Ellis 1 American chestnut genome.
- Had a short video made explaining the American Chestnut Research and Restoration Project in a "Nutshell": https://www.youtube.com/watch?v=ANSn1Mw2LbM&feature=youtu.be.

Propagated and planted over 600 T1 transgenic Darling 58 American chestnut trees

In 2016, our team produced eight T1 trees expression the oxalate oxidase (OxO) enzyme. These trees are the offspring of controlled pollinations done with Darling 58 transgenic pollen to non-transgenic Mother trees. In 2017, we established two of these eight lines in tissue culture. This year, we rooted, acclimatized, and planted over 600 from these two T1 lines. Many of these trees will be grown for distribution once we have regulatory approval.

Pollination Workshop

As we prepare for the release of our transgenic trees, we are considering all options for how to distribute the plant material. These options include sending out seedlings, scions, and pollen. To help those interested in receiving pollen, we put on a workshop to instruct attendees how to pollinate American chestnut female flowers with transgenic pollen. Nineteen people attended the workshop and activities included learning pollen theory, hands-on pollination of Mother Trees with transgenic pollen, making and using pollination bags, a demo of collecting American chestnut pollen from a bee hive, a demo on grafting, and a harvesting demo. We intend to run this workshop biennially for those who are interested in receiving transgenic pollen.

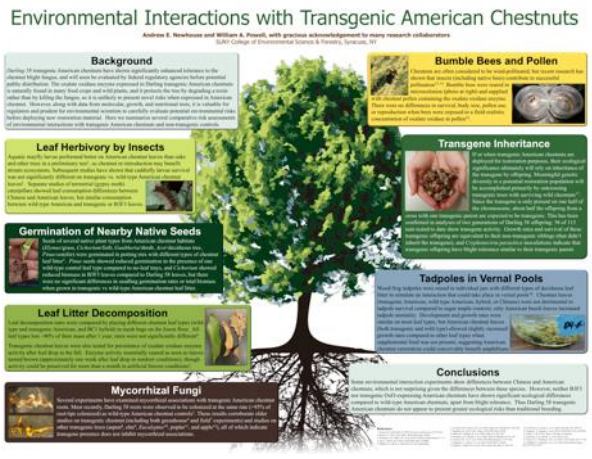
T1 transgenic Darling 58 American chestnut tree assays

A team of faculty, students, and staff inoculated first generation transgenic American chestnut crosses (T1s) with a highly virulent form of the blight fungus. Two tests were performed, one with small stem potted seedlings, and one with two-year-old planted trees. For the potted plants, 36 Darling 58s, 16 non-transformed (NT) American chestnuts, and eight Chinese chestnuts were inoculated. The height of the cankers was significantly different between D58, Chinese, and NT American. Girdling was also significant; after 29 days, ALL NT American stems were girdled, while NO D58 stems were girdled. This demonstrates the inheritance of blight tolerance.

For the planted T1 seedling inoculations, three Darling 58 T1's, one Darling 54 T1, and four full-sibling non-transgenic controls were inoculated. After 30 days, the canker area was significantly different, however, there was no wilt seen on the susceptible controls. This is still preliminary and we're waiting to see what happens over the winter.

Preparing USDA Submission

In September, we've submitted the 188 page first draft of our petition for non-regulated status and 2700 pages supporting documentation for a completeness review by the USDA. The documentation has taken a long time to prepare due to the many components that need to be included. Many tests were done to make sure the new transgenic tree was not significantly different than the non-transgenic controls. You can see a summary of these tests at: https://www.esf.edu/chestnut/poster.htm.



The USDA will review the documentation for completeness and let us know if we need to include anything else before the data review begins.

Cross pollinated Mother trees with transgenic American chestnut pollen

This year, we harvested **1701** nuts from crosses with Darling 58 T1 pollen. So far, we've cored 75 nuts from the Fert9T2 mother tree, and 33 were 0x0 positive (expected 37.5, chi-square indicates we're statistically within the expected half range). We're confident most of these were pre-bagged on time before female flowers ripened, so we shouldn't be seeing much if any open pollination. Together, with the D58 pollinations we checked in 2017, **we've cored a total of 115 nuts from D58 crosses, and 57 were 0x0 positive**: almost exactly half, as expected! The remaining will be tested as seedlings in the spring.

Expanding our seed orchard

One of our field sites was established as a seed orchard to produce transgenic nuts. When originally established, it was big enough to plant 50 transgenic American chestnuts and 50 Mother trees. Over the past summer, we've increased the size of the orchard by 2/3rds by expanding the fence, irrigation, and removing unwanted rocks. The irrigation system was also updated with a new pump, new piping, and new seals.

American Chestnut Research and Restoration Project in a "Nutshell"

To help with public outreach and increase interest in American chestnut restoration, a short 3-minute video was made explaining the American Chestnut Research and Restoration Project. Please share widely.

It can be viewed at: https://www.youtube.com/watch?v=ANSn1Mw2LbM&feature=youtu.be

Sequenced the Ellis 1 American chestnut

Working with collaborators at HudsonAlpha Institute for Biotechnology (https://hudsonalpha.org) and TACF (https://www.acf.org), the entire Ellis 1 American chestnut genome has been sequenced. 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 on right) The Ellis cell line was used to produce the first blight tolerant 'Darling' American chestnut trees. This Ellis 1 genome sequence will provide a significant resource for current and future research on the American chestnut and related species.

Publications

Goldspiel, H. B., Newhouse, A. E., Powell, W. A., & Gibbs, J. P. (2018). Effects of transgenic American chestnut leaf litter on growth and survival of wood frog larvae. *Restoration Ecology*, *0*(0). https://doi.org/10.1111/rec.12879

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